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THE EFFECT OF DIET ON VASCULAR RESPONSES TO MENTAL STRESS

by

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ABSTRACT

Stress is increasingly prevalent in society. There is evidence that mental stress is related to an increased risk for cardiovascular disease, and can trigger myocardial infarction and stroke. Vascular responses to stress have been implicated as an underlying mechanism. Stress also influences health behaviours, such as dietary choices and physical activity. Specific nutrients and engagement in physical activity have been shown to influence the vasculature, yet their effect on peripheral and cerebral vascular function in the context of mental stress is unclear. A scoping review first established literature investigating the impact of dietary interventions on vascular responses to mental stress (Chapter 2). Following a repeatability study (Chapter 3) for the primary assessment of endothelial function (brachial flow-mediated dilatation) and ultrasound assessment of cerebral vasculature (common carotid artery blood flow), this thesis investigated the effect of saturated fat and flavonoid-rich cocoa interventions on vascular responses during stress and endothelial function following mental stress in a healthy population (Chapters 4 – 6). The relationship between stress and health behaviours was then explored in a free-living environment (Chapters 7 – 8). The findings of this thesis reported saturated fat consumption to impair the recovery of endothelial function following stress and attenuate cerebral oxygenation during stress. Furthermore, consuming flavonoid-rich cocoa with saturated fat counteracted the negative impact of fat consumption on the stress-induced decline in endothelial function. However, flavonoid-rich cocoa did not influence cerebral oxygenation during stress following fat consumption. In a free-living environment, the data suggests perceived stress positively associated with flavonoid consumption. Similarly, stress-related psychological outcomes (positivity and fatigue) were positively related to

consumption of fat, saturated fat, and sugar. Stress was not associated with engagement in physical activity yet engaging in physical activity associated with improved perceptions of coping with stress and feeling more on top of things. The findings of this thesis suggest that health behaviours adopted during periods of stress may worsen or mitigate the impact of stress on vascular function and modify how we psychologically cope with stress. Future work is required to understand how to drive behaviour change to gain the most vascular protection during stress or, alternatively, investigate the potential of chronic dietary and exercise interventions to build vascular resilience during periods of heightened stress.

Keywords: Stress, Vascular function, Diet, Physical activity

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Definition
AIC	Akaike's information criterion
Akt	Protein kinase B
ANOVA	Analysis of variance
ASL	Arterial spin labelling
BMI	Body mass index
BP	Blood pressure
Ca^{2+}	Calcium
CAD	Coronary artery disease
CBF	Cerebral blood flow
CCA	Common carotid artery
CCK	Cholecystokinin
CCO	Cytochrome-c-oxidase
CHD	Coronary heart disease
CI	Confidence intervals
CO	Cardiac output
CONSORT	Consolidated Standards of Reporting Trials
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EDHF	Endothelium derived hyperpolarizing factor
eNOS	Endothelial nitric oxide synthase
EPIC	European Prospective Investigation into Diet and Cancer
ET-1	Endothelin-1
FBF	Forearm blood flow
FETA	FFQ EPIC Tool for Analysis
FFQ	Food frequency questionnaire
FMD	Flow-mediated dilatation
FVC	Forearm vascular conductance
HFC	High-flavanol cocoa
HFM	High-fat meal
HHb	Deoxyhaemoglobin
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRV	Heart rate variability
ICC	Intraclass correlation coefficient
ICG	Impedance cardiogram
IL-6	Interleukin-6
IPAQ	International physical activity questionnaire

Abbreviation	Definition
L-NMMA	NG-monomethyl-L-arginine
LFC	Low-flavanol cocoa
LFM	Low-fat meal
LL	Log likelihood
MAP	Mean arterial pressure
MET	Metabolic equivalent
MI	Myocardial infarction
MMP-9	Matrix metalloproteinase 9
MRI	Magnetic resonance imaging
NHS	National health service
NIRS	Near infrared spectroscopy
NO	Nitric oxide
nTHI	Total haemoglobin normalised to the initial value
O_2Hb	Oxyhaemoglobin
P13K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PA	Physical activity
PASAT	Paced auditory serial addition task
PEP	Pre-ejection period
PFC	Pre-frontal cortex
PGI_2	Prostacyclin
PICOT	Population, Intervention, Comparison/Control Group, Outcome,
PKA	Protein kinase A
POMS	Profile of mood states
PR	Pulse rate
PSS	Perceived stress scale
PUFA	Polyunsaturated fatty acids
PWV	Pulse wave velocity
ROS	Reactive oxygen species
Sat	Saturated
SBP	Systolic blood pressure
SD	Standard deviation
SV	Stroke volume
TAG	Triglycerides
TCD	Transcranial doppler
TMD	Total mood disturbance
TNF-α	Tumour necrosis factor alpha
TOI	Total oxygenation index
TPR	Total peripheral resistance
TXA_2	Thromboxane
UK	United Kingdom
VCAM1	Vascular cell adhesion molecule 1
η_p^2	Partial eta squared

1. General Introduction

We live in an increasingly stressful society, with three out of four adults reporting to have felt so stressed they are unable to cope (Mental Health Foundation, 2018b). Stress has been associated with an increased risk for cardiovascular diseases (CVD), and the vasculature has been suggested to play a role. Stress has been reported to influence health behaviours, such as diet and physical activity (PA), which can also impact vascular function. Therefore, the possible clinical consequences of regular stress exposure are substantial.

This introduction will provide a background to the evidence of the associations between stress and CVD, measures of vascular function, as well as health behaviours, followed by an overview of the aims of the thesis.

1.1. Cardiovascular diseases: Incidence and prevalence

CVD are the leading cause of death globally (World Health Organisation, 2020), and since 1997 the number of people living with heart and circulatory diseases has doubled (British Heart Foundation, 2024). The cardiovascular system consists of the heart and blood vessels (vasculature), and there is a range of conditions that may affect the circulatory system, collectively termed CVD (Farley et al., 2012). These include coronary heart disease (CHD) which affects blood vessels supplying the heart, as well as cerebrovascular disease which refers to blood vessels supplying the brain. Furthermore, peripheral arterial disease affects blood vessels supplying the arms and legs, and atherosclerosis is a build-up of fatty plaque within the coronary arteries (Scarborough et al., 2010). Another form of CVD is myocardial infarction (MI), which is an acute event caused by an arterial blockage preventing perfusion of the heart, estimated to cause 100,000 hospital admissions each year in the UK (British Heart

Foundation, 2024). Another acute event is a stroke, which is most commonly caused by an arterial blockage which prevents blood flow to the brain (ischaemic) or by bleeding in or around the brain (haemorrhagic), which is the biggest cause of severe disability in the UK and twice as likely in people with CHD or who have had a MI (British Heart Foundation, 2024). Therefore, the societal and economic impact of CVD is significant.

1.2. Vascular function

The vasculature plays an important role in CVD, and it is therefore not surprising that the development and progression of CVD has been related to endothelial dysfunction (Hadi et al., 2005). The endothelium is a dynamic organ which lines the vascular system and controls vascular function and blood flow to different organs (Sandoo et al., 2010). The vascular wall of a blood vessel is made up of three layers: the intima (inner layer), the tunica media (middle layer) and the tunica externa (outer layer) (Levick, 2003). Endothelial cells are located on the intima of all vessels but show morphological and physiological differentiations depending on the vessel, i.e., arterial (macrovascular), or venous or capillary (microvascular) (Ghitescu and Robert, 2002).

Endothelial cells respond to various hormones, neurotransmitters, and vasoactive factors to maintain vascular homeostasis (Sandoo et al., 2010). For example, these can be vasodilatory factors such as nitric oxide (NO), prostacyclin (PGI_2), and endothelium derived hyperpolarising factor (EDHF) or vasoconstrictive factors such as endothelin-1 (ET-1) and thromboxane (TXA_2). NO is a key endothelium-dependent vasodilator which plays an important role in the maintenance of basal vasodilator tone of the blood vessels (Vallance et al., 1989). NO is produced in the vasculature under

the influence of endothelial nitric oxide synthase (eNOS), which converts the amino acid L-arginine to NO (Palmer et al., 1988). Increased intracellular levels of calcium (Ca^{2+}) activates eNOS (Bucci et al., 2000), which then causes eNOS phosphorylation (Butt et al., 2000), initiated by shear stress and protein kinases (Boo et al., 2002). On the other hand, ET-1 is a vasoconstrictor which is decreased by NO (Alonso and Radomski, 2003). The balance between NO and ET-1 production is crucial in maintaining endothelial function (Cardillo et al., 2000). Inflammation is another mechanism which can reduce NO availability, with inflammatory proteins (C-reactive protein (CRP) and Tumour Necrosis Factor alpha (TNF-α)) downregulating the expression of eNOS and activating ET-1 (Wadley et al., 2013). Whilst inflammation upregulates inducible NOS (iNOS), the NO produced from iNOS is toxic and susceptible to oxidative reactions (Guzik et al., 2003). Inflammation is paralleled by higher levels of oxidative stress, and reactive oxygen species (ROS) can scavenge NO from the endothelium, which also drives endothelial dysfunction (Wadley et al., 2013). Therefore, disruption or an imbalanced control of these factors leads to endothelial dysfunction, which is an early indicator for CVD (Widmer and Lerman, 2014).

1.3. Assessments of vascular function

Direct assessment of vascular function in the coronary arteries is invasive and carries significant risk to the participant. Therefore, vascular function is usually assessed in the peripheral circulation, as a close correlation between peripheral and coronary endothelial function has been reported (Anderson et al., 1995). Most assessments of vascular function involve measuring the dilation of blood vessels in response to a

stimulation, and impaired vasodilation indicates poorer endothelial function (Sandoo et al., 2010). The two main techniques used to assess vascular function in the periphery, and utilised in this thesis, are flow mediated dilatation (FMD) and forearm blood flow (FBF). These techniques are used to examine various peripheral vascular beds, as FMD assesses macrovascular function (brachial artery) whilst FBF assesses microvascular function (forearm resistance vessels). This thesis also uses techniques to examine cerebrovascular function, again including a measure of macrovascular function (common carotid artery blood flow), and microvascular function (oxygenation of pre-frontal cortex).

FMD of the brachial artery is the most common assessment of endothelial function. This technique uses duplex doppler ultrasound to image the brachial artery and measure changes in arterial diameter following a temporary occlusion of the vessel. The occlusion (induced by a cuff on the forearm inflated to 220 mmHg for 5 minutes) reduces blood flow and induces tissue ischaemia. When the cuff is released, there is a sudden increase in blood flow (reactive hyperaemia) which exerts shear stress on the endothelial cells and stimulates NO release. The subsequent vasodilation following cuff release is measured, and the maximum percentage change in arterial diameter relative to baseline diameter is calculated to provide an FMD score. FMD has been shown to be a good surrogate marker of NO bioavailability (Sandoo et al., 2010). A lower FMD indicates poor endothelial function, and this has been related to CVD risk. For example, a meta-analysis reports that a 1 % decrease in FMD associates with a 9 – 13 % increase in risk of future cardiovascular events (Inaba et al., 2010, Green et al., 2011). The protocol to accurately assess FMD is standardised, and is influenced by the experience of the sonographer, protocol and set-up, as well as participant

preparation and external influences (Thijssen et al., 2019). Therefore, rigorous sonography training and a repeatability study is required prior to using FMD in research (described in Chapter 3).

FBF is a commonly used assessment of vasodilatory responses within the blood vessels of the forearm, measured by venous occlusion plethysmography. This assessment stops venous return from the forearm (using a cuff on the upper arm, inflated to 40 mmHg for 5 seconds at a time), while allowing arterial inflow. Blood enters the forearm but venous occlusion prevents blood returning back to the heart, which results in a linear increase in forearm volume proportional to the incoming arterial blood flow (Sandoo et al., 2010). The increase in forearm volume with each 5 second inflation is assessed by a mercury in rubber strain-gauge plethysmograph placed around the most muscular part of the forearm (Sandoo et al., 2010). An increase in the length of the strain-gauge represents an increase in FBF, and hence, vasodilation. Several mechanisms are thought to contribute to this vasodilation, such as activation of cholinergic vasodilator nerves (Blair, 1959), β-adrenergic receptors (Halliwill et al., 1997) and the sympathetic nervous system following increases in adrenaline for example (Black and Garbutt, 2002). However, the most consistent mechanism for forearm vasodilation is NO, which is disrupted by inflammation and vasoconstrictive factors (Joyner, 2001). An advantage of FBF is that it can be assessed in response to a specific stimulus such as exercise, reactive hyperaemia, mental stress, and pharmacological stimuli known to influence vasodilation/constriction (Joyner, 2001).

Given the prevalence of cerebrovascular diseases and stroke, this thesis also includes investigations into the cerebral vasculature, using near infrared spectroscopy (NIRS)

of the prefrontal cortex and blood flow through the common carotid artery (CCA). NIRS uses spatially resolved spectroscopy to measure changes in chromophore concentrations of oxyhaemoglobin and deoxyhaemoglobin, providing measures of oxygen saturation, and overall prefrontal cortical haemodynamics (Davies et al., 2015). Another assessment of the cerebrovasculature uses duplex doppler to measure blood flow and diameter of the CCA (Thomas et al., 2015), an extracranial artery which supplies the brain. The combination of prefrontal cortex oxygenation (intracranial) and CCA blood flow (extracranial) assessments are complementary and provide a more complete picture of the cerebral vasculature. A repeatability study of CCA blood flow is also presented in Chapter 3. Cerebral oxygenation and blood flow provide an indication of cerebral blood flow (CBF), which is affected by cerebral perfusion pressure and cerebrovascular resistance (Suppan et al., 2022). Importantly, CBF is regulated by intrinsic (autonomic, smooth muscle cells, myogenic, and cerebral metabolism) and systemic (blood pressure and cardiocirculatory parameters, blood gases, and ventilation) factors to ensure maintenance of cerebral perfusion (Koep et al., 2022).

1.4. Mental stress

An early definition of stress that is still commonly used states that “stress is the nonspecific response of the body to any demand made upon it” (Selye, 1974, p. 137), which encompasses both physically and psychologically-induced stress. This thesis will focus on mental stress and thus a more specific definition of psychological stress used in this thesis is the feeling which arises when the demands of a situation outweigh the individual’s ability to cope with these demands (Fink, 2016). This feeling can be

acute and chronic, both of which have been linked to cardiovascular health and wellbeing (Steptoe and Kivimäki, 2012, Schneiderman et al., 2005). Acute stress incorporates a single instance of stress which is short in duration and often in response to an external stimulus, whilst chronic stress can be defined as the prolonged exposure to stress which can relate to a number of long-term stimuli such as sustained work pressure, major life changes or illness (Chu et al., 2022). The mechanisms by which acute stress and chronic stress impact health are thought to be different. For example, acute stress induces a cascade of changes in the cardiovascular, nervous, endocrine, and immune systems, which are generally adaptive in young, healthy individuals. Yet, these physiological responses can become maladaptive if the stress response is continually activated (i.e., chronic stress), or in individuals who are biologically vulnerable (Schneiderman et al., 2005). Furthermore, both acute and chronic stress can worsen psychological health, shown by their association with the onset of major depression (Hammen et al., 2009). Importantly, higher levels of chronic stress not only predict higher levels of acute stress, but can amplify the impact of acute stress on mental health (Hammen et al., 2009).

This thesis will focus only on acute mental stress, as it has been identified as a trigger for MI (Strike and Steptoe, 2005). Epidemiological studies have reported this relationship between stressful events and MI. For example, during the Iraqi missile war in Croatia, the frequency of MI and MI-related mortality increased in civilians of Zagreb city (Bergovec et al., 1992). Furthermore, another study reported a significant increase in the number of admissions for MI during the week after the Northridge earthquake (Leor and Kloner, 1996). Even sports matches have been suggested to trigger MI, shown by a 25 % increase in the risk of admission for acute MI the day England lost to

Argentina in the football World Cup penalty shoot-out and up to two days later (Carroll et al., 2002). A similar rise in MI-related deaths was reported when supporters' local club team lost at home (Kirkup and Merrick, 2003). However, winning seems to have an opposite effect, with lower MI-related mortality in French men on the day France won the Football World Cup compared to other days (Berthier and Boulay, 2003). In each of these studies, an increase in emotional stress was implicated.

The mechanisms underlying the stress-induced triggering of MI have been explored. One epidemiological study assessed serological markers of inflammation and vasoconstriction in those admitted for MI during the Football World Cup, and reported higher levels of inflammatory markers (TNF-α), adhesion molecules (vascular cell adhesion molecule-1, VCAM1), and vasoconstrictors (ET-1) compared to a group of patients admitted for MI during the World Cup with no emotional stress and compared to a healthy control group (Wilbert-Lampen et al., 2010). These mechanisms have been explored in more detail using controlled laboratory studies, using mental stress-induced myocardial ischaemia as an outcome measure. Mental stress-induced ischaemia in patients with coronary artery disease (CAD) (Strike and Steptoe, 2003) which can predict ambulatory ischaemia (Blumenthal et al., 1995) and future cardiac events (Babyak et al., 2010). Therefore, mental stress-induced myocardial ischaemia in laboratory settings has been suggested as an appropriate proxy measure of MI and provides an opportunity to explore factors that could contribute to the triggering of MI in a controlled experimental setting. This has shown that mental stress-induced ischaemia is related to vascular responses during stress. For example, mental stress-induced ischaemia is associated with an attenuated vasodilatory response to stress in

CAD patients (Burg et al., 2009) and increased vascular resistance in CVD patients (Goldberg et al., 1996, Jain et al., 1998).

It is well documented that mental stress induces immediate increases in heart rate (HR) and blood pressure (BP), driven by activation of the sympathetic nervous system and withdrawal of the parasympathetic nervous system (Turner, 1994). Physical stress (e.g., exercise) also induces cardiovascular changes, yet whilst in response to exercise the increase in HR and BP is metabolically necessary, the metabolic demand of undergoing mental stress does not justify these cardiovascular perturbations (Carroll et al., 2009). Acute stress can be induced passively, for example using a cold pressor test (immersing a limb in cold water), or actively, such as using mental arithmetic, public speaking or interview, and Stroop tasks. These active mental stress tasks have been developed to enhance the stressfulness and subsequent physiological response, using elements of social evaluation, competition, time pressure and increasing difficulty (Veldhuijzen van Zanten et al., 2002). As a result, stress tasks which are not provocative enough to induce a sufficient physiological response have been criticised.

As well as a cardiovascular response (i.e. increases in HR and BP), stress also increases peripheral vasodilation (measured by FBF in this thesis) (Joyner and Halliwill, 2000), and notably, this vasodilatory response to stress is attenuated in populations at risk of CVD, such as obesity (Hamer et al., 2007), and clinical populations with heart failure (Middlekauff et al., 1997) or systemic inflammation (Veldhuijzen van Zanten et al., 2008). An attenuated vasodilatory response is also reported in healthy individuals with poorer endothelial function, as shown by an increased vascular resistance to stress (Sherwood et al., 1999). NO has been

established to contribute to the rise in FBF during mental stress (Dietz et al., 1994), as blocking NO production results in an attenuated vasodilatory response (Cardillo et al., 1997). The increase in shear stress on the vessel wall during stress activates eNOS to cause NO-mediated dilation, which is required to relieve the pressure on the vascular wall (Puzserova and Bernatova, 2016b). Inflammation has also been reported to mediate NO production, as experimentally induced inflammation (vaccination/eccentric exercise) resulted in an attenuated vasodilatory response during stress (Paine et al., 2013a, Paine et al., 2014).

Acute stress follows a time course whereby increased sympathetic nervous activity and vasodilatory responses occur immediately after stressor onset (Poitras and Pyke, 2013). FBF can be assessed in response to a stimulus, thus providing a measure of these vasodilatory responses during stress. Due to the level of standardisation and control required for the FMD assessment, FMD cannot be assessed during mental stress. However, acute mental stress continues to impact the vasculature following the stressful event, with elevations in cortisol peaking 20 – 40 minutes post-stress (Poitras and Pyke, 2013). Cortisol is the primary effector of the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the physiological response to mental stress in combination with sympathetic nervous system activity (Poitras and Pyke, 2013). Therefore, FMD can be used to capture stress-induced changes to endothelial function following the stressful event, when haemodynamic have returned to baseline.

Mental stress has been shown to lead to transient declines in endothelial function (measured by FMD) post-stress (Poitras and Pyke, 2013). The first study to present this reported a 2.2 % decline in brachial FMD at 30 minutes post-stress, which

remained impaired for 90 minutes, but returned to baseline after 240 minutes following stress (Ghiadoni et al., 2000). Since, a number of studies have demonstrated a similar stress-induced decline in FMD in healthy subjects from 10 – 90 minutes post-stress (Broadley et al., 2005, Spieker et al., 2002, Jambrik et al., 2005, Lind et al., 2002). However, some studies have presented no change in brachial FMD following stress (Dyson et al., 2006, Harris et al., 2000). Furthermore, the impact of mental stress on endothelial function in clinical populations is less understood. Populations with hypercholesterolemia (Gottdiener et al., 2003) and Type 2 Diabetes Mellitus (Ghiadoni et al., 2000) with lower baseline endothelial function did not present a further impairment in FMD, yet a stress-induced impairment in brachial FMD was reported in postmenopausal women with a history of Type 2 Diabetes Mellitus (Wagner et al., 2012). Therefore, baseline endothelial function may influence the effect of stress on FMD.

The mechanisms driving an impairment in endothelial function following stress are connected to the vascular responses during stress. As such, stress induces immediate increases in corticotropin-releasing hormone (CRH), the major regulatory hormone of the HPA axis released from hypothalamus, followed by increases in cortisol released from the adrenal cortex. These, as well as increased sympathetic nervous activity and inflammatory markers in response to stress, likely affect endothelial function following stress (Poitras and Pyke, 2013). For example, CRH has been shown to stimulate ET-1 and cell adhesion molecules (Wilbert-Lampen et al., 2006), having a vasoconstrictive effect on the vasculature. Similarly, the increase in cortisol likely contributes to the impairment in endothelial function as pharmacological inhibition of cortisol production prevented mental stress-induced endothelial dysfunction (Broadley et al., 2005).

Furthermore, cortisol reactivity during stress has been correlated with changes in FMD following stress (Plotnick et al., 2017a). Cortisol may impact endothelial function directly by inhibiting eNOS (Wallerath et al., 1999), and indirectly by enhancing ROS production (Iuchi et al., 2003) and ET-1 (Spieker et al., 2002), shown to reduce NO bioavailability. Pro-inflammatory cytokines which are also upregulated during mental stress have been similarly reported to inhibit NO production (Clapp et al., 2004). Therefore, NO is a proposed mechanism in both vascular responses during stress and endothelial (dys)function following mental stress.

Acute mental stress has been identified as a trigger for stroke (Prasad et al., 2020). Research investigating the effect of mental stress on the cerebral vasculature is limited. However, chronic stress has been shown to affect the functional processing of the pre-frontal cortex (PFC) and impair attention control (Liston et al., 2009). Furthermore, acute mental stress increases cerebral blood velocity (Shoemaker et al., 2019), and PFC tissue oxygenation (Nagasawa et al., 2020), indicative of an increased cerebral blood flow. As with the periphery, systemic increases in BP and cardiac output, and NO-mediated vasodilation are suggested to mediate the increase in cerebral blood velocity and perfusion during stress (Bonvento et al., 1994, Iadecola and Zhang, 1994, Shoemaker et al., 2019). Crucially, vascular dysfunction may attenuate the cerebrovascular response to stress, as an impaired cerebral blood flow response to stress was reported in people with hypertension (Naqvi and Hyuhn, 2009). Furthermore, blunted blood velocity to the middle cerebral artery has been associated with a higher CVD risk score (Perdomo et al., 2020). However, one study presented no correlation between endothelium-dependent vasodilation in the internal carotid

artery and brachial artery (Carr et al., 2020), and therefore the association between peripheral and cerebral vascular function is not fully understood.

In summary, given the relationship between poorer peripheral and cerebral vascular responses during stress, endothelial dysfunction following stress, and cardiovascular events, there is a need to explore interventions which could reduce the negative impact of mental stress on vascular function and health.

1.5. Stress and health behaviours

Stress has been reported as a risk factor for obesity, with a review of longitudinal studies reporting stress to associate with increased adiposity (Wardle et al., 2011). Unfortunately, obesity also increases the risk for CVD (Powell-Wiley et al., 2021), with poor diet accounting for 32 % of the risk for CVD (British Heart Foundation, 2024). Stress can directly influence weight gain, shown by a slower rate of fat oxidation during stressful periods (Kiecolt-Glaser et al., 2015). Furthermore, stress indirectly relates to obesity through changes in behaviour. For example, a review reports higher stress to associate with less healthy dietary behaviours as well as increased body weight (Moore and Cunningham, 2012). Therefore, stress-related eating has received significant attention in research. Specifically, a systematic review and meta-analysis includes a considerable number of studies that show a significant association between stress and increased consumption of unhealthy foods and decreased consumption of healthy foods (Hill et al., 2021). A similar pattern has been reported in cross-sectional and prospective studies (Newman et al., 2006, Roberts et al., 2014b, Oliver and Wardle, 1999), as well as laboratory studies which reported that participants opted for high-fat and high-sugar foods over fruit following stress (Zellner et al., 2006).

One mechanism underlying stress-induced eating relates to reward theory. This theory postulates that during stressful periods, changes in glucocorticoids (e.g., cortisol) and corticotropin-releasing factor activate areas of the brain associated with reward, inducing a drive to eat high-energy and highly palatable foods (Cottone et al., 2009). Consumption of these foods then triggers a hedonic experience, creating a positive feedback loop whereby highly palatable foods are perceived as especially rewarding during periods of stress (Hill et al., 2021). Furthermore, stress-induced eating is driven by habits, making food choices during stressful periods automatic and uncontrolled (Pool et al., 2015). Thus, the frequent consumption of highly palatable foods (i.e., saturated fat and simple sugars) characteristic of a western diet (Gao et al., 2021), perhaps exaggerates this stress-induced change to poor diet in western populations (such as the UK). Importantly, overweight individuals are more affected by stress-induced overeating (Cotter and Kelly, 2018), creating a viscous cycle between stress and obesity.

There is evidence that stress can influence other health behaviours. For example, a review investigated the relationship between stress and PA and reports 76.4 % of studies showed stress prospectively predicted lower levels of PA (Stults-Kolehmainen and Sinha, 2014). However, some studies reviewed (17.2 %) report a positive association between stress and PA, suggesting PA can be used as a coping mechanism during stress (Stults-Kolehmainen and Sinha, 2014). PA is beneficial for cardiovascular health (Nystoriak and Bhatnagar, 2018), cognition, and mental health (Lubans et al., 2016). More specifically, exercise training can induce improvements in both conduit and resistance artery NO-mediated function (assessed via FBF and FMD) (Green et al., 2004) and physical inactivity is associated with enhanced vasoconstrictor

tone and rapid changes in arterial structure (Thijssen et al., 2010). Indeed, physical inactivity is a known modifiable risk factor for CVD (British Heart Foundation, 2024). Therefore, stress directly relates to CVD and can indirectly lead to poor health behaviours (unhealthy diet and reduced PA) which also increases the risk of obesity, and subsequent CVD.

1.6. The impact of diet on vascular function

1.6.1. Saturated fat

One nutrient consumed in excess during stressful periods is saturated fat. Saturated fatty acids are simple linear chains of singly-bonded carbon atoms and whilst their structure is simple (Figure 1.1), saturated fatty acids have a diverse effect on health (Dayrit, 2023). Fatty acids are major components of triacylglycerols (triglycerides, TAG), phospholipids, and other complex lipids. Triglycerides, and subsequently fatty acids, are the main contributors to dietary fat in humans, shown to contribute to CVD and metabolic diseases such as Type 2 Diabetes, inflammatory diseases, and cancers (Calder, 2015). Specifically, saturated fat has been reported to impair brachial endothelial function (1.0 – 5.6 % decreases in FMD) for up to 8 hours post-consumption in healthy and clinical populations (Vogel et al., 1997, Rendeiro et al., 2016, Fard et al., 2000). The effect of saturated fat on microvascular blood flow remains to be determined. One study reported an increase in FBF following the fatty meal, which were associated with postprandial changes in triglycerides, insulin, and high-density lipoprotein (HDL) cholesterol (Raitakari et al., 2000). However, this study found no effect of fat consumption on FMD, which is contradictory to other literature. The mechanisms by which saturated fat impacts vascular function have not been fully

elucidated. However, hypertriglyceridemia following fat consumption has been shown to stimulate ET-1, ROS, and inflammatory markers, which together reduce endothelium-derived NO (Bae et al., 2001, Steinberg et al., 1997, Tsai et al., 2004).

The effect of saturated fat on the cerebral vasculature is not well understood. Cognitive impairments have been evidenced following a chronic high-fat diet in rats (Winocur and Greenwood, 2005), and a review reports an association between increased fat and greater incidence of dementia in older adults (Kalmijn, 2000). Evidence of acute fat consumption in humans is limited. However, one study reported no change in cerebral perfusion or conductance following fat consumption (Patik et al., 2018), whilst another found a decrease in cerebral blood flow to the hypothalamus following fat consumption (Frank et al., 2012). As with the peripheral vasculature, fat-induced increases in inflammation and oxidative stress have been suggested to play a role in the effect of fat on the cerebral vasculature (Freeman et al., 2014).

The effect of saturated fat on peripheral and cerebral vascular function in the context of mental stress is not known, but given the stress-induced drive to consume fatty foods, and the comparable modes by which stress and fat can impair endothelial function, the consequence on health could be significant and warrants investigation.

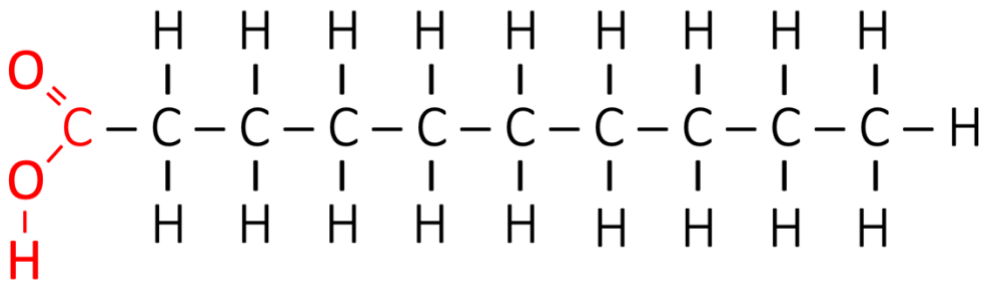


Figure 1.1 Chemical structure of a saturated fatty acid (Akoh and Min, 2008)

1.6.2. Plant-derived flavonoids

Flavonoids are naturally-occurring small polyphenolic compounds which are found in fruits, vegetables, red wine, chocolate, and teas (Manach et al., 2004). Flavonoids can be further divided, based on the degree of oxidation of the heterocyclic ring, the hydroxylation pattern of the ring structure, and the substitution in the three-position, into subclasses (Rendeiro et al., 2012). The main subclasses are anthocyanins (mostly found in red wine and berries), flavanols (in green tea, red wine, and cocoa), flavonols (in onions, leeks, and broccoli), flavones (in parsley and celery), isoflavones (in soya), and flavanones (in citrus fruits and tomatoes). The dietary group of flavonoids investigated in this thesis are cocoa flavanols (Figure 1.2). Cocoa flavanols have received particular attention. This is due to their positive effect on Kuna Indians (population living off the coast of Panama), who consume more raw cocoa which associated with a lower cardiovascular mortality and age-dependent hypertension compared to those who migrated to urban Panama City (Corti et al., 2009, Hollenberg et al., 2009). This hypothesis was confirmed by higher urinary flavanol and NO metabolites in Kuna Indians living on the island compared to in the urban city (Schroeter et al., 2006). Experimental studies have examined the effects of cocoa flavanols on different aspects of health, and specifically endothelial function. Chronic intake of cocoa flavanols has been reported to improve brachial FMD in healthy young and older adults, and at-risk populations (Sansone et al., 2015, Heiss et al., 2015, Heiss et al., 2010, Balzer et al., 2008). Similarly, acute ingestion of cocoa flavanols has been shown to increase endothelial function within 1 – 3 hours of intake, increasing brachial FMD by 1.4 – 5.7 % in healthy adults (Schroeter et al., 2006, Sansone et al., 2017, Monahan et al., 2011, Faridi et al., 2008, Vlachopoulos et al., 2005), and by 1.5

– 3.8 % in diabetics (Balzer et al., 2008), hypertensives (Grassi et al., 2005), and CAD patients (Heiss et al., 2010). Furthermore, two-week cocoa flavanol consumption was also reported to increase forearm blood flow (assessed via strain gauge plethysmography) in healthy individuals (Heiss et al., 2015) and brachial artery blood flow (Muniyappa et al., 2008) in individuals with hypertension, yet a 6-week cocoa flavanol intervention did not influence FBF responses in patients with CAD (Farouque et al., 2006). Flavanol metabolites, and specifically (-)-epicatechin, are shown to peak in the blood at 2 hours post-intake (Manach et al., 2004, Monahan et al., 2011). This is coupled by increases in NO species, shown to mediate the beneficial effects of high-flavanol cocoa on endothelial function (Schroeter et al., 2006). Dietary flavanols have also been shown to improve cerebral cortical oxygenation, cerebral blood velocity, and cognition (Gratton et al., 2020, Sorond et al., 2008), yet the association between polyphenol consumption and cognitive benefits is not unanimous (Lamport and Williams, 2021). However, little is known about the effect of flavonoids on the peripheral and cerebral vasculature in the context of mental stress. Given the benefits of these compounds in the fasted and postprandial state, they may be particularly useful in the context of mental stress, when the vasculature is more vulnerable.

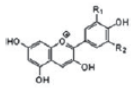
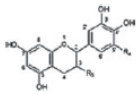
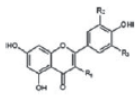
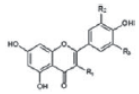
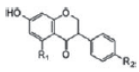
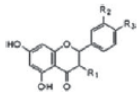
Group	Functional groups	Structural formula	Examples	Food sources
Anthocyanins	R1 = H, OH, OCH3; R2 = H, OH, OCH3		Pelargonidin, cyanidin, delphinidin, petunidin, malvidin, paeonidin	Red wine, berries
Flavanols	R1 = gallate, OH R2 = H; OH		Catechin, epicatechin, epigallocatechin, epigallocatechin gallate	Green tea, cocoa
Flavonols	R1 = OH, R2 = H, OH, OCH3; R3 = H, OH		Quercetin, kaempferol, myricetin, isorhamnetin	Onion, Brocolli
Flavones	R1 = H; R2 = H, OH; R3 = H		Luteolin, apigenin	Parsley, Celery
Isoflavones	R1 = OH; R2 = H, OH		Genistein, daidzein	Soya
Flavanones	R1 = H; R2 = H, OCH3; R3 = H, OH		Naringenin, hesperetin	Citrus fruits, tomatoes

Figure 1.2 Structure of the main flavonoids present in the human diet (Rendeiro et al., 2012)

1.7. Overall summary of background

An acute episode of mental stress can result in a transient decline in endothelial function, and this has been implicated as a mechanism by which stress can trigger MI and stroke and increase the risk for CVD. During periods of stress, maladaptive behaviours are adopted, such as poor diet and lower levels of PA. These behaviours increase the risk of CVD, and the vasculature has been implicated in this increased risk. Consumption of saturated fat can also impair endothelial function, whilst flavonoid-rich foods can improve endothelial function. A schematic of these relationships is displayed in Figure 1.3. Given that stress can change the pattern of consumption of these nutrients, it is important to understand whether saturated fat will exacerbate the effect of stress on endothelial function, and whether flavonoid-rich foods can rescue this effect.

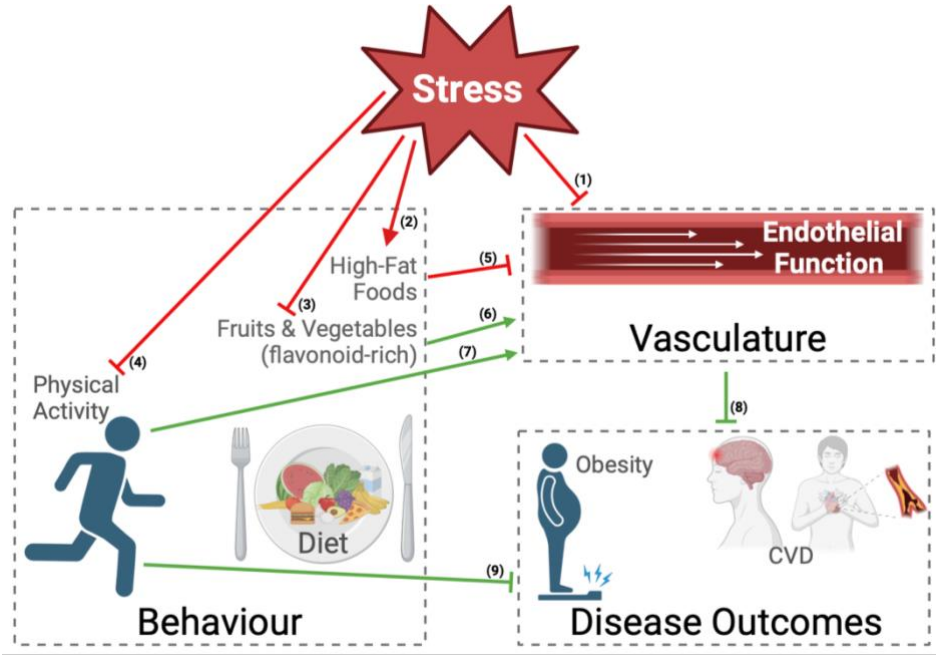


Figure 1.3 Schematic of the relationship between stress, endothelial function, behaviour, and disease outcomes

Stress impairs endothelial function (1) and leads to increased high-fat foods (2), reduced fruit and vegetables (3) and reduced physical activity (4). High-fat foods can also impair endothelial function (5), whilst flavonoid-rich food (6) and physical activity (7) can improve endothelial function. Healthy endothelial function (8) and physical activity (9) can prevent disease outcomes such as obesity and CVD. Created with BioRender.com.

1.8. Aims of this thesis

The primary aim of this thesis is to assess the impact of health behaviours (namely diet) on vascular function in the context of mental stress. Firstly, a scoping review was undertaken to identify literature investigating the impact of dietary interventions on vascular responses to mental stress (Chapter 2). Chapter 3 includes a repeatability study for the primary assessment of endothelial function (brachial FMD) and an ultrasound assessment of cerebral vasculature (CCA blood flow). Chapter 4, Chapter 5, and Chapter 6 address my primary aim and use randomised, crossover, counterbalanced, acute intervention designs to investigate the impact of a high-fat meal on peripheral vascular responses (Chapter 4) and cerebral oxygenation (Chapter

5), during and following mental stress, and whether a flavonoid intervention (Chapter 6) can rescue the fat and stress-induced impairments in peripheral vascular function and cerebral oxygenation. Chapters 4 and 5 include the same participants. It was hypothesised that saturated fat consumption will further exacerbate the impact of mental stress on vascular function, and that flavonoid-rich cocoa will attenuate the stress and fat-induced decline in vascular function. Using such a design allows for an extremely controlled setting by which we can administer an acute nutritional intervention, deliver a laboratory stress task which induces significant cardiovascular responses, and assess changes in the peripheral and cerebral vasculature. Furthermore, we can maintain a level of control over participants' diet and PA before and during each session, and are able to control for factors such as menstrual cycle phase, shown to influence vascular function (Thijssen et al., 2011). Therefore, this improves the reliability and reproducibility of our findings. The second aim of this thesis is to explore the relationship between stress and health behaviours (e.g., diet and PA) in an ecologically valid, real-life setting. Chapter 7 and Chapter 8 use a daily diary methodology to investigate the within-person and between-person associations between stress and diet (Chapter 7) and stress and PA (Chapter 8), in the same group of participants. It was hypothesised that during stressful days, participants would increase their consumption of fat and sugar, and decrease their consumption of fibre and flavonoids. Furthermore, we hypothesised that whilst stress may associate with reduced PA behaviour, engagement in PA can associate with reduced perceptions of stress and a greater ability to cope with stress. These questionnaire-based studies were conducted during the COVID-19 pandemic. The findings of this thesis and future recommendations are summarised in Chapter 9.

2. A Scoping Review Investigating the Effect of Dietary Interventions on Vascular Function in the Context of Mental Stress

2.1. Abstract

Episodes of acute mental stress have been shown to increase the risk for cardiovascular diseases, potentially via stress-induced impairments in vascular function. During stressful periods, individuals are likely to consume more unhealthy foods and fewer fruits and vegetables. Certain nutrients can have a negative or positive impact on the vasculature, yet their effect on vascular function in the context of mental stress is unclear. In this scoping review, comprehensive database searches were carried out to identify studies investigating the effect of diet on vascular function in the context of mental stress. Searches identified 523 articles for screening, of which 20 were selected for data extraction based on the inclusion criteria of including a component of diet AND a stress protocol AND a vascular assessment. Dietary interventions included nutrients which could impair vascular responses to stress (*n* = 8), healthy dietary nutrients (*n* = 9), and whole dietary changes (*n* = 3), and a range of vascular outcome measures and stress protocols were used. Preliminary evidence suggests that fat consumption can impair the recovery of vascular function following stress, whilst cocoa flavanols and Vitamin C may be protective during stress. The findings are mixed and limited, yet this review identifies current gaps in the literature and provides recommendations for future research to investigate how dietary choices can modify the impact of stress on vascular health.

2.2. Introduction

Stress is extremely prevalent, with a UK survey demonstrating that almost three-quarters of adults report to feel so stressed they are unable to cope (Mental Health Foundation, 2018b). Unfortunately, mental stress is detrimental to health, being a risk factor contributing to mechanisms underlying many disease states (Bairey Merz et al., 2002, Toda and Nakanishi-Toda, 2011b). For example, episodes of acute mental stress can trigger myocardial infarction (Carroll et al., 2002, Leor and Kloner, 1996) and stroke (Prasad et al., 2020), and hence, increase the risk for cardiovascular diseases (CVD). A decline in vascular function has been suggested as a likely mechanism underlying the relationship between stress and CVD (Paine et al., 2012). For example, stress-induced myocardial ischaemia has been associated with attenuated peripheral vasodilatory responses (Burg et al., 2009) and increased vascular resistance (Jain et al., 1998) during stress. Furthermore, transient declines in endothelial function (measured by brachial flow mediated dilatation, FMD) are evidenced following exposure to acute mental stress in young, healthy adults (Ghiadoni et al., 2000, Lind et al., 2002, Spieker et al., 2002). This impairment in FMD is clinically relevant as a 1 % decline in brachial FMD corresponds to a 13 % increase in CVD risk (Inaba et al., 2010) and impairments in FMD are associated with increased risk of cerebrovascular events, such as stroke (Santos-García et al., 2011).

The endothelium regulates blood flow through the release of vasodilatory and vasoconstrictive factors. Nitric oxide (NO) is an endothelium-dependent vasodilator, which plays a key role in maintaining vascular homeostasis (Sandoo et al., 2010). Stress affects the vascular actions of NO, via downregulation of endothelial nitric oxide

synthase (eNOS) expression, exaggerated release of the vasoconstrictor endothelin-1 (ET-1), and activation of pro-inflammatory cytokines and glucocorticoids (Toda and Nakanishi-Toda, 2011b). Furthermore, experimentally-induced inflammation has been reported to attenuate the vasodilatory response to mental stress (Paine et al., 2013a), and this has been paralleled by reduced NO availability and increased oxidative stress (Clapp et al., 2004). Therefore, stress disrupts the control of these factors, increasing inflammation and oxidative stress, which further reduces NO bioavailability and elicits stress-induced endothelial dysfunction (Wadley et al., 2013).

Importantly, stress-induced changes in behaviour can also negatively influence health (Hill et al., 2021). During stressful periods, individuals are likely to overeat and consume more unhealthy foods (such as fat and sugar) and fewer fruits and vegetables (Newman et al., 2006, Roberts et al., 2014b, Oliver and Wardle, 1999, Zellner et al., 2006, Gardiner et al., 2021). Crucially, consumption of certain foods can have a negative or positive impact on the vasculature. For example, brachial FMD is impaired for 8 hours following consumption of a high-fat meal (Rendeiro et al., 2016, Jackson et al., 2007). Fat-induced increases in triglycerides (TAG) and C-reactive protein (CRP) have been shown to stimulate ET-1, inflammatory markers (Tsai et al., 2004) and oxidative stress (Bae et al., 2001), which subsequently reduces endothelium-derived NO (Man et al., 2020) and impairs endothelial function. Similarly, high-sodium diets are evidenced to reduce NO-mediated endothelial function via increased reactive oxygen species (ROS) and increased endothelial stiffness and damage (Patik et al., 2021). However, diets rich in flavonoids, which are widely present in plants (e.g., fruits, vegetables, tea), have been shown to improve endothelial function (Monahan et al., 2011, Sansone et al., 2015, Li et al., 2013), by reducing inflammatory biomarkers and

ET-1, and increasing NO bioavailability (Loke et al., 2008, Cassidy et al., 2015). Therefore, there is commonality in the mechanisms by which stress and diet can influence the vasculature. Given the independent effects of stress and diet on vascular function, the foods that individuals choose to eat during periods of psychological stress may modify and even exacerbate the impact of stress on the vascular system. However, the effect of diet on vascular function in the context of mental stress has not been well established.

Therefore, this review aimed to explore literature investigating the impact of dietary interventions on the relationship between stress and vascular health. Specifically, we conducted a scoping review of randomised controlled trials which assessed the impact of macronutrients, micronutrients and food bioactives on biomarkers of vascular function (e.g., brachial FMD and forearm blood flow) in healthy, at-risk, and populations with diseases.

2.3. Methods

This review utilised a systematic search strategy to provide an overview of the available literature.

2.3.1. Literature search methodology

A formal literature search was the primary method of identifying relevant manuscripts. The search strategy is detailed below. The electronic databases MEDLINE, EMBASE, and APA PsychInfo were searched to identify articles published relating to diet, mental stress, and vascular function. Database searches included publications from the first available date to January 2024. Specific terms used were ‘stress’ / ‘‘psychological’ or

‘mental’ or ‘laboratory’ or ‘social’ or ‘daily’ stress’ / ‘stress ‘reactivity’ or ‘task’’ AND ‘vascular function’/‘endothelial function’/ ‘flow-mediated dilatation’ / ‘‘blood’ or ‘peripheral’ or ‘forearm’ flow’ / ‘vascular stiffness’ / ‘vascular reactivity’ / ‘nitric oxide’ / ‘plethysmography’ / ‘impedance’ / ‘‘pulse wave analysis’ or ‘velocity’ / ‘augmentation index’ AND ‘diet’ / ‘polyphenols’ / ‘flavonoids’ / ‘antioxidants’ / ‘vitamins’ / ‘feeding and eating disorders’ / ‘dietary restraint’ / ‘eating behaviour’. A manual search of article reference lists was conducted as a secondary method of identifying relevant texts.

2.3.2. Inclusion criteria

Inclusion criteria were developed through researcher discussion and guided by the Population, Intervention, Comparison/Control Group, Outcome, and Time (PICOT) framework (Higgins and Green, 2011). This inclusion criteria covered population (human), intervention (dietary intervention and stress exposure/component) and outcome (vascular function assessment). Importantly, research articles needed to include a component of diet AND stress AND a vascular assessment, to be included. Exclusion criteria included animal studies, and publications such as book chapters, conference abstracts or posters.

2.3.3. Study selection and data extraction

The literature search was carried out by one reviewer (RB), using the three databases and the defined keywords, through Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). All citations identified in the search were independently screened by two reviewers (RB, CR) based on the title and abstract, to assess their suitability for inclusion. Disagreements regarding eligibility of studies were resolved by discussion and consensus with a third reviewer (JVvZ).

Included title and abstracts were then screened for their full texts. Full text extraction was carried out by RB and inspected by CR. All procedures were in line with published guidelines for writing a scoping review (Tricco et al., 2018).

All studies were quantitative and have a variety of cross-sectional, within-subject, and between-subject designs, as well as a range of acute and chronic data collection periods. To give a comprehensive overview of the available literature, this review reports data from studies using a variety of different designs. A range of dietary interventions, stress tasks, and vascular outcomes were assessed. We firstly summarised randomised controlled trials that investigate a negative impact of nutrients on biomarkers of vascular health, followed by studies that examine a positive impact of nutrients on assessments of vascular health. Finally, we report how changes in whole dietary patterns may impact vascular responses to stress.

2.4. Findings

A total of 523 articles were found, of which 30 articles fulfilled inclusion criteria for full-text review. A further 10 articles were excluded following full-text review, meaning 20 articles were included in this review. The most common reason for exclusion at abstract level was not including all three components (i.e., diet, stress, and vascular outcome). At full-text level, the most common reasons for exclusion were not fulfilling full-text criteria and not including a vascular outcome (see Figure 2.1). Of the 20 articles, *n = 8* included studies incorporated a dietary intervention which could impair vascular responses to stress (e.g., saturated fat and salt), *n = 9* included a healthy dietary intervention (e.g., Vitamin C, cocoa flavanols, nitrate, Emblica Officinalis, and healthy fats) and *n = 3* reported on whole dietary interventions (e.g., hypocaloric, and

postprandial). An overview of the included studies investigating the influence of diet on vascular responses to mental stress are reported in Table 2.1 – Table 2.3, separate for each group of dietary interventions.

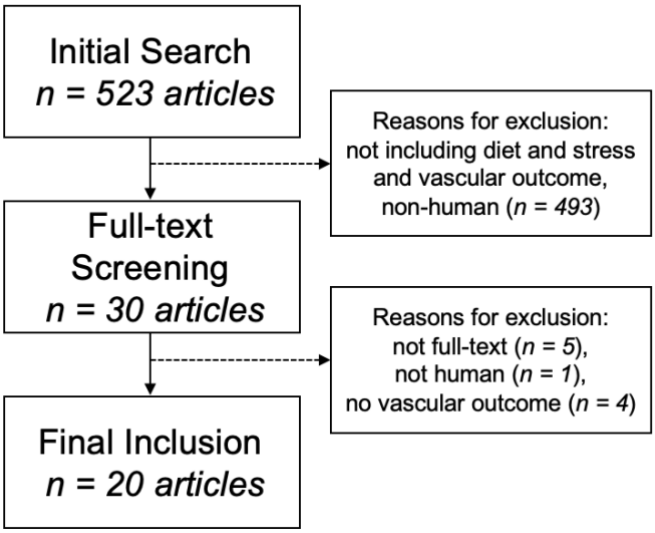


Figure 2.1 Flow chart of the literature search

2.4.1. Saturated fat and salt

Table 2.1 provides an overview of studies which investigated the impact of saturated fat and salt on vascular outcomes in the context of stress, revealing the potential of these nutrients to impair vascular responses to stress.

2.4.1.1. Saturated fat

Six studies investigated the acute effects of fat consumption and used a crossover design (Baynham et al., 2023b, Baynham et al., 2023a, Gowdak et al., 2010, Poitras et al., 2014, Jakulj et al., 2007, Kiecolt-Glaser et al., 2017). All studies included a high-fat intervention with comparable fat quantity (ranging from 42 g – 60 g fat), but considerable differences in the levels of saturated fat (ranging from 16 g – 40 g

saturated fat). Studies also differed in the control intervention, with most studies using a calorically matched low-fat meal (ranging from 0 g – 11 g fat, 0 – 9 g saturated fat) (Baynham et al., 2023b, Baynham et al., 2023a, Poitras et al., 2014, Jakulj et al., 2007), whilst others used a high-oleic sunflower oil meal (Kiecolt-Glaser et al., 2017) or no meal (fasted state) (Gowdak et al., 2010).

This literature suggests that saturated fat worsens vascular function, specifically by impairing vasodilatory responses (as measured by FBF) (Gowdak et al., 2010), increasing vascular resistance (total peripheral resistance, TPR) (Jakulj et al., 2007), and attenuating cortical oxygenation (as measured by Near-Infrared Spectroscopy) (Baynham et al., 2023a) during stress. However, the effect of saturated fat on vasodilatory responses during stress was mixed: Baynham et al. (2023b) reported no effect of fat consumption on FBF, whilst Gowdak et al. (2010) showed reduced vasodilation to stress following consumption of a high-fat meal. One notable methodological difference between these two studies is the control intervention used: Baynham et al. (2023b) compared a high-fat meal with a low-fat meal (calorically and macronutrient matched, except for fat) across two visits, whilst Gowdak et al (2010) compared it to a fasted state assessed on the same day. Therefore, the reduced FBF in this study could be mainly driven by a postprandial effect, or due to an order effect of testing. Similarly, differences in the stress task and length (3-minute Stroop vs 8-minute paced auditory serial addition task, PASAT) and sample (females only vs males and females) between these studies may also contribute to this inconsistency.

Some studies also evaluated the impact of fat consumption on vascular function following stress and showed no effect on FMD 10 minutes post-stress (Poitras et al.,

2014), but a delayed recovery in FMD 90 minutes post-stress (Baynham et al., 2023b). The lower level of saturated fat (54 g fat: 16 g sat fat) delivered in Poitras et al. (2014) may explain the absence of FMD impairment, compared to levels delivered in Baynham et al. (2023b) (57 g fat: 35 g sat fat). The timing of FMD assessments may also explain these differences, given the stress-induced decline in FMD is mainly present between 30 – 90 minutes post-stress (Ghiadoni et al., 2000, Lind et al., 2002, Spieker et al., 2002).

It is worth noting the variability in the magnitude of the cardiovascular reactivity to stress, as some studies induced large heart rate (HR) responses (25 – 30 bpm increase) (Baynham et al., 2023b, Baynham et al., 2023a, Poitras et al., 2014) whereas others reported more modest responses (5 – 10 bpm increase) (Gowdak et al., 2010, Jakulj et al., 2007), which may modify the impact of fat on the vasculature during/following stress. HR reactivity to laboratory stress has been related to HR responses in real life (Johnston et al., 2008, Davig et al., 2000), with HR increases of 15 – 40 bpm in response to real-life stress (Johnston et al., 2008, Matthews et al., 1986, Davig et al., 2000).

The studies presented above examined vascular responses to a laboratory-based mental stress task. Kiecolt-Glaser and colleagues (2017) had a different approach, where they assessed the impact of stress experienced the previous day on the effect of acute high-fat meals on a serological marker of vascular function, vascular cell adhesion molecule 1 (VCAM1). They showed that saturated fat consumption increased the levels of VCAM1. However, when participants reported to experience stress the prior day (average 1.2 stressors), the effect of saturated fat on VCAM1 was no longer

detectable, suggesting that stress alone may have heightened this serological marker of inflammation, masking the effects of fat. Therefore, this study suggests a comparable activation of VCAM1 following a stressful day and saturated fat consumption.

In summary, there is evidence that consumption of at least 35 g of saturated fat can impair vascular function in the periphery and brain during mental stress (which induces 25 – 30 bpm increases in HR) and delay the recovery of endothelial function specifically at 90 minutes post-stress. However, there are inconsistencies between studies likely due to different stress tasks (and subsequent HR reactivity), timing of vascular assessments post-stress, and control interventions used.

2.4.1.2. Salt

Two studies investigated the impact of a 6 – 7 day salt load on vascular responses to mental stress and reported no effect on forearm vasodilation (FBF) (Dishy et al., 2003) or microvascular endothelial function (as measured by laser Doppler flowmetry) (Stupin et al., 2021), compared to a low-salt diet. Neither study reported salt intake to influence resting blood pressure (BP) or BP and HR reactivity to stress. However, the stress task used in these studies induced very modest HR responses (1 – 4 bpm decreases and 9 – 11 bpm increases in HR, in Stupin et al., 2021 and Dishy et al., 2003, respectively), lower than suggested HR responses to real life stress (Johnston et al., 2008), which may limit the interpretation of the data presented. Overall, these studies suggest that salt consumption does not influence vascular function in response to stress.

Table 2.1 Impact of saturated fat and salt interventions on vascular responses to mental stress

	Study	Design	Participants	Diet intervention	Stress protocol	Vascular outcome	Vascular findings
Saturated fat	Baynham (2023a)	ACUTE, randomised, unblinded, within-subject (crossover)	21 healthy males and females (11M) Age: 22.1 ± 2.7 years, BMI: 23.6 ± 3.1 kg/m^2	High-fat (56.5g, 35.1g sat. fat) vs low-fat (11.4g, 5.6g sat. fat) meal	8 min PASAT	NIRS (cortical oxygenation), CCA blood flow	HFM attenuated cortical tissue oxygenation during stress. No change in CCA blood flow.
	Baynham (2023b)	ACUTE, randomised, unblinded, within-subject (crossover)	21 healthy males and females (11M) Age: 22.1 ± 2.7 years, BMI: 23.6 ± 3.1 kg/m^2	High-fat (56.5g, 35.1g sat. fat) vs low-fat (11.4g, 5.6g sat. fat) meal	8 min PASAT	FMD, FBF, FVC	HFM delayed recovery of FMD 90 min post-stress. HFM did not impact FBF responses to stress.
	Gowdak (2010)	ACUTE, unrandomised, unblinded, within-subject (crossover)	24 females: n=14 homozygous for Gln27 allele (age: 41 ± 3 years, BMI: 23 ± 1kg/m^2), n=10 homozygous for Glu27 allele (age: 40 ± 2 years, BMI: 24 ±1 kg/m^2)	Fasted (0g, 0g sat. fat) then high-fat (62g, 40g sat. fat) meal (same day)	3 min Stroop colour word test	FBF, FVC	HFM reduced FBF responses to stress but did not affect resting FBF compared to fasted state.
	Poitras (2014)	ACUTE, unrandomised, unblinded, within-subject (crossover)	10 healthy men, Age: 23.2 ± 3.3 years, BMI: 24.4 ± 2.4 kg/m^2	High-fat (54g, 16g sat. fat) vs low-fat (0g, 0g sat.fat) meal	10 min mental arithmetic and speech tasks	FMD	HFM had no impact on FMD post-stress.

Salt	Jakulj (2007)	ACUTE, randomised, unblinded, within-subject (crossover)	30 healthy students (18F), Age: 18 – 25 years BMI: 22.8 ± 3.9 kg/m^2	High-fat (42g, 16.5g sat.fat) vs low-fat (1g, 0.8g sat. fat) meal	5 min mental arithmetic, 5 min public speaking	TPR	HFM increased TPR reactivity to stress compared to LFM.
	Kiecolt-Glaser (2017)	ACUTE, randomised, double-blind, within-subject (crossover)	58 healthy women, n=38 breast cancer survivors (age: 52.1 ± 7.3 years, BMI: 28.8 ± 5.3kg/m^2), n= 20 control (age: 55.0 ± 10.2 years, BMI: 26.7 ± 4.1 kg/m^2)	High-saturated fat (60g, 16.84g palmitic, 13.5g oleic) vs high-oleic sunflower oil (60g, 8.64g palmitic, 31.21g oleic)	Daily stressful events (prior day via Daily Inventory of Stressful Events)	VCAM1	VCAM1 was higher following saturated fat meal compared to high oleic sunflower oil meal. Prior day stressors removed this condition effect.
	Dishy (2003)	6-DAY, randomised, unblinded, within-subject (crossover)	25 normotensive males and females (15 M), Age: 34 ± 2 years, BMI: 27.1 ± 1 kg/m^2	High-salt (400 mmol/day) vs low-salt (10 mmol/day) for 6 days	Stroop task (on 5th day, length not specified)	FBF (assessed during first 2 min of stress task)	High salt did not influence FBF responses to mental stress
	Stupin (2021)	7-DAY, non-randomised, unblinded, within-subject (crossover)	47 healthy adults (19F), Age: 21 ± 2 years, BMI: 24 ± 3 kg/m^2 (approx.)	Low-salt (3.75g/day) followed by High-salt (14.7g /day). 7 days each.	7 min mental arithmetic (true or false to long arithmetic)	Microvascular endothelial function (post-occlusive reactive hyperaemia)	No difference in microvascular reactivity to mental stress in low or high salt condition

Sat: saturated, BMI: body mass index, NIRS: near infrared spectroscopy, CCA: common carotid artery, FBF: forearm blood flow, FMD: flow-mediated dilatation, TPR: total peripheral resistance, VCAM1: vascular cell adhesion molecule 1, HFM: high-fat meal, LFM: low-fat meal, PASAT: paced auditory serial addition task.

2.4.2. Vitamin C, Cocoa flavanols, Nitrate, Emblica Officinalis fruit, and Healthy fats

Table 2.2 provides an overview of studies which investigated the influence of healthy dietary interventions hypothesised to improve vascular function in the context of mental stress.

2.4.2.1. Vitamin C

Chronic (45 days) Vitamin C supplementation increased vasodilatory (FBF) responses to mental stress (Dantas et al., 2011). In addition, acute (30 minute) Vitamin C supplementation reduced vascular remodelling marker (MMP-9) in response to stress (Batista et al., 2020), an important biomarker for CVD (Sundström and Vasan, 2006). Furthermore, Vitamin C improved FMD 60 minutes post-stress in one study (Rocha et al., 2023a), yet had no effect on FMD 30- and 90-minutes following stress in another study (Plotnick et al., 2017a). Whilst both studies used males only, Rocha et al. (2023) recruited overweight/obese men, as opposed to healthy men investigated in Plotnick et al. (2017b). Although the evidence is limited, this suggests that the benefit of Vitamin C may be heightened in overweight individuals with poorer endothelial function, compared to healthy young adults (Kajikawa and Higashi, 2022). Another disparity between these studies is the administration of Vitamin C, as Rocha et al. (2023) used an intravenous administration, which is more readily bioavailable, compared to administration of Vitamin C capsules, which had no effect on FMD (Plotnick et al., 2017a). Plotnick et al. (2017b) further reported that a correlation between cortisol reactivity and changes in FMD was abolished by Vitamin C consumption, suggesting that individuals with higher cortisol reactivity to mental stress may experience a greater benefit in vascular function from Vitamin C supplementation during stress. In summary,

three out of four studies suggest that Vitamin C improves vascular responses to stress. However, most included only males and overweight/obese participants, so future research should investigate its impact in female and healthy weight populations.

2.4.2.2. Cocoa flavanols, nitrate & Emblica officinalis fruit

Flavonoids and nitrate are food bioactives that have been extensively shown to improve endothelial function (FMD) by increasing NO availability (Rodriguez-Mateos et al., 2015). A total of three studies were included in this review investigating their potential benefits in the context of mental stress. High-flavanol cocoa improved vasodilatory (FBF) responses during stress and attenuated the decline in FMD following stress in young healthy males (Baynham et al., 2021). Furthermore, a 2-week supplementation of Emblica officinalis fruit extract (Indian gooseberry, rich in polyphenols) attenuated the stress-induced increase in arterial stiffness (augmentation pressure and index) (Usharani et al., 2017).

On the other hand, nitrate supplementation did not influence vasodilatory (FBF) responses during stress in males from black ethnicity (Akins et al., 2021). One would hypothesise that greater benefits would be seen in individuals from black ethnicity, given that they have reduced vasodilatory response to stress (Cardillo et al., 1998). However, this was a very small study ($n = 9$), likely underpowered, which limits the interpretation of results.

2.4.2.3. Healthy fats

Two studies in this review investigated the effect of healthy fats (polyunsaturated fatty acids, PUFAs, and more specifically omega-3 fatty acids) on the vasculature in the

context of mental stress. One study evidenced 6-week supplementation of diets rich in polyunsaturated fat to reduce TPR at rest and during stress (West et al., 2010). Furthermore, West et al. (2010) report 6-weeks PUFA supplementation to improve FMD, yet this was only assessed in a small proportion of participants and not in the context of mental stress. A cross-sectional study investigated the effect of diets rich in omega-3 fatty acids, and report that individuals who consume high levels of fish (> 70 g baked fish at least 3 – 4 times/week) had a reduced pulse wave velocity (PWV, an index of arterial stiffness) response to stress, but no difference in TPR responses, compared to non-fish eaters (< 70 g baked fish 1 – 2 times/week) (Matsumura et al., 2012). Given the small sample used in this study (*n = 12* fish eaters, *n = 13* controls) and the self-reported assessment of fish consumption (via the FFQ), drawing conclusions from these findings is challenging. Furthermore, as neither TPR nor PWV provide a direct assessment of vascular function, the effect of healthy fats on vascular function is unclear. Future work should address whether PUFAs, particularly delivered via fish consumption may be beneficial to vascular function in the context of stress.

Table 2.2 Impact of positive dietary interventions on vascular responses to mental stress

	Study	Design	Participants	Diet intervention	Stress protocol	Vascular outcome	Vascular findings
Vitamin C	Plotnick (2017b)	ACUTE, randomised, double-blind, within-subject (crossover)	15 healthy men Age: 21 ± 2 years, BMI: 22.1 ± 2.0 kg/m^2	Vitamin C (2 x 500mg capsules, 1 hour apart) vs placebo (2 x 500mg lactose powder capsules)	5 min Trier Social stress test	FMD	Vitamin C did not influence FMD. Correlation between cortisol reactivity and FMD, which was abolished by Vitamin C
	Batista (2020)	ACUTE, randomised, single-blind, within-subject (crossover)	14 overweight/obese males, Age: 27 ± 7 years, BMI: 29.7 ± 2.6 kg/m^2	Vitamin C (3g diluted in 500ml 0.9% NaCL) vs placebo (500ml 0.9% NaCL) intra-venously for 30 min	5 min Stroop colour word test	MMP-9 (vascular modelling biomarker)	Vitamin C infusion reduced MMP-9 activity at rest and during stress
	Rocha (2023)	ACUTE, randomised, double-blind, within-subject (crossover)	15 overweight/ obese men, Age: 27 ± 7 years, BMI: 29.8 ± 2.6 kg/m^2	Vitamin C (3g diluted in 500ml 0.9% NaCL) vs placebo (500ml 0.9% NaCL) intravenously for 30 min vs AT1R blockade (orally)	5 min Stroop colour word test	FMD	Vitamin C increased FMD 60 min post-stress. AT1R block increased FMD 30 min post-stress. FMD decreased 30 min post-stress following placebo.
	Dantas (2011)	45-DAYS, randomised, double-blind, randomised, between-subject (parallel)	21 obese male and female children (gender unknown), Age: 10.9 ± 0.4 years, BMI: 29 ± 1 kg/m^2 (+ 8 age-matched lean controls, BMI: 18 ± 1 kg/m^2)	Vitamin C (500mg/day) (n=11) vs placebo (n=10) for 45 days	3 min Stroop colour word test	FBF, FVC	Vitamin C supplementation increased FBF and FVC at rest and during stress

Cocoa flavanols	Baynham (2021)	ACUTE, randomised, double-blind, within-subject (crossover)	30 healthy men, Age: 22.1 ± 2.7 years, BMI: 23.6 ± 3.1 kg/m^2	High-flavanol (150 (-)-epicatechin) vs low-flavanol (< 4mg (-)-epicatechin) cocoa	8 min PASAT	FMD, FBF, FVC	HFC attenuated decline in FMD 30 min post-stress. HFC increased FBF at rest and during stress.
Nitrates	Akins (2021)	ACUTE, randomised, double-blind, within-subject (crossover)	9 black males, Age: 23 ± 3 years, BMI: 26.8 ± 4.2 kg/m^2	140 ml of NO^-_3-rich beverage (Beet-it-sport; 12.8mmol NO^-_3) vs NO^-_3-depleted placebo (0.0055mmol NO^-_3)	3 min mental arithmetic (subtract 7 or 13 from 3-digit number)	FBF (duplex Doppler – brachial artery)	Nitrate supplementation did not influence FBF responses to stress
Emblica Officinalis	Usharani (2017)	2-WEEK, randomised, double-blind, within-subject (crossover)	12 healthy males, Age: 24.75 ± 2.01 years, Height: 164.81 ± 7.01cm, Weight: 57.81 ± 5.57kg (BMI: 21.3 kg/m^2)	2 x capsules of standardised Emblica Officinalis Fruit extract (250mg)/day vs placebo for 2 weeks (2-w/k washout)	Computerised psychometric performance tests (5 min)	Augmentation index, augmented aortic pressure	Fruit extract attenuated stress-induced increase in augmentation index and pressure
Healthy fats	West (2010)	6-WEEK, randomised, unblinded, within-subject (crossover)	20 hyper-cholesterolemic adults (gender split unknown), Age: 49.3 ± 1.7 years, BMI: 28.9kg/m^2	Average American (AA) (SFA: 12.7g, PUFA: 8.7g) vs Linoleic acid (LA) (SFA: 8.5g, PUFA: 16.4g) vs Alpha Linolenic (ALA) (SFA: 8.2g, PUFA: 17.2g) diet.	3 min Speech stressor	FMD (n=12 only), TPR	High PUFA (LA and ALA) diets reduced TPR at rest and during stress. FMD increased in ALA diet only (not in context of stress)
	Matsumura (2012)	CROSS-SECTIONAL, between-subject, matched groups	25 healthy students, n=12 fish eaters (2M, age: 21.4 ± 3.7 years, BMI: 21.6 ± 2.7kg/m^2) n=13 controls (2M, age: 21.9 ± 3.1, BMI: 20.8 ± 3.1kg/m^2)	Fish eaters (>70g fish 3-4/week) vs controls (<70g) for 12 months. Assessed via FFQ	5 min mental arithmetic (subtract 13 from 5000)	TPR, PWV, cardio finger vascular index	PWV response to stress lower in fish eaters. No differences in TPR.

Vit: vitamin, NO: nitric oxide, BMI: body mass index, FMD: flow mediated dilatation, MMP-9: matrix metalloproteinase-9, FBF: forearm blood flow, FVC: forearm vascular resistance, TPR: total peripheral resistance, PWV: pulse wave velocity, HFC: high-flavanol cocoa, AA: Average American, LA: linoleic acid, ALA: alpha-linolenic acid, SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids, FFQ: food frequency questionnaire, NO^{-}_{3}: Nitrate, NaCL: Sodium Chloride.

2.4.3. Whole dietary interventions

Three studies were included in this review that investigated whole-diet changes and how this may impact vasculature in the context of mental stress (Table 2.3).

2.4.3.1. Hypocaloric diet

Two studies investigated the impact of a chronic (16 week) hypocaloric diet and exercise training on vasodilatory responses to mental stress in obese women (Tonacio et al., 2006b) and obese children (Ribeiro et al., 2005b). The findings are consistent, whereby a chronic hypocaloric diet alone had no effect on vasodilatory (FBF) responses at rest or during stress. However, when a chronic hypocaloric diet was paired with chronic exercise training (3 x 60 minutes supervised exercise sessions/week for 16 weeks), vasodilatory activity was improved at rest and during stress (Tonacio et al., 2006b, Ribeiro et al., 2005b). Therefore, we cannot determine whether it is the exercise intervention driving these benefits, or the interaction of the exercise intervention with the hypocaloric diet.

2.4.3.2. Postprandial

One study specifically explored the effect of eating a meal on responses to stress in the arteries related to the digestive system, assessed by Doppler ultrasound flowmetry (Someya et al., 2010). The diet intervention included solid food containing 6.5 g protein, 16.8 g fat, and 30 g carbohydrate (300 kcal total). They reported that, in a fasted state, mental stress induced vasoconstriction in the superior mesenteric artery (which mainly supplies the small intestine) but not the celiac artery (which mainly supplies the stomach). However, immediately following the meal, blood velocity

increased in both arteries at both rest and during stress, suggesting that the vasodilatory effect of meal ingestion on this vasculature overrides the vasoconstrictive effect of mental stress. These studies specifically focus on the vasculature around the digestive system, so it is not known how this relates to peripheral vasculature. Importantly, these findings suggest that food intake alone can impact arterial blood flow, which may have implications for control conditions used, particularly in a fasted state.

Table 2.3 Impact of changes to diet on vascular responses to mental stress

	Study	Design	Participants	Diet intervention	Stress protocol	Vascular outcome	Vascular findings
Hypocaloric	Tonacio (2006)	16 WEEKS, randomised, unblinded, between-subject (parallel)	44 obese women Age: 33 ± 1 years, BMI: Diet – 34 ± 1 kg/m^2, Diet + exercise – 33 ± 1 kg/m^2	Hypocaloric diet: reduction of 600Kcal/day (n=22) vs Hypocaloric diet + exercise training (n=22). For 16 weeks	3 min Stroop colour word test	FBF, FVC	Hypocaloric diet had no effects on FBF responses at rest or during stress. Hypocaloric diet + exercise increased FBF responses at rest and during stress.
	Ribeiro (2005)	16 WEEKS, randomised, unblinded, between-subject (parallel)	39 obese children Age: 10 ± 0.2 years, BMI: 29 ± 0.3 kg/m^2 (+ 10 age-matched lean controls, BMI: 17 ± 0.5kg/m^2)	Hypocaloric diet: 1400Kcal/day (n=18) vs Hypocaloric diet + exercise training (n=21). For 16 weeks	4 min Stroop colour word test	FBF, FVC	Hypocaloric diet had no effects on FBF responses at rest or during stress. Hypocaloric diet + exercise increased FBF responses at rest and during stress.
Postprandial	Someya (2010)	ACUTE, randomised, unblinded, within-subject (crossover)	10 healthy males and females (6M) Age: 24 ± 2 years, Height: 165 ± 9 cm, Weight: 57 ± 9 kg (BMI: 20.9 kg/m^2)	Postprandial (6.5g protein, 16.8g fat, 30g carbs, 300 kcal) vs fasted	5 min colour word test	Blood flow and vascular conductance (coeliac and superior mesenteric artery)	Mental stress exerts different effects on digestive arteries under fasting but not postprandial conditions.

BMI: body mass index, FBF: forearm blood flow, FVC: forearm vascular conductance.

2.5. Discussion

2.5.1. General findings

To our knowledge, this is the first review to examine the impact of dietary interventions on the vasculature in the context of mental stress. This review includes studies reporting different dietary interventions such as those thought to impair vascular function (e.g., saturated fat and salt), healthy interventions (e.g., Vitamin C, cocoa flavanols, nitrate, Emblica officinalis fruit, and healthy fats), and overall changes to diet (e.g., hypocaloric). The findings are mixed and very limited, and differences between interventions, vascular assessments, and study design make direct comparisons between studies challenging. This discussion will identify current gaps in the literature and provide recommendations for future research aimed to address how dietary choices can modify the impact of stress on vascular function.

2.5.2. Dietary interventions

Differences in the method of delivery of nutrients, doses of nutrients supplemented, the control/placebo used, and the duration of intervention are all key aspects to take into consideration when investigating the impact of diet on vascular responses to mental stress, and these are likely contributing to inconsistent findings in the papers reviewed here. For example, as previously mentioned, the administration of Vitamin C intravenously reduced MMP-9 activity (Batista et al., 2020) and increased FMD 60 minutes post-stress (Rocha et al., 2023a), yet intake of Vitamin C in capsules had no effect on FMD (Plotnick et al., 2017a). Vitamin C recycles the enzyme BH_4, which synthesises eNOS at the endothelial cell plasma membrane, generating NO (May and Harrison, 2013) and inducing vasodilation of the endothelium. Delivering Vitamin C in

capsule form requires the intestine to absorb it before it can be transported through the circulation to the endothelium, whereas intravenous supplementation of Vitamin C will result in quicker delivery of this nutrient to endothelial cells (May and Harrison, 2013) which hence impacts its response. Whilst this may be more efficient, intravenous delivery of nutrients is not realistic or ecologically valid from a nutritional context. Vitamin C supplementation in capsule form did increase vasodilatory responses to stress (Dantas et al., 2011), yet this was a 45 day intervention, showing the chronic effect of Vitamin C on vascular health. Furthermore, the nutrient dose may also influence its effect on endothelial function. For example, 16 g saturated fat did not affect FMD following stress (Poitras et al., 2014), yet 35 g saturated fat impaired the recovery of FMD following stress (Baynham et al., 2023b). A previous review reported that approximately 50 g fat is sufficient to impact endothelial function (Jackson et al., 2007). As both study interventions were > 50 g fat, perhaps future research should prioritise the saturated fat content over total fat content. Furthermore, it is important to consider whether the nutritional doses delivered are compatible with a typical meal or snack that could be consumed by individuals during stressful periods. Similarly, West et al. (2010) presents the effect of different fats (saturated vs polyunsaturated) on vascular function in the context of stress, which could also be an area for future investigation.

The control/placebo condition is also a critical aspect to take into consideration. For example, Someya et al. (2010)'s data suggests that just eating can differentially affect blood flow to digestive system arteries. Although it is not clear how consuming a meal can impact blood flow in peripheral arteries, this should be taken into account when using the fasted state as a control. Furthermore, cortisol reactivity is suggested as a

potential mechanism underlying stress-induced changes in FMD. Cortisol also has a postprandial effect (Kirschbaum and Hellhammer, 1994), which is another reason why an adequate control should be used in comparison to the dietary intervention investigated.

The duration of the dietary intervention also likely influences its effect on vascular function in the context of stress. Notably, in this review all studies delivering fat interventions were acute, and both salt loading studies used 6 – 7-day interventions, and there were varied lengths of Vitamin C, cocoa flavanols, nitrate and Emblica officinalis interventions, ranging from acute to 45 days. One study assessed 12-month fish consumption (Matsumura et al., 2012), yet it should be acknowledged that this was self-reported and not an intervention. Therefore, based on the current findings, it is difficult to comment on the most effective duration of a dietary intervention in the context of vascular responses to mental stress. Both studies investigating the effect of a 16-week hypocaloric diet also included exercise training, and only the combination of diet and exercise training improved vascular function in response to stress (Tonacio et al., 2006b, Ribeiro et al., 2005b). Therefore, future research should also explore the combined effect of dietary interventions with physical activity, as this may prove an additive stimulus to protect vascular function during periods of stress.

2.5.3. Vascular assessments

Differences in the assessment of vascular function plays a role in the disparity of findings in this review. FBF during mental stress was the most commonly used technique, reported to be increased by Vitamin C supplementation (Dantas et al., 2011), high-flavanol cocoa consumption (Baynham et al., 2021), hypocaloric diet and

exercise training (Tonacio et al., 2006b, Ribeiro et al., 2005b) and impaired by fat consumption in one study (Gowdak et al., 2010) but not another (Baynham et al., 2023b), and not affected by nitrate supplementation (Akins et al., 2021). Vasodilatory responses to stress were assessed by different techniques: for example, Baynham et al. (2021) used venous occlusion plethysmography, assessing blood flow across all vessels in the forearm (microvasculature) whereas Akins et al. (2021) used duplex Doppler of the brachial artery, assessing blood flow in just the brachial artery (macrovasculature). The assessment of macrovasculature function (e.g., FMD) and microvasculature function (e.g., FBF) have different advantages and the use of duplex Doppler can be challenging during stress. Few studies used assessments of both the macro- and microvasculature in the context of stress, and diet may have differential effects on these. For example, flavanols have been shown to improve brachial FMD (macrovasculature) (Schroeter et al., 2006), yet their effect on the microvasculature is less clear. Some studies report an increased blood flow in the forearm microcirculation (measured by venous occlusion plethysmograpghy) (Heiss et al., 2015, Baynham et al., 2021), yet others revealed no changes in microcirculatory parameters (assessed by laser Doppler fluxmetry) (Hammer et al., 2015), or forearm reperfusion rate or oxygenation (measured by NIRS) (Santos et al., 2023) following flavanol intake.

Only one study focused on changes in cerebral circulation during stress, reporting fat intake to attenuate the stress-induced increase in cortical oxygenation, with no further effects detected on resting carotid artery blood flow following stress (Baynham et al., 2023a). Again, including assessments of macrovasculature, for example common/internal carotid artery blood flow and middle cerebral artery blood velocity during stress, as well as investigating changes in the microvasculature (NIRS) should

be a target of future research. Similarly, these assessments should be included following the stressful stimulus, given that fat induced changes in the microvasculature during stress, but not in the macrovasculature following stress. Thus, perhaps fat consumption influences the cerebral circulation only during a mental stimulus. Given the links between stress and stroke (Prasad et al., 2020) and cardiovascular and cerebrovascular health (Samieri et al., 2018), future work should include cerebrovascular assessments, and investigate the effect of stress and nutrients on cerebral blood flow.

FMD was the second most commonly used assessment of endothelial function and this review reports inconsistent findings of the effect of fat consumption on FMD following stress. However, the timing of the FMD assessment post-stress needs to be taken into careful consideration, as the literature clearly suggests stress-induced impairments in FMD from 30 – 90 minutes post-stress (Poitras and Pyke, 2013). So, this might be a key time window to focus on when investigating dietary interventions. Additionally, FMD measurements immediately after the stress task (e.g., 10 minutes) (Poitras et al., 2014), may be affected by elevations in sympathetic nervous system activity from the stress episode (Brindle et al., 2014). Therefore, the timing of the FMD assessment in relation to the stress task as well as the dietary intervention should be a consideration for future research.

Some studies in this review assessed the vasculature using indirect assessments of arterial stiffness; namely PWV, augmentation index and TPR. For example, one study presented an increased augmentation index during acute stress (Usharani et al., 2017). However, augmentation index reflects resting vasomotor tone and is only

partially dependent on NO, providing a measure of systemic arterial stiffness (Wilkinson et al., 2002). As acute mental stress would not be expected to influence systemic and functional arterial stiffness, this finding is surprising and hence, future work should focus on assessing arterial stiffness in both acute and chronic stress. Furthermore, some studies use TPR to assess vascular function during stress (Jakulj et al., 2007, West et al., 2010). However, TPR assesses whole body vascular responses and has previously shown to be unrelated to peripheral vascular responses (FBF) during stress (Paine et al., 2013b). Therefore, future work investigating TPR should also include direct assessments of vasodilation.

Finally, few studies assessed serological markers of vascular function. One study investigated VCAM1 (Kiecolt-Glaser et al., 2017), a marker which plays a key role in inflammation. Given the increase in inflammatory markers following stress (Marsland et al., 2017), which reduce NO bioavailability and contribute to impairments in vascular function (Toda and Nakanishi-Toda, 2011b), similar markers of vascular function and inflammation, in combination with functional assessments of vascular function, should be included in future research. Another study assessed a vascular modelling biomarker (MMP-9) (Batista et al., 2020). Notably, chronic stress activated MMP-9 in a rat hypothalamus (Kucharczyk et al., 2016), and this relates to subsequent impairments in rat memory and cognition (van der Kooij et al., 2014). Therefore, future studies should investigate these mechanisms (Li et al., 2022), as well as moderators of NO, inflammation and oxidative stress, which may play a role in the impact of nutrients on vascular function during stress.

2.5.4. Stress protocols

The studies reviewed here also differed in the way stress was induced. Many of the laboratory tasks used (e.g., Stroop colour word, trier social stress, mental arithmetic, and speech tasks) have been shown to reflect a similar psychological stress stimulus to everyday life (Dickerson and Kemeny, 2004), with real life stressors often producing larger responses to those seen in the laboratory (Zanstra and Johnston, 2011). Different types of stress task have been shown to activate the hypothalamic-pituitary-adrenal (HPA) axis and cardiovascular system. For example, mental arithmetic tasks elicit substantial HR and BP reactivity and haemoconcentration (Veldhuijzen Van Zanten et al., 2004), and reaction time tasks elicit a large sympathetic response (Brindle et al., 2014). Furthermore, speech tasks are reported to induce larger BP responses to other stressful stimuli, yet HR responses remain fairly consistent across stress tasks (Brindle et al., 2014). The magnitude of the physiological response is influenced by the provocativeness and competitiveness of the task, as the more provocative the task the more compelling the haemoconcentration response (Veldhuijzen Van Zanten et al., 2004), and competition increases cardiovascular perturbations (Veldhuijzen van Zanten et al., 2002). Similarly, elements of social evaluation during speech tasks can also elicit heightened physiological reactivity (Bosch et al., 2009). Finally, the duration of the task can also influence the physiological response, possibly by moderating the task's provocativeness. Therefore, the discrepancy in the duration of each stress protocol (3 – 10 minutes) may also play a role in the vascular responses to stress. Importantly, these elements of increasing difficulty, punishment, competition, and reward are used to enhance the stressfulness of the task and physiological reactivity to the task (Veldhuijzen van Zanten et al., 2002),

and are easier to implement with some tasks compared to others. Therefore, future studies should incorporate such elements to induce reliable responses. Given these differences, it is difficult to compare the included studies, yet the reported cardiovascular responses to these tasks in the reviewed studies are important. Mental stress induces immediate increases in HR and BP (increased sympathetic activation, withdrawal of parasympathetic activation), which is accompanied by arterial vasodilation (Dietz et al., 1994), required to relieve the stress-induced pressure on the vascular wall (Puzserova and Bernatova, 2016b). Attenuation of this vasodilatory response is characteristic of impaired vascular function yet, if the initial activation of the autonomic nervous system (e.g., < 5 bpm increase in HR, as reported in some studies) is not demonstrated, the subsequent vascular response could be compromised. Hence, if the stress task does not induce sufficient sympathetic activation, the effect of nutrient/food bioactive consumption on vascular responses to mental stress cannot be ascertained.

2.5.5. Populations

It should be acknowledged that studies reported in this review included a range of populations, such as healthy participants, obese children, overweight/obese adults, hypercholesterolemic adults, breast cancer survivors, and adults homozygous for Gln27/Glu27 allele. For example, both studies investigating the effect of a hypocaloric diet on vascular responses to mental stress used obese populations (Tonacio et al., 2006b, Ribeiro et al., 2005b). Given that obesity can lead to a blunted vasodilatory response to mental stress (Hamer et al., 2007), perhaps there is greater benefit of a hypocaloric diet and exercise training improving vascular responses to stress in this

population. This is supported by studies investigating Vitamin C supplementation, as the three studies reporting Vitamin C to improve vascular function during/following stress included overweight/obese samples (Dantas et al., 2011, Rocha et al., 2023a, Batista et al., 2020) yet the study reporting no effect of Vitamin C included a healthy sample (Plotnick et al., 2017a). However, it should be noted that 75 % of these studies used male only samples, and so the effect of Vitamin C on female vascular function should be examined. On the other hand, most studies investigating the effect of saturated fat and salt on vascular function in the context of stress included healthy samples, again highlighting a gap in the research area. Finally, only one study in this review investigated vascular responses to stress across different ethnicities (Akins et al., 2021). Some ethnicities are at higher risk of stress and CVD (Brothers et al., 2019, Albert et al., 2017), and in particular, black (Cardillo, 1998) and South Asian (Ormshaw et al., 2018) populations present impaired vascular responses to stress. Therefore, these populations should be of interest for future studies, as they might benefit the most from healthier food choices during stress or be most affected by unhealthy food choices during stress.

2.5.6. Recommendations for future studies

The 20 included studies in this review illustrates the potential for nutrients to modify the effect of stress on vascular function. However, to get a detailed understanding of the effects of different nutrients on vascular responses mental stress more research is needed. The following summary of recommendations (Table 2.4) aim to address gaps in the current literature.

2.5.7. Limitations

This review is not without limitations. Firstly, in some of the included studies, investigating the impact of a particular nutrient on vascular function in the context of mental stress was not the primary aim, and thus, the protocol and methodology were not designed to sufficiently explore this research question. Secondly, this review limited the included studies to those disseminated in English, which may have overlooked additional research. Finally, due to a low number of studies, statistical analysis, meta-analyses, and risk of bias of the data was inappropriate. As such, an inherent limitation of scoping reviews is the focus on breadth rather than depth of information (Tricco et al., 2018). However, this review helps identify gaps in the literature to aid the direction of future research, rather than ascertain the exact effect of each nutrient on vascular responses to mental stress.

Table 2.4 Summary of recommendations

Dietary interventions
The minimum level of nutrients delivered to influence vascular function should be investigated, to improve future recommendations of food choices and quantities.
Research should explore the effect of different lengths of nutrient interventions on vascular function in the context of mental stress.
Vascular assessments
Vascular assessments should include a measure of micro- and microvasculature, such as vasodilatory responses during stress (e.g., FBF) as well as endothelial function following stress (e.g., FMD).
Research should investigate the effect of nutrients and stress on cerebral micro- and macrovascular function (e.g., using transcranial Doppler, NIRS, duplex Doppler of internal carotid artery).
Assessment of mechanistic markers which underlie the stress-induced and nutrient-induced changes in vascular function should be explored in combination with vascular measures (e.g., inflammation, NO, cortisol, ET-1, oxidative stress markers).

Stress protocols

Future research should use stress tasks which will induce a sufficient cardiovascular response, representative of everyday stress (e.g., by including elements of increasing difficulty, competition, social evaluation, punishment, and reward).

Populations

Studies should include a range of participants, with different health statuses, across a range of ages and weights, from different ethnicities, and include investigation of both male and female participants.

2.6. Conclusion

Stress is widespread in our societies and often unavoidable. The consequences of stress on the vasculature are significant. This review highlights that food choices during stress can modify the consequences of stress on vascular health; with preliminary evidence of fat consumption impairing the recovery of vascular function following stress, yet cocoa flavanols and Vitamin C being protective during stress. There is insufficient evidence to conclude the extent to which nutrients can influence vascular function in the periphery and brain, in the context of different stressful stimuli. However, this review provides a foundation for future research through identification of gaps in the current literature, to inform dietary strategies to facilitate healthier food choices during stress and subsequently protect vascular health

3. Repeatability Study

3.1. Abstract

Endothelial function can be assessed non-invasively using flow-mediated dilatation (FMD). Despite concerns about its reproducibility, highly reliable FMD measurements can be achieved in healthy participants following appropriate training. The aim of this study was to assess the reproducibility of brachial FMD, as well as common carotid artery (CCA) blood flow. 10 healthy males and females visited the laboratory on 3 separate occasions, to assess inter-day reliability (visit 1, 2, and 3) in brachial FMD and CCA blood flow assessments. During each laboratory visit, ultrasound assessments of FMD and CCA, and brachial blood pressure, were performed twice to assess intra-day reliability (measurements A and B). Coefficient of variation (CV) and intraclass correlation coefficient (ICC) estimates revealed good reproducibility for intra-day (CV: 5.49 ± 3.40 %, ICC: 0.92) and inter-day (CV: 10.87 ± 9.18 %, ICC: 0.64) brachial FMD. Excellent reproducibility was demonstrated for brachial artery diameter (intra-day CV: 1.30 ± 1.15 %, ICC: 0.99, inter-day CV: 1.78 ± 1.46, ICC: 0.99), yet there was greater variability for brachial artery shear rate (intra-day CV: 28.23 ± 15.43 %, ICC: 0.43, inter-day CV: 19.49 ± 18.18, ICC: 0.44). Excellent reproducibility was demonstrated for CCA diameter (intra-day CV: 1.93 ± 1.50 %, ICC: 0.89, inter-day CV: 2.37 ± 2.03 %, ICC: 0.83), and moderate reliability for CCA shear rate (intra-day CV: 11.79 ± 9.28 %, ICC: 0.65, inter-day CV: 12.27 ± 9.59, ICC: 0.61). Bland Altman plots display no systemic bias, with all data points within 95 % limits of agreement. This study reports a high reproducibility for measurements of macrovascular endothelial function (brachial FMD), and extracranial blood flow (CCA ultrasound). Therefore, these techniques can be reliably applied in this thesis.

3.2. Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally (World Health Organisation, 2021) and include any disease that affects the heart or blood vessels. Furthermore, hypertension, diabetes and inflammatory diseases are strongly associated with CVD (Wang et al., 2021), and have significant prevalence and impact worldwide. An underlying characteristic of CVD, as well as these closely associated diseases, is a disruption to vascular homeostasis. Endothelial cells, located on the intima of the blood vessels, regulate vascular function by continuously responding to stimuli and releasing vasoactive factors accordingly (Sandoo et al., 2010). Nitric oxide (NO) is an endothelium-dependent vasodilator which has an important role in the maintenance of vascular tone. Therefore, the endothelium is a dynamic organ involved in the maintenance of vascular homeostasis via the regulation of vasodilatory and vasoconstrictive factors. Disruption of these processes, for example due to a damaged endothelium, is a marker of endothelial dysfunction, which subsequently increases the risk of CVD (Cines et al., 1998).

Direct assessment of endothelial function in the coronary arteries is highly invasive and carries risks. Therefore, endothelial function is most commonly assessed in the peripheral circulation, as there is a close association between peripheral and coronary endothelial function (Anderson et al., 1995). Furthermore, assessment of peripheral endothelial function is regarded a good predictor of CVD risk (Rossi et al., 2008, Gokce et al., 2003). Microvascular endothelial function can be assessed using venous occlusion plethysmography (detailed in general introduction), and other techniques such as iontophoresis, laser doppler imaging and nailfold capillaroscopy. Whereas the

gold-standard assessment for macrovascular endothelial function is flow-mediated dilatation (FMD) of the brachial artery (Sandoo et al., 2010), which reflects endothelium-dependent, NO-mediated dilation of the artery. FMD has been shown to correlate with future CVD risk, with a 1 % decrease in FMD associating with a 13 % increase in CVD risk (Inaba et al., 2010).

FMD is non-invasive and uses duplex ultrasound to examine changes in brachial artery diameter following a transient ischaemia (Thijssen et al., 2019). FMD is expressed as the percentage change in vessel diameter from post-cuff release relative to baseline. The diameter measurements are quantified by ultrasound imaging of the vessel, using automated edge-detection software. There are many advantages of FMD, namely being non-invasive and highly sensitive; with small changes in vascular diameter eliciting large FMD responses. However, given the impact of such small changes in arterial diameter, attention must be paid to technical and biological factors that may influence the assessment (Thijssen et al., 2019). Therefore, the major limitation of FMD is its need for standardisation. There are many environmental and participant-related factors which can affect endothelial function, such as food and alcohol intake, smoking, supplements, drugs, physical activity, mental stress, and the menstrual cycle (Thomas et al., 2015) and hence, the effect of these should be minimised. Furthermore, there are operator-dependent factors, including protocol recommendations, ultrasound technique, and analysis guidelines. Therefore, researchers have published evidence-based recommendations and expert consensus to standardise the FMD protocol and improve its assessment (Thijssen et al., 2019). There is large variability in studies which have failed to adhere to FMD recommendations and guidelines, with an observed coefficient of variation (CV) of up to 51 % (Charakida et al., 2013). A

significant cause of low reproducibility in FMD assessments is sub-optimal operator training. However, following standardised training, FMD in healthy participants can be highly reproducible (CV: 11.6 – 16.1 %) (Ghiadoni et al., 2012). Therefore, there are current standards that stipulate vascular researchers to conduct their own repeatability study for assessments of brachial FMD. Current recommendations suggest sonographers are qualified for measurements when their CV for repeated scans were < 2% for brachial artery diameter and < 15% for brachial artery FMD (Thijssen et al., 2019). It has also been suggested that > 100 scans are required to gain competency in FMD assessments (Corretti et al., 2002). Therefore, these recommendations and evidence-based guidelines have been followed.

Cerebral vascular function can also be measured with duplex ultrasound. Measures of extracranial (and intracranial) blood flow can provide new insight into the mechanisms of cerebral blood flow regulation (Thomas et al., 2015). Therefore, the common carotid artery (CCA) will also be scanned using the same duplex ultrasound used for FMD. This yields measurements of artery diameter and blood velocity through the CCA, allowing calculation of extracranial blood flow. Furthermore, researchers report a similar approach to improve the reproducibility of carotid duplex ultrasound, with recommendations of > 150 scans of each vessel of interest (ideally > 50 % with feedback from a skilled operator), and a CV of < 10 % between days, prior to independent data collection (Thomas et al., 2015). Therefore, as with the brachial FMD assessment, the same standardisation and reproducibility study will be carried out for cerebral blood flow assessments.

Therefore, the aim of this chapter was to assess the reproducibility of brachial FMD and CCA blood flow measurements, and to ensure an adequate inter-and intra-day variability for both assessments. This will certify reliable and trustworthy FMD and CCA blood flow findings in the proceeding studies of this thesis.

3.3. Methods

3.3.1. Participants

A total of ten male and female participants (5 female) were recruited from the University of Birmingham through email and verbal advertisements. All participants were healthy, between 18 and 45 years old, and free from any known cardiovascular, neurological, and metabolic diseases. Participants were asked to abstain from alcohol consumption and vigorous exercise for 24 hours, and to avoid caffeine consumption for 12 hours, prior to each visit. Where this was not possible, participants were asked to remain consistent between visits.

3.3.2. Protocol

All participants visited a quiet, temperature-controlled laboratory at the same time of day, on three separate occasions, at least 24 hours apart, to assess inter-day reliability (Visit 1, 2, and 3). Where possible, female participants were tested on consecutive days to reduce fluctuations in menstrual cycle hormones. Upon arrival, all laboratory procedures were explained. Following this, height and weight were measured, and general health was assessed using a general health questionnaire. Participants were then asked to rest supine for 15 minutes, prior to blood pressure measurements. Following this, ultrasound assessments of brachial FMD and CCA were performed, in

this order. Brachial blood pressure, FMD and CCA were performed again 45 minutes later to assess intra-day reliability (measurements A and B).

3.3.3. Assessments

3.3.3.1. Blood pressure

Systolic and diastolic blood pressure (SBP, DBP) and pulse rate (PR) were recorded using an automated oscillometric blood pressure monitor (Omron HEM-705CP), with a cuff attached to the left upper arm, following 15 minutes rest in the supine position. Three consecutive measurements were taken and averaged for each time point.

3.3.3.2. Brachial FMD

Flow-mediated dilatation (FMD) was used to assess endothelial function of the brachial artery. A 15–4 Mhz (15L4 Smart MarK^{TM}; Terason, MA, USA) transducer was attached to a Terason Duplex Doppler System (Usmart 3300 NexGen Ultrasound; Terason). This has a wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Pisa, Italy), which allows for continuous measurement of diameter and blood velocity throughout the FMD assessment. The brachial artery was imaged longitudinally, 5 – 10 cm proximal to the antecubital fossa. A brachial cuff was placed around the forearm and, following a 1-minute baseline, this was inflated to 220 mmHg for 5 minutes, to cause ischaemia. Subsequently, the rapid cuff deflation caused reactive hyperaemia, and the image was recorded continuously for 5 minutes post-pressure release. This is in accordance with established guidelines (Thijssen et al., 2019). All file images were analysed offline. Peak diameter was defined as the largest diameter obtained after occlusion is released. The FMD response was calculated as

the relative diastolic diameter change between baseline and peak diameter. Resting arterial diameter and shear rate was also estimated based on a time-average across the first minute of the recording.

3.3.3.3. CCA blood flow

Duplex doppler ultrasound was used to assess common carotid artery (CCA) diameter and shear rate. A 15–4 Mhz (15L4 Smart MarK^{TM}; Terason, MA, USA) transducer was attached to a Terason Duplex Doppler System (Usmart 3300 NexGen Ultrasound; Terason). This combines a wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Pisa, Italy), which allows for continuous measurement of artery diameter and shear rate. Participants were asked to turn their head and neck slightly to the left side. Then, a two-minute recording of the right CCA was obtained. All file images were analysed offline. Analysis allows estimation of resting arterial diameter and shear rate based on a time-average across two minutes of the recording.

3.3.4. Statistical Analyses

Separate 3 visit (Visit 1, Visit 2, Visit 3) analyses of variance (ANOVAs) were conducted on all physiological data, at timepoints A and B.

For the vascular assessments only (brachial FMD, brachial diameter, brachial shear rate, CCA diameter and CCA shear rate), intra and inter-day variability was assessed using the coefficient of variation (CV) and intraclass correlation coefficient (ICC). Intra- and inter-day reliability of brachial FMD was visualised using Bland-Altman plots.

CV was calculated as: $CV = \left(\frac{SD}{\bar{x}}\right) \times 100$

Where SD is standard deviation of the sample and $\bar{x}$ is the sample mean. CVs are reported for intra-day reliability (timepoints A vs B for Visits 1, 2, 3, separately) and inter-day reliability (Visits 1 vs 2, 1 vs 3, and 2 vs 3, for both timepoints A and B, separately). The lower the % reported, the lower the variability between assessments (higher reproducibility).

ICC estimates are reported for the same vascular assessments, and include separate ICC and 95 % confidence intervals for each intra-day comparison (timepoints A vs B for visits 1, 2, 3, separately), and combined timepoints for inter-day comparisons (Visits 1 vs 2, 1 vs 3, 2 vs 3). ICC estimates and their confidence intervals (CI) were calculated using SPSS (version 28), based on a mean-rating (two measurements), absolute agreement, two-way mixed-effects model (Koo and Li, 2016). ICC values < *0.5*, between *0.5 – 0.75*, between *0.75 – 0.9*, and > *0.9* are indicative of poor, moderate, good, and excellent reliability, respectively.

Bland-Altman plots were produced to give a graphical representation of brachial FMD assessments only. A mean difference between intra-day values (combined across visits, timepoints A vs B) and inter-day values (Visits 1 vs 2, 1 vs 3, 2 vs 3) were calculated, with 95% limits of agreement, using the formula:

mean difference ± 2 × SD of difference between 2 scores.

A one-way repeated measures ANOVA assessed the difference in inter-day CVs for brachial FMD between males and females.

P < .05 was deemed significant. Data is Mean ± SD in text and tables. Missing data (*n - 1*) is reported in *n* values, due to poor FMD image quality and CCA malfunction.

3.4. Results

3.4.1. Participant Characteristics

Participant baseline characteristics (*n* = 10) are reported in Table 3.1. Three visit ANOVAs revealed no significant differences between PR (p =.198), SBP (p =.698) and DBP (p =.277) at timepoint (A) across the three visits.

Table 3.1 Mean ± SD Resting participant characteristics

Baseline Characteristics	Visit 1 (A)	Visit 2 (A)	Visit 3 (A)
Age (years)	24.20 ± 4.37	/	/
BMI (Kg/m^2)	22.97 ± 3.77	/	/
PR (bpm)	61 ± 6	63 ± 6	64 ± 8
SBP (mmHg)	118 ± 7	116 ± 8	118 ± 3
DBP (mmHg)	73 ± 8	70 ± 7	70 ± 5

n = 10. BMI: body mass index, PR: pulse rate, SBP: systolic blood pressure, DBP: diastolic blood pressure.

3.4.2. Vascular Assessments

Brachial FMD and CCA parameters are displayed in Table 3.2. Three visit ANOVAs revealed no significant differences in brachial diameter (*n = 9*), brachial shear rate (*n = 9*), brachial FMD (*n = 9*), CCA diameter (*n = 9*) and CCA shear rate (*n = 9*) between visits, at both timepoints A and B.

Table 3.2 Mean ± SD Vascular assessments at timepoint A and B for Visits 1, 2 & 3

Vascular assessments	Visit 1		Visit 2		Visit 3		*Main effect across visits for each timepoint**	
Time point	(A)	(B)	(A)	(B)	(A)	(B)	*(A)*	*(B)*
Brachial diam. (mm)	3.90 ± 0.68	3.86 ± 0.68	3.90 ± 0.71	3.85 ± 0.69	3.85 ± 0.70	3.82 ± 0.73	*p=.820*	*p=.663*
Brachial SR (s^{-1})	146.19 ± 85.19	112.54 ± 83.80	166.85 ± 68.94	106.09 ± 45.28	159.77 ± 46.45	103.97 ± 27.03	*p=.135*	*p=.646*
Brachial FMD (%)	5.65 ± 1.97	5.63 ± 1.59	6.17 ± 1.79	6.11 ± 2.17	5.56 ± 1.63	5.71 ± 1.95	*p=.503*	*p=.098*
CCA diam. (mm)	6.97 ± 0.41	6.91 ± 0.48	6.75 ± 0.58	6.89 ± 0.50	6.94 ± 0.42	6.94 ± 0.54	*p=.073*	*p=.951*
CCA SR (s^{-1})	177.39 ± 33.38	161.04 ± 43.28	192.71 ± 31.66	176.20 ± 40.09	167.62 ± 27.96	167.64 ± 51.63	*p=.069*	*p=.481*

n = 9.*One-way (Visit 1, Visit 2, Visit 3) ANOVAs between visits at timepoint A and B separately. *p <.05.* CCA: common carotid artery, FMD: flow-mediated dilatation, diam: diameter, SR: shear rate.

3.4.3. Intra-day variability of vascular assessments

Table 3.3 presents intra-day variability for vascular assessments. The average intra-day CV for brachial FMD was 5.49 %, suggesting low variability. This is reinforced by ICC estimates of 0.92 (95 % CI: 0.83-0.96), indicating excellent intra-day reliability. Furthermore, the intra-day CV for brachial artery diameter was 1.30 % and ICC estimates were 0.99 (95 % CI: 0.98-1.00), again suggesting sufficient reproducibility. Brachial artery shear rate has greater variability, with an intra-day CV of 28.23 % (ICC – 0.43, CI: 0.07-0.7). Similarly, the intra-day CV for CCA diameter and shear rate was 1.93 %, and 11.79 %, respectively. ICC estimates show excellent reproducibility for CCA diameter (ICC – 0.89, CI: 0.78-0.95) and moderate reliability for CCA shear rate (ICC – 0.65, CI: 0.37-0.82).

3.4.4. Inter-day variability of vascular assessments

Table 3.3 presents inter-day variability for vascular assessments. The average inter-day CV for brachial FMD was 10.87 %, which is within the range of adequate reproducibility (Thijssen et al., 2019). The inter-day ICC range was 0.52-0.72, indicating moderate reliability. The inter-day CV for brachial diameter was 1.78 %, and ICC estimates indicate excellent reliability. Similarly, CCA diameter had a CV of 2.37 %, and good reliability using ICC estimates. As with intra-day measures, inter-day shear rate for both arteries have more variability, with CVs of 19.49 % and 12.27 % for brachial and carotid arteries, respectively.

A one-way repeated measures ANOVA revealed a significant sex difference in inter-day variability of brachial FMD ($p = .022$). Whilst intra-day CVs are similar (F – 3.94 %, M – 6.73 %), the inter-day CV for females was 8.73 % greater compared to males (F – 15.72 %, M – 6.99 %). Furthermore, the lowest inter-day ICC for brachial FMD in females is between visits 1 and 3, possibly driven by fluctuations in menstrual cycle hormones. Importantly, the CV for brachial diameter is adequate and similar between males and females (intra-day: F – 1.26 %, M – 1.33 %, inter-day: F – 1.72 %, M – 1.82 %), and therefore inter-day fluctuations in brachial FMD are not driven by changes in brachial diameter. In summary, there is a greater inter-day variability of brachial FMD in females compared to males, but this is not driven by changes in brachial diameter.

Table 3.3 Intra-and inter-day variability of brachial and common carotid assessments

		Brachial FMD (%)	Brachial diam. (mm)	Brachial SR (S^{-1})	CCA diam. (mm)	CCA SR (S^{-1})	*BA*	*CCA*
Intra-day variability		CV ± SD (%)	CV ± SD (%)	CV ± SD (%)	CV ± SD (%)	CV ± SD (%)	*N*	
Visit 1	(1A vs 1B)	5.24 ± 2.09	1.09 ± 0.73	26.09 ± 20.23	1.77 ± 1.65	12.80 ± 13.75	*9*	*10*
Visit 2	(2A vs 2B)	5.57 ± 4.86	1.37 ± 1.44	30.66 ± 12.04	1.88 ± 1.60	11.02 ± 4.72	*9*	*9*
Visit 3	(3A vs 3B)	5.66 ± 3.25	1.42 ± 1.27	27.94 ± 14.01	2.15 ± 1.25	11.55 ± 9.37	*9*	*9*
Average		**5.49 ± 3.40**	**1.30 ± 1.15**	**28.23 ± 15.43**	**1.93 ± 1.50**	**11.79 ± 9.28**		
Inter-day variability		CV ± SD (%)	CV ± SD (%)	CV ± SD (%)	CV ± SD (%)	CV ± SD (%)		
Time-point A	(1A vs 2A)	11.56 ± 9.87	1.74 ± 1.01	27.70 ± 21.83	2.55 ± 2.54	11.87 ± 8.39	*9*	*10*
	(2A vs 3A)	12.35 ± 10.76	1.88 ± 1.48	24.60 ± 24.92	3.78 ± 3.23	10.27 ± 8.68	*9*	*9*
	(1A vs 3A)	9.17 ± 10.50	1.56 ± 1.60	21.58 ± 17.20	1.86 ± 1.36	11.53 ± 9.26	*9*	*9*
Time-point B	(1B vs 2B)	8.16 ± 8.45	1.75 ± 1.49	11.47 ± 9.61	2.16 ± 1.90	13.80 ± 9.96	*9*	*9*
	(2B vs 3B)	13.30 ± 10.22	1.62 ± 1.46	16.27± 14.90	2.02 ± 1.58	11.39 ± 14.13	*9*	*9*
	(1B vs 3B)	10.70 ± 5.26	2.12 ± 1.72	15.31 ± 20.58	1.84 ± 1.56	14.75 ± 7.10	*9*	*10*
Average		**10.87 ± 9.18**	**1.78 ± 1.46**	**19.49 ± 18.18**	**2.37 ± 2.03**	**12.27 ± 9.59**	*9*	*9*
Intra-day variability		ICC (CI)	ICC (CI)	ICC (CI)	ICC (CI)	ICC (CI)		
Visit 1	(1A vs 1B)	0.90 (0.41-0.98)	0.99 (0.98-0.99)	0.21 (-0.49-0.74)	0.86 (0.53-0.96)	0.55 (0.08-087)	*9*	*10*
Visit 2	(2A vs 2B)	0.96 (0.85-0.99)	0.99 (0.96-1.00)	0.86 (-0.49-0.97)	0.96 (0.84-0.99)	0.69 (0.11-0.92)	*9*	*9*
Visit 3	(3A vs 3B)	0.90 (0.62-0.98)	0.99 (0.97-1.00)	0.45 (-0.26-0.84)	0.88 (0.55-0.97)	0.74 (0.20-0.94)	*9*	*9*
Combined (A vs B)		**0.92 (0.83-0.96)**	**0.99 (0.98-1.00)**	**0.43 (0.07-0.70)**	**0.89 (0.78-0.95)**	**0.65 (0.37-0.82)**	*27*	*28*
Inter-day variability		ICC (CI)	ICC (CI)	ICC (CI)	ICC (CI)	ICC (CI)		
Time-points combined	1 vs 2	0.67 (0.29-0.86)	0.99 (0.97-1.00)	0.50 (0.06-0.78)	0.84 (0.63-0.93)	0.55 (0.14-0.80)	*18*	*19*
	2 vs 3	0.72 (0.37-0.89)	0.98 (0.95-0.99)	0.42 (-0.05-0.73)	0.78 (0.50-0.91)	0.75 (0.45-0.90)	*18*	*18*
	1 vs 3	0.52 (0.06-0.79)	0.99 (0.96-1.00)	0.39 (-0.08-0.72)	0.87 (0.69-0.95)	0.52 (0.11-0.79)	*18*	*18*
Average		**0.64**	**0.99**	**0.44**	**0.83**	**0.61**	*18*	*18*

CV: coefficients of variation, ICC: intra-class coefficients, SD: standard deviation, CI: confidence intervals, BA: brachial artery, CCA: common carotid artery, FMD: flow-mediated dilatation, diam: diameter, SR: shear rate.

3.4.5. Bland Altman Plots

Bland Altman plots for intra and inter-day variance of brachial FMD are reported in Figure 3.1. The plots present the difference between days (y axis) and the mean of both days (x axis). The plots display no systematic bias, and all data points are within 95% limits of agreement, with greater agreement for intra-day variance compared to inter-day variance.

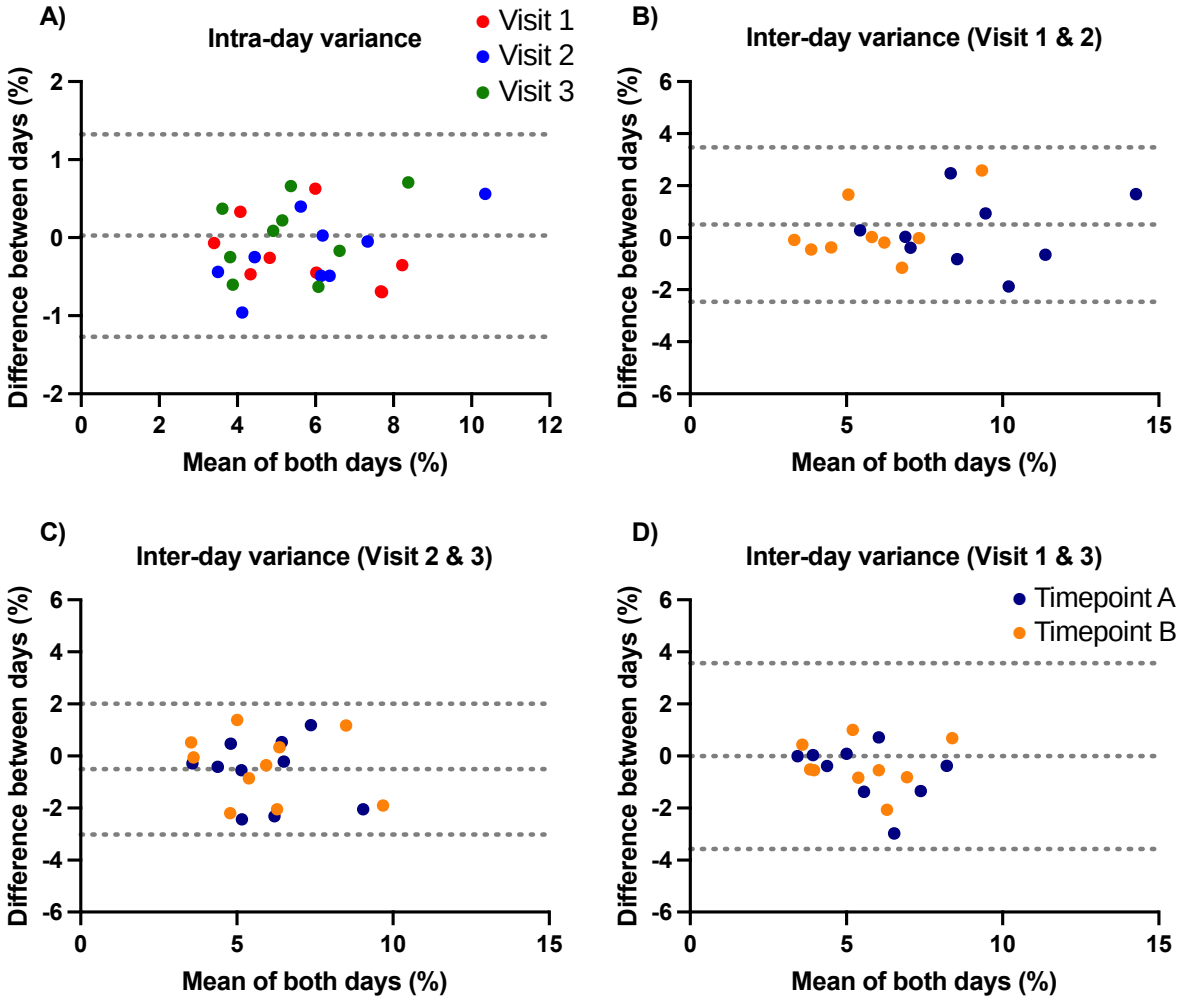


Figure 3.1 Bland-Altman plots of the intra-day (A) and inter-day (B, C, D) variability of brachial FMD

n = 9. Dotted lines indicate the mean of the difference and the limits of agreement (± 2 standard deviations).

3.5. Discussion

Assessment of macrovascular endothelial function using brachial FMD, and assessment of CCA diameter and velocity, demonstrated good reproducibility, with sufficient measurement agreement within and between days. Several studies have examined reproducibility of brachial FMD, and the intra-day (CV – 5.49 ± 3.40 %) and inter-day (CV – 10.87 ± 9.18 %) reproducibility in this study, is in line with previous work. For example, Ghiadoni et al. (2012) similarly found no significant differences between FMD values within and between days, and presented an intra-day CV of 9.9 ± 8.4 % and an inter-day CV of 12.9 ± 11.6 % in a healthy population. Furthermore, the dal-VESSEL trial evidenced reproducibility of FMD assessments in patients with CVD at 19 clinical centres, and presented an inter-day CV of 15.6 % (Charakida et al., 2013). All these trials followed strict technical FMD guidelines, and unsurprisingly, the greater the adherence to these guidelines, the less measurement error in brachial FMD (Greyling et al., 2016). This justifies the need to conduct a reproducibility study. As previously mentioned, sonographers are deemed qualified for FMD measurements when the CV for repeated scans are < 2 % for brachial artery diameter and < 15 % for brachial FMD (Ghiadoni et al., 2012, Thijssen et al., 2019, Charakida et al., 2013). In the present study, the inter-day CV was 1.78 ± 1.46 % for brachial diameter and 10.87 ± 9.18 % for brachial FMD, thus demonstrating sufficient sonography and allowing future FMD data in this thesis to be reproducible.

Technical recommendations for the use of duplex ultrasound to assess extracranial blood flow has been published by Thomas et al. (2015), and these were strictly followed for CCA measurements in the present study. Thomas et al. (2015) outlined

the need for highly skilled and practiced operators to obtain acceptable quality images, evidenced by > 150 scans prior to data collection, and sufficient reproducibility (CV < 10 % between days). A formal training process and this repeatability study ensured that > 150 scans of both carotid and brachial vessels were obtained prior to data collection. Furthermore, this study presents an intra-day CV of 1.93 ± 1.50 % and inter-day CV of 2.37 ± 2.03 % for CCA diameter, suggesting excellent reproducibility.

There are many factors affecting vascular assessments using ultrasound, with the need for highly skilled operators being a major limitation. However, a poor reproducibility can still be present with competent sonography. For example, manual diameter inspection of the vascular wall during analysis increases the variability due to observer error (Thijssen et al., 2019). However, recent developments have allowed automated edge-detection software, as used in the present study, which has greatly improved reproducibility and lowered intra-observer variation. Furthermore, brachial FMD and CCA blood flow are susceptible to environmental and biological variations (Thijssen et al., 2019). These include diet, alcohol, caffeine, exercise, medications, time of day and menstrual cycle phase (Thomas et al., 2015). In the present study, participants were asked to remain consistent in these behaviours. However, as consistency is impossible to control, for future studies in this thesis, Thomas and colleagues' guidelines will be followed: participants avoiding alcohol, caffeine and strenuous exercise for > 12 hours, and fasted for > 6 hours, prior to testing. Similarly, experiments will be conducted at the same time of day. Furthermore, Thomas et al. (2015) state that pre-menopausal females should be tested during the same phase of the menstrual cycle and, if compared with males, this should be between days 1 and 7 of menstruation. In the present repeatability study, menstrual cycle phase was not

controlled for, but visits were scheduled on consecutive days, where possible. As shown by the significantly larger inter-day CV in brachial FMD in females (15.72 %) compared to males (6.99 %), it is likely that menstrual hormones played a role in the variation of brachial FMD. Similarly, this is supported by a lower ICC value between visits 1 and 3, which were 5 days apart in some cases, permitting scope for large hormone fluctuations. Therefore, this indicates the need to test females during the early-follicular phase (when oestrogen and progesterone are low), to minimise this effect. Future studies in this thesis will ensure females are tested accordingly.

The reviewed evidence of brachial FMD (Thijssen et al., 2019) and CCA blood flow (Thomas et al., 2015) reproducibility presents the importance of conducting a repeatability study. This chapter evidences a high reproducibility for measurements of macrovascular endothelial function using brachial FMD, and extracranial blood flow using CCA ultrasound. Therefore, these techniques can be reliably applied in future research presented in this thesis.

4. Fat Intake Impairs the Recovery of Endothelial Function Following Mental Stress in Young Healthy Adults

4.1. Abstract

Introduction: Mental stress has been identified as a trigger of cardiovascular events. A single episode of stress can induce acute impairments in endothelial function in healthy adults. Importantly, during stressful periods, some individuals change their eating behaviour, for example by increasing their consumption of high-fat foods, which is also known to negatively impact endothelial function. Therefore, this study examined whether consumption of a high-fat meal would further exacerbate the negative effect of mental stress on vascular function.

Methods: In a randomised, counterbalanced, cross-over, postprandial intervention study, 21 healthy males and females ingested a high-fat (56.5 g fat) or a low-fat (11.4 g fat) meal 1.5 hours before an 8-minute mental stress task (Paced-Auditory-Serial-Addition-Task, PASAT). Plasma triglyceride (TAG) concentration was assessed pre- and post-meal. Forearm blood flow (FBF), blood pressure (BP), and cardiovascular activity were assessed pre-meal at rest and post-meal at rest and during stress. Endothelial function, measured by brachial flow-mediated dilatation (FMD) was assessed pre-meal and 30 and 90 minutes following mental stress.

Results: Plasma TAG concentration was significantly increased following the high-fat meal compared to the low-fat condition. Mental stress induced similar increases in peripheral vasodilation, BP, and cardiovascular activity, and impaired FMD 30 minutes post-stress, in both conditions. FMD remained significantly impaired 90 minutes following stress in the high-fat condition only, suggesting that consumption of fat attenuates the recovery of endothelial function following mental stress.

Discussion: Given the prevalence of changes in eating behaviour during stressful periods among some young adults, these findings may have important implications for dietary choices to protect the vasculature during periods of stress.

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4.2. Introduction

Stress is extremely prevalent in today's society, with 74 % of the population stating having felt so stressed they are unable to cope (Mental Health Foundation, 2018b). Stress has also been linked with both poor physiological and psychological health (O'Connor et al., 2021). For example, epidemiological studies have shown that when a population is hit by stressful events such as earthquakes, war, and even losing key football matches, there is an increased incidence of myocardial infarction (Bergovec et al., 1992, Carroll et al., 2002, Leor and Kloner, 1996). Laboratory studies have shown that mental stress can induce myocardial ischaemia (Strike and Steptoe, 2003), and that laboratory-based stress-induced myocardial ischaemia is related to ambulatory ischaemia (Krantz et al., 2001). Although, the underlying mechanisms are not yet fully understood, impairments in vascular function have been implicated as a possible mechanism. For example, those who experience mental stress-induced myocardial ischaemia also have an attenuated peripheral vasodilatory response during stress (Burg et al., 2009), as well as increased vascular resistance (Goldberg et al., 1996, Jain et al., 1998).

It has been well established that mental stress evokes increases in heart rate and blood pressure, driven by activation of the sympathetic nervous system and withdrawal of the parasympathetic nervous system (Turner, 1994). Mental stress also impacts the vasculature, and this sympathetic and parasympathetic activation is associated with a nitric oxide (NO)-mediated increase in peripheral vasodilation during mental stress (as measured by forearm blood flow; FBF) (Paine et al., 2013a, Puzserova and Bernatova, 2016a). Importantly, stress-induced vasodilation is attenuated in populations at risk of

cardiovascular disease (CVD), such as obesity (Hamer et al., 2007). Furthermore, mental stress can trigger a transient, but clinically significant, decline in endothelial function (as measured by brachial flow-mediated dilatation; FMD) from 15 to 90 minutes following stress in young healthy adults (Poitras and Pyke, 2013, Inaba et al., 2010). Potential mechanisms have been suggested to involve stress-induced increases in cortico-releasing hormone (CRH), cortisol, and pro-inflammatory cytokines (Poitras and Pyke, 2013), as well as up-regulation of oxidative stress (Wadley et al., 2014); all of which can attenuate NO-production and result in endothelial dysfunction (Toda and Nakanishi-Toda, 2011a).

Stress can also influence physical health indirectly through changes in behaviour (Hill et al., 2021) and adoption of maladaptive coping mechanisms (O'Connor et al., 2021). Importantly, stress can impact eating patterns in some individuals, with studies reporting 42 % of individuals to consume more, and more often unhealthy foods (i.e., high-fat and sugar) during stressful periods (Newman et al., 2006, Roberts et al., 2014a, Oliver and Wardle, 1999). For instance, one study presents that young adults are more likely to choose foods with higher levels of fat following stress compared to a no stress condition (Zellner et al., 2006). Crucially, fat intake can negatively impact the vasculature: brachial FMD is reported to be impaired for 8 hours following consumption of a high-fat meal in healthy and clinical populations (Rendeiro et al., 2016, Jackson et al., 2007, Fard et al., 2000). Hypertriglyceridemia and hyperglycaemia following fat consumption (Bae et al., 2001, Steinberg et al., 1997) have been shown to stimulate the vasoconstrictor endothelin-1 (ET-1), reactive oxygen species (ROS) and inflammatory markers (Bae et al., 2001, Tsai et al., 2004), which subsequently reduce endothelium-derived NO (Man et al., 2020). Reduced NO

production is implicated as a major mechanism driving fat-induced endothelial dysfunction. Furthermore, impaired resting endothelial function has been associated with poorer vascular responses to stress (Sherwood et al., 1999). As such, it is likely that increased fat intake during stress further aggravates the effect of stress on the vasculature. Given the high prevalence of fat consumption during stressful periods it is important to determine the full impact of such interactions on human vascular health.

To our knowledge, only one study has previously attempted to address this question using a model of repeated stress, but possibly due to a relatively low number of participants (N = 10) and timing of FMD measurements, did not show effects of stress and fat separately on FMD or an interaction between stress and fat (Poitras et al., 2014). The current study aimed to investigate the effect of a high-fat meal on peripheral (FBF) blood flow as well as endothelial function (FMD) in healthy adults in the context of a mental stress challenge. We hypothesised that a high-fat meal will impair peripheral blood flow during stress and exacerbate mental stress-induced endothelial dysfunction, compared to a low-fat meal.

4.3. Methods

4.3.1. Participants

Twenty-one participants (11 male, 10 female) were recruited via email and poster advertisements. Females were tested during the same phase of the menstrual cycle (early follicular, days 1-5 of menstruation) to control for the influence of menstrual hormones. Participants were between 18 and 45 years old. Exclusion criteria were: (i) smokers, (ii) consumption of > 21 units alcohol per week, (iii) acute illness/infection, (iv) history of cardiovascular, respiratory, metabolic, liver, inflammatory diseases, or

blood-clotting disorders, (v) allergies or food intolerances, (vi) weight reducing dietary regiment or dietary supplements, and (vii) long-term medication or antibiotics in the previous 3 months. Participants were awarded course credit marks when applicable. Ethical approval was obtained from the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN17_1755D), and all participants gave written informed consent prior to participation in the study.

4.3.2. Habitual Dietary Intake

Habitual dietary intake was assessed using the validated European Prospective Investigation into Diet and Cancer (EPIC) Norfolk Food Frequency Questionnaire (FFQ) (Bingham et al., 2001). Participants recalled their usual dietary intake over the previous 12 months, with 131 different food items, on a 9-point scale (never or less than once per month, 1-3 per month, once a week, 2-4 per week, 5-6 per week, once a day, 2-3 per day, 4-5 per day, and 6+ per day). The FFQ EPIC Tool for Analysis (FETA) was used to calculate nutrient data (Mulligan et al., 2014). The following nutrients are reported in this study: energy (kcal), fat (g), saturated fat (g), carbohydrate (g), sugars (g), fibre (g), protein (g) and portions of fruit and vegetables (calculated as 1 portion corresponding to 80 g), to give a general view of habitual dietary intake.

4.3.3. Study Design

The study design was a randomised, counterbalanced, cross-over, postprandial intervention study (Figure 4.1). Participants visited the laboratory twice, at least a week apart for males and approximately one month apart for females. Participants were asked to refrain from food for 12 hours and from alcohol, vigorous exercise, and caffeine 24 hours before each testing session. Each session commenced at

approximately 8 AM, and firstly, compliance with pre-visit requirements were checked. Participants were then instrumented with equipment to measure cardiovascular activity. Following this, participants rested in a supine position for 20 minutes before pre-intervention (Baseline) measurements were taken: i) brachial FMD, ii) FBF, iii) cardiovascular activity (beat-to-beat blood pressure [BP], heart rate [HR], heart rate variability [HRV] and pre-ejection period [PEP]); iv) blood sample (to measure plasma triglycerides [TAG] concentration). Following these assessments, participants consumed either a high-fat meal (HFM) or a low-fat meal (LFM). Participants then rested for 1.5 hours during which they completed lifestyle questionnaires (only habitual dietary data reported, session 1) and had the option to complete their own work or watch a nature documentary. Subsequently, FBF and cardiovascular activity were measured during an 8-minute rest (Rest) and during an 8-minute mental stress – Paced-Auditory-Serial-Addition-Task (PASAT) (Stress). During each 8-minute assessment, FBF was measured during minutes 2, 4, 6, and 8. BP, HR, PEP and HRV were analysed for these minutes. Brachial FMD was measured 30 minutes and 90 minutes following stress. A second blood sample to measure TAG concentration was taken 45 minutes following stress. A trained researcher carried out all measurements and analyses. Both sessions lasted 5 hours and participants were debriefed following completion of both visits (Figure 4.2).

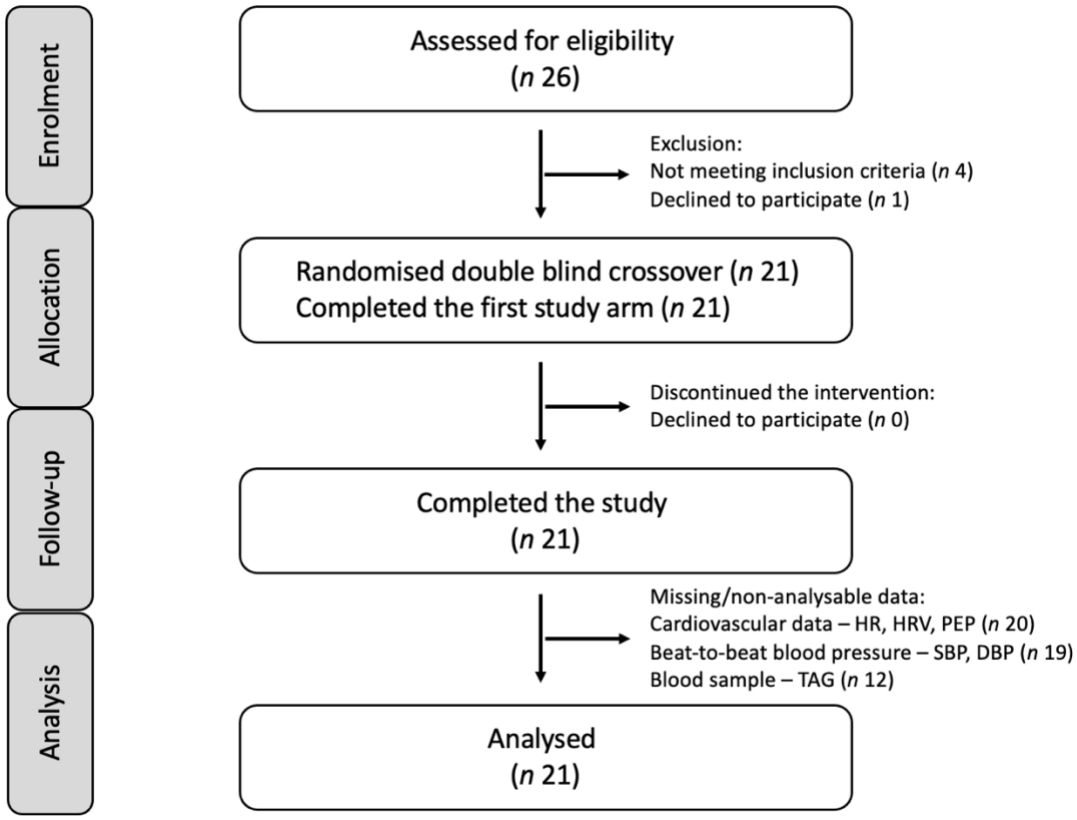

Figure 4.1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram for postprandial intervention study

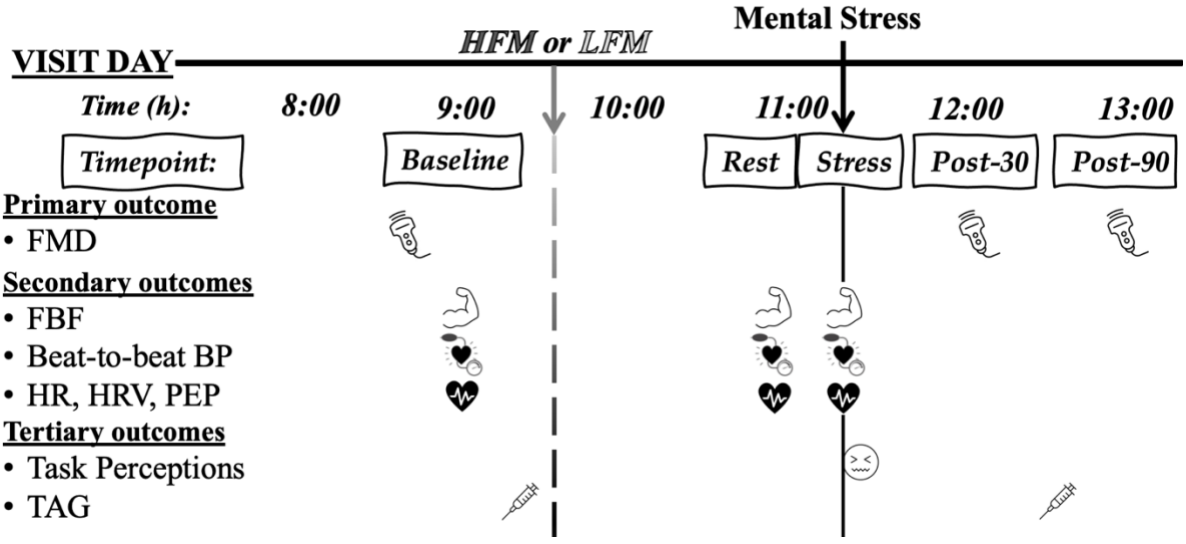

Figure 4.2 Experimental study design

4.3.4. High-and Low-Fat Interventions

The HFM and LFM were prepared just before consumption, and all fresh ingredients were bought within 24 hours of each testing session. The meals were calorie matched, with the HFM containing 56.5 g fat and the LFM containing 11.4 g fat (Rendeiro et al., 2016) (Table 4.1). All other nutrients were as closely matched as possible, except for carbohydrate as a higher level of carbohydrate in the LFM was necessary to match caloric intake. Participants were asked to consume the meal within 20 minutes. Seven participants were not able to finish the low-fat meal and 2 participants were not able to finish the high-fat meal, but no adverse side effects were reported. Whilst it was impossible to blind experimenters and volunteers to the interventions during the visits, these were blinded during all data analyses.

Table 4.1 Nutrient composition of the high-fat and low-fat meals

Meal type	High-Fat Meal[1]	Low-Fat Meal[2]
Nutrient composition:		
Energy (Kcal)	891.0	886.0
Fat (g)	**56.5**	**11.4**
Saturated fat (g)	**35.1**	**5.6**
Carbohydrate (g)	65.0	160.1
Sugars (g)	20.2	19.4
Fibre (g)	2.4	5.9
Protein (g)	29.9	33.3
Salt (g)	2.0	2.5

[1] This meal consists of 2 butter croissants with 10 g salted butter, 1.5 slices of cheese and 250 ml whole milk. [2] This meal consists of 4 slices of white bread with 30 g Philadelphia light spread, 90 g so-organic cornflakes and 250 ml semi-skimmed milk.

4.3.5. Blood Sampling and Plasma Triglycerides Analysis

Blood samples were collected to assess fasting and post-meal plasma TAG concentration. Blood samples were collected in EDTA-coated 10 ml tubes by a trained phlebotomist, from the antecubital vein of the arm. The samples were immediately centrifuged at 5000 rpm for 10 minutes at 4 ^{O}C to separate the plasma. 1000 ul of plasma was pipetted into 1 aliquot for TAG analysis, and stored at -80 ^{O}C for future assessment. Plasma was later analysed using commercially available kits for TAG concentration (Triglyceride Kit, Randox, London, United Kingdom), using an automated photometric clinical chemistry analyser RC Daytona+ (Randox). Samples were analysed in duplicates, with a coefficient of variation (CV) of 0.44 %.

4.3.6. Mental Stress Task

The mental stress task used was the 8-minute PASAT, shown to have good test-retest reliability and to induce a physiological response (Paine et al., 2013a, Ginty et al., 2013, Veldhuijzen van Zanten et al., 2005). The PASAT requires participants to add two sequentially presented single-digit numbers (1-9), adding the number presented to the previous number they heard. The delivery of the numbers became quicker, with time intervals reducing every 2 minutes; from a 2.8 s interval to 2.4 s, 2.0 s, and finally 1.6 s. Participants were filmed and asked to watch themselves on the screen, which they were told would be evaluated by 2 independent body language assessors. An experimenter marked the participants' responses, whilst sounding a loud aversive buzzer-noise at standard intervals once every 10 answers: either following an incorrect response or at the end of the 10-number block. The participants were told they were in direct competition with other participants and lost points for each incorrect answer.

These elements of social evaluation, punishment, and competition have been used previously (Baynham et al., 2021) and have been shown to enhance the provocativeness of the task (Veldhuijzen van Zanten et al., 2002). Immediately following the PASAT, an experimenter asked the participant to verbally rate how difficult, stressful, competitive, and enjoyable they found the task, and to what extent they were trying to perform well, scored on a 7-point scale ranging from 0 ‘not at all’ to 6 ‘extremely’. Following completion of both visits, participants were informed about the deception of the task.

4.3.7. Cardiovascular Activity

4.3.7.1. Impedance Cardiography

The Ambulatory Monitoring System, VU-AMS5s (TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands) was used to continuously record an electrocardiogram (ECG) and impedance cardiogram (ICG) to measure heart rate (HR, bpm), heart rate variability (HRV, ms – a measure of parasympathetic activity) and pre-ejection period (PEP, ms – a measure of sympathetic activity) in line with published guidelines (de Geus et al., 1995, Sherwood et al., 1990). The VuAMS5fs was connected to 7 Ag/AgCl spot electrodes (Invisatrace, ConMed Corpo- ration; Largo, FL, USA). ECG electrodes were placed below the right clavicle, between the lower 2 ribs on the right side, and at the apex of the heart on the left lateral margin of the chest. ICG electrodes were placed at the top end of the sternum at the suprasternal notch and at the bottom of the sternum at the xiphoid process, and on the spine, 3 cm above and 3 cm below the upper and lower electrodes, respectively. Analyses were undertaken offline using VU-DAMS software with manual inspection and correction of ECG and averaged ICG data, used

to derive HR, HRV, and PEP, averaged for each minute of assessment. HRV was calculated from beat-to-beat ECG data as the square root of the mean of the sum of the squared successive differences in cardiac inter-beat intervals. PEP was defined as the time between Q-wave onset and commencement of systole (Sherwood et al., 1990, de Geus et al., 1995).

4.3.7.2. Beat-to-Beat Blood Pressure

Beat-to-beat arterial BP was measured using a Finometer (Finapres Medical Systems; Amsterdam, The Netherlands), with a cuff around the intermediate phalanx of the middle finger. Continuous data was recorded via a Power1401 (CED, Cambridge, UK) connected to a computer programmed in Spike2. Data was analysed for the same minutes as FBF was recorded and averaged for each minute of assessment. Analyses were undertaken offline whereby each file was visually inspected, and systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were obtained.

4.3.8. Forearm Blood Flow

Forearm blood flow was measured using venous occlusion plethysmography. A mercury-in-silastic strain gauge was connected to a plethysmograph (ECG, Hokanson; Jacksonville, WA, USA), producing an output voltage with frequency 0-25 Hz. The plethysmograph signal was digitised at 100 Hz with 16-bit resolution, via a Power1401 (CED) connected to a computer programmed in Spike2, as previously described by Paine et al. (2013). One congestion cuff was placed around the wrist (TMC7, Hokanson), and inflated for 1 minute to supra-systolic blood pressure (> 220 mmHg). Another congestion cuff was placed around the brachial region of the upper arm

(SC12, Hokanson), and inflated for 5 seconds to above venous pressure (40 mmHg), every 15 seconds providing 3 blood flow measurements each minute. Blood flow analysis and calibration were undertaken offline using Spike2 (CED). Each increase in limb circumference is identified as a slope, which were averaged to yield a mean blood flow per minute (Paine et al., 2013a). Forearm vascular conductance (FVC) was calculated by dividing FBF by MAP per minute of assessment.

4.3.9. Flow-Mediated Dilatation

Flow-mediated dilatation was used to assess endothelial function of the brachial artery. A 15–4 Mhz (15L4 Smart MarK^{TM}; Terason, MA, USA) transducer was attached to a Terason Duplex Doppler System (Usmart 3300 NexGen Ultrasound; Terason). This has a wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Pisa, Italy), which allows for continuous measurement of diameter and blood velocity throughout the FMD assessment. Following 20 minutes of supine rest, the brachial artery was imaged longitudinally, 5-10 cm proximal to the antecubital fossa. A brachial cuff was placed around the forearm and, following a 1-minute baseline, this was inflated to 220 mmHg for 5 minutes, to cause ischaemia. Subsequently, the rapid cuff deflation caused reactive hyperaemia, and the image was recorded continuously for 5 minutes post-pressure release. This is in accordance with established guidelines (Thijssen et al., 2010). All measurements were undertaken by the same trained researcher, who demonstrates sufficient reproducibility in brachial FMD measurements (coefficient of variation: intra-day 5.49 %, inter-day 10.87 %). All file images were analysed by the same trained researcher, blinded to condition and measurement details. Peak diameter was defined as the largest diameter obtained

after occlusion is released. The FMD response was calculated as the relative diastolic diameter change between baseline and peak diameter. Resting arterial diameter was also estimated based on a time-average across the first minute of recording.

4.3.10. Statistical Analysis

All statistical analyses were conducted using IBM SPSS software (version 25). The cardiovascular and FBF measurements during pre-intervention baseline, rest, and stress were averaged separately to provide a mean pre-intervention baseline, rest, and stress value for each outcome. Pre-intervention baseline measures (FMD, FBF, HR, SBP, DBP, TAG), task perceptions and PASAT scores were compared using a 2 condition (HFM, LFM) repeated measures analysis of variance (ANOVA). Plasma TAG concentration was analysed using a 2 condition (HFM, LFM) by 2 time (baseline, 2hr post-meal) repeated measures ANOVA. Subsequently, a series of 2 condition (HFM, LFM) by 3 time (baseline, rest, stress) repeated measures ANOVAs were conducted to analyse the cardiovascular and FBF variables. FMD (including resting arterial diameter) was analysed using a 2 condition (HFM, LFM) by 3 time (baseline, post-30, post-90) repeated measures ANOVA. Where appropriate, pairwise comparisons using Bonferroni correction were conducted to investigate significant effects in more detail. All analyses were also conducted with sex as a between-subject variable, yet there were no significant interaction effects of sex. All values reported in text, tables, and graphs are mean ± SD. Occasional missing data are reflected in the reported 'n' values, and include n-1 due to VU-AMS malfunction, n-2 due to Finapres malfunction and n-9 due to participants not willing to have a blood sample or missed sample time-points. Seven participants did not finish the meal. All statistical tests were repeated excluding

these 7 participants. The results were broadly similar to the analyses with the full sample; therefore, it was decided to include all participants to maximise power. For all analyses, significance was set at $\alpha < 0.05$.

4.4. Results

4.4.1. Participant Characteristics

Participant characteristics are presented in Table 4.2. Participants were aged 20 to 30 years old, with a healthy body mass index (BMI) and identified as white European ethnicity (*n = 19*) or Asian ethnicity (*n = 2*). Pre-intervention baseline FMD, FBF, HR, BP, and TAG concentration were similar in both conditions (*n = 2*1, Table 4.2).

Table 4.2 Mean ± SD participant pre-intervention baseline characteristics in the high-fat meal and low-fat meal condition

Participant characteristics	High-fat meal	Low-fat meal	p value*
N	21 (M:11, F:10)		/
Age (years)	22.1 ± 2.7		/
BMI (kg/m^2)	23.62 ± 3.1		/
FMD (%)	5.62 ± 1.33	5.50 ± 1.32	.474
FBF (mm/100ml/min)	2.47 ± 0.98	2.21 ± 0.60	.240
HR (bpm)	59.11 ± 8.70	59.26 ± 7.71	.736
SBP (mmHg)	123.11 ± 22.23	118.82 ± 15.68	.487
DBP (mmHg)	56.03 ± 12.49	52.05 ± 9.02	.234
TAG (mmol/l)	0.78 ± 0.30	0.79 ± 0.28	.956

n = 21. N: number, BMI: body mass index, FMD: flow-mediated dilatation, FBF: forearm blood flow, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, TAG: triglycerides. *P value from ANOVAs.

4.4.2. Habitual Dietary Intake

Table 4.3 displays participant's estimated daily intake of key nutrients and the percentage of participants exceeding or not meeting daily recommendations, as suggested by the National Health Service (NHS). The average daily intake of fat was 59.42 ± 18.85 g (23.8 % of participants exceeding the recommended daily intake) and saturated fat was 21.30 ± 6.53 g (19.0 % of participants exceeding the recommended daily intake). Fat and saturated fat consumption was similar between males and females. The average intake of fruit and vegetables was 5.71 ± 3.32 portions per day, with females consuming almost 1 extra portion per day. However, 38.1 % of participants did not meet the daily recommendations of 5 portions of fruit and vegetables per day. 100 % of participants did not meet the suggested recommendations for fibre intake and exceeded the recommendations for sugar intake.

Table 4.3 Mean ± SD estimated daily intake of key nutrients

Nutrients	Sample average	% of participants over/under recommended daily intake
Energy (Kcal)	1576.48 ± 418.93	N/A
Fat (g)	59.42 ± 18.85	23.8% over
Saturated fat (g)	21.30 ± 6.53	19.0% over
Carbohydrate (g)	185.50 ± 57.45	N/A
Sugars (g)	87.27 ± 42.51	100% over
Fibre (g)	14.09 ± 5.72	100 % under
Protein (g)	74.34 ± 25.07	N/A
Portions of fruit & vegetables*	5.71 ± 3.32	38.1% under

Recommendations – fat: < 70g/day, saturated fat: < 30g/day (male) / < 20g/day (female), sugar: < 30g/day, fibre: > 30g/day, fruit & vegetables: > 5 portions/day. *1 portion = 80g. (NHS guidelines).

4.4.3. Plasma triglycerides

A 2 condition (HFM, LFM) × 2 time (baseline, 2 hr post-meal) ANOVA revealed an overall time effect (*n = 12*, p <.001), condition effect (*n = 12*, p <.001) and a condition × time interaction effect (*n = 12*, p <.001) for TAG concentration (Figure 4.3). Post-hoc analyses revealed that TAG concentration was significantly higher after the high-fat meal compared to the low-fat meal (p <.001), and significantly higher 2hr post-meal compared to pre-intervention baseline (p <.001). Further exploration of the interaction effect revealed that there was no significant difference in TAG concentration between conditions at pre-intervention baseline (p =.956), but TAG concentration was significantly higher following the high-fat meal compared to the low-fat meal at 2 hr post-meal (p <.001).

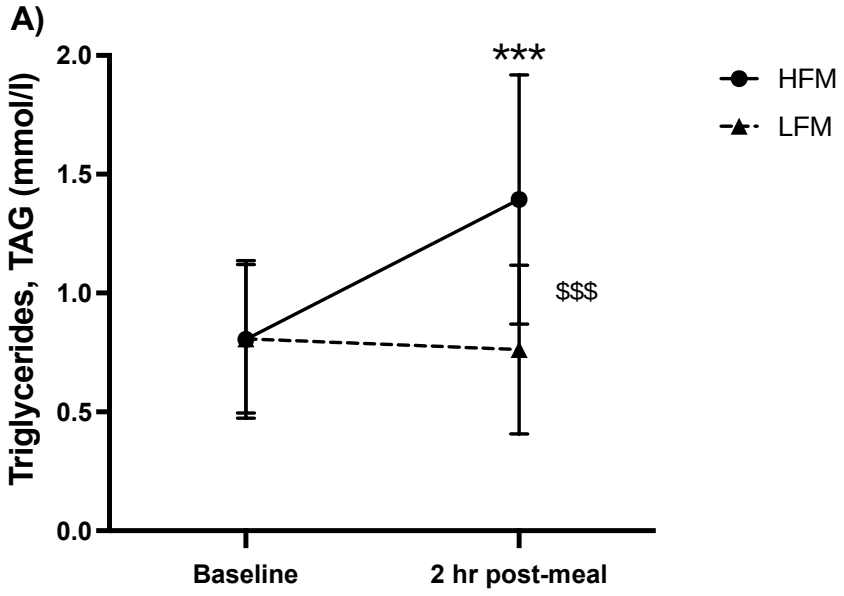

Figure 4.3 Time course of TAG concentration [A] at baseline and 2 hr post-meal

Data are presented as Mean ± SD. *n = 12*. * significantly different compared to baseline in high-fat meal condition, $ significantly different between conditions, *** / $$$ p<.001. HFM: high-fat meal, LFM: low-fat meal.

4.4.4. Mental stress task ratings

Separate two condition (HFM, LFM) ANOVAs revealed no significant difference in task performance (PASAT score) or task perceptions between high-fat and low-fat conditions (*n = 21*). Participants perceived the task as similarly difficult, stressful, competitive, enjoyable, and tried to perform well to the same extent after both high-fat and low-fat meals (*n = 21*, Table 4.4).

Table 4.4 Mean ± SD task performance (PASAT) and ratings

Task Ratings	High-fat meal	Low-fat meal	p value*
PASAT Score	141 ± 34	138 ± 35	.544
Perceived difficulty	4.81 ± 0.60	4.71 ± 0.72	. 576
Perceived stressfulness	4.90 ± 0.94	4.66 ± 0.73	.204
Perceived competitiveness	4.33 ± 1.20	3.86 ± 1.35	.135
Perceived enjoyment	1.95 ± 1.16	1.48 ± 1.08	.125
Perception of trying to perform well	5.00 ± 0.89	5.14 ± 0.96	.419

n = 21. Note: maximum score for PASAT is 228, task ratings are scored from 0 – 6. PASAT: paced-auditory-serial-addition-task. *P value from ANOVAs.

4.4.5. Cardiovascular activity

Separate 2 condition (HFM, LFM) × 3 time (baseline, rest, stress) ANOVAs revealed an overall time effect for HR (*n = 20*, p <.001), PEP (*n = 20*, p <.001), HRV (*n = 20*, p <.001), SBP (*n = 19*, p <.001) and DBP (*n = 19*, p <.001) (Figure 4.4). Post-hoc analyses revealed that HR was significantly higher during rest compared to baseline (p <.001) and increased further during stress (p <.001). HRV was significantly lower during stress compared to baseline and rest (p's <.001). Compared to baseline, PEP was significantly lower during rest (p <.001), with a further decrease during stress (p

<.001). Both SBP and DBP were significantly higher during stress compared to both baseline (SBP: p <.001, DBP: p =.002) and rest (p's <.001) and no significant differences were found between pre-intervention baseline and rest (SBP: p =.492, DBP: p =.152). There were no significant condition or condition × time interaction effects for HR (condition: p =.301, interaction: p =.562), HRV (condition: p =.773, interaction: p =.913), PEP (condition: p =.854, interaction: p =.608), SBP (condition: p =.463, interaction p =.882) or DBP (condition: p =.269, interaction: p =.620).

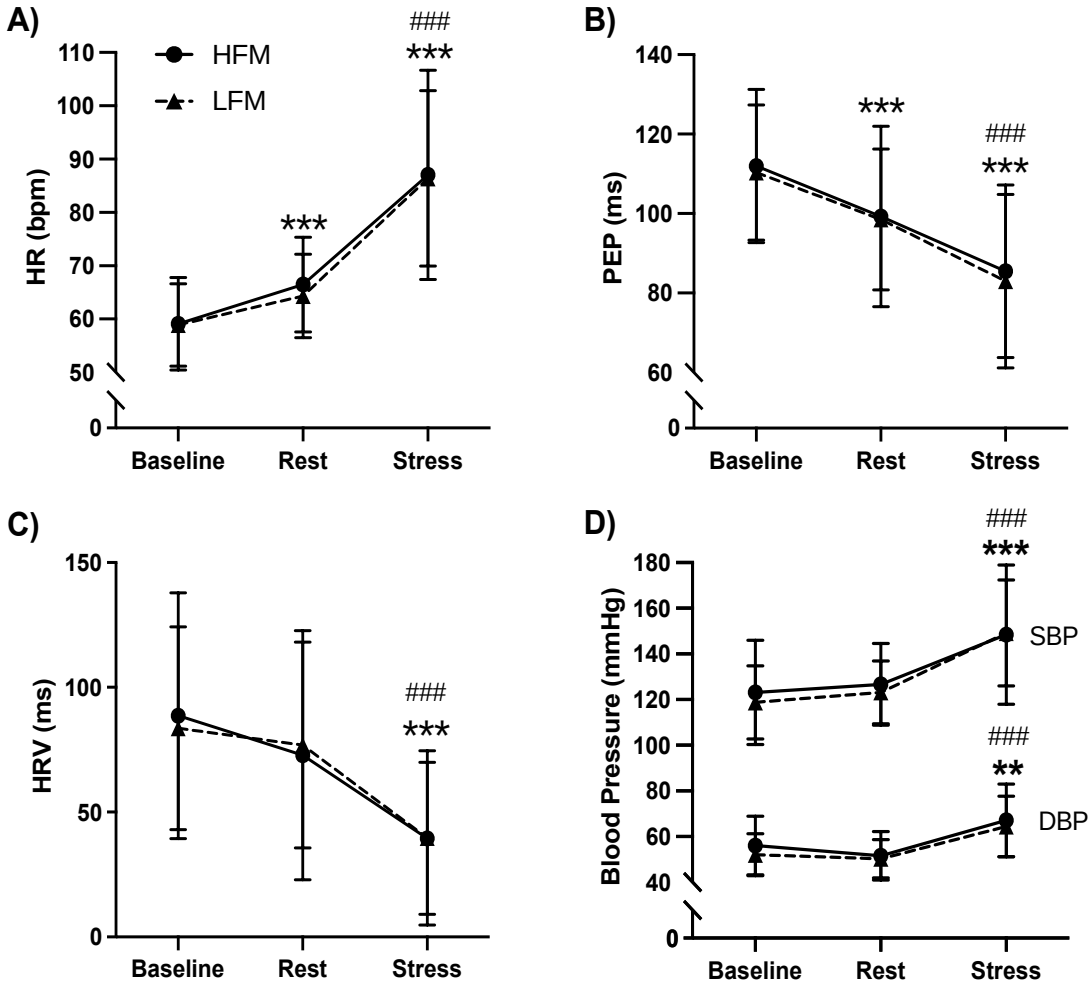

Figure 4.4 Time course of cardiovascular activity (HR [A], PEP [B], HRV [C], BP [D] during baseline, rest and stress following either a high-fat or low-fat meal

Data are presented as Mean ± SD. *n = 20* [A, B, C] / *n = 19* [D]. * significantly different from baseline, # significantly different from rest, *** / ### p<.001, ** p<.01. HR: heart rate, HRV: heart rate variability, PEP: pre-ejection period, SBP: systolic blood pressure, DBP: diastolic blood pressure, HFM: high-fat meal, LFM: low-fat meal.

4.4.6. Forearm blood flow during acute mental stress

A 2 condition × 3 time ANOVA revealed an overall time effect for FBF (*n = 21*, p <.001) and FVC (*n = 19*, p =.007) (Figure 4.5). Post-hoc analyses revealed that FBF was significantly higher during stress compared to both baseline (p <.001) and rest (p <.001). Similarly, FVC was significantly higher during stress compared to baseline (p =.023) but not rest (p =.062). In summary, FBF and FVC significantly increased during stress. There were no condition nor condition × time interaction effects for FBF (condition: p =.357, interaction: p =.136) or FVC (condition: p =.432, interaction: p =.188).

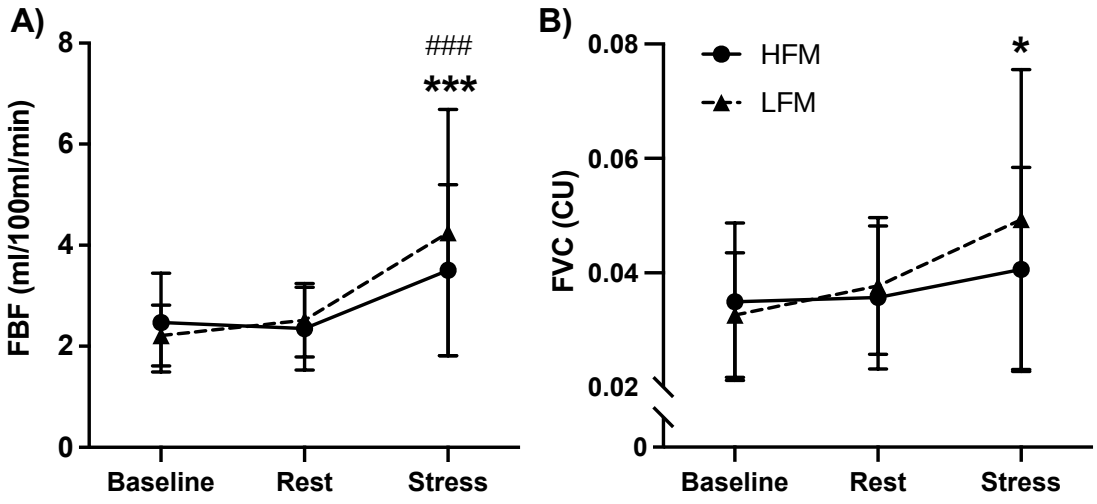

Figure 4.5 Time course of forearm blood flow (FBF [A] & FVC [B]) during baseline, rest and stress following either a high-fat or low-fat meal

Data are presented as Mean ± SD. *n = 21* [A] / 19 [B]. * significantly different from baseline, # significantly different from rest. *** / ### p<.001, * p<.05. FBF: forearm blood flow, FVC: forearm vascular conductance, HFM: high-fat meal, LFM: low-fat meal.

4.4.7. Flow-mediated dilatation following mental stress

Brachial FMD following mental stress is reported in Figure 4.6 (*n = 21*). A 2 condition × 3 time ANOVA revealed a significant time effect for brachial FMD (p <.001). Post-hoc analyses showed that FMD at 30 minutes post-stress was significantly lower compared to both baseline (p <.001) and 90 minutes post-stress (p =.001), and FMD at 90 minutes post-stress was lower compared to baseline (p =.048). Furthermore, there was a significant condition × time interaction effect for brachial FMD (p =.008) (Figure 4.6). Further exploration of this interaction effect revealed that FMD was significantly lower 90 minutes post-stress in the high-fat condition compared to the low-fat condition (p =.018). Examination of the time effects in both conditions separately, showed that in the high-fat condition, there was no significant difference in FMD between 30 minutes and 90 minutes post-stress (p =.134), but both were different from baseline (p <.001, p =.003, respectively). In the low-fat condition, FMD was significantly lower at 30 minutes post-stress compared to both baseline (p =.008) and 90 minutes post-stress (p <.001), but there was no difference between baseline and 90 minutes post-stress (p =1.000). In other words, in the high-fat condition, FMD remained significantly lower up to 90 minutes post-stress, whereas in the low-fat condition, FMD was no longer significantly different from baseline 90 minutes post-stress. There was no significant condition effect for brachial FMD (p =.085).

Brachial arterial diameter (*n = 21*), positive blood flow (*n = 21*), and negative blood flow (*n = 19*) are reported in Table 4.5. There was no significant effect of condition (p =.123), time (p =.316) or condition × time interaction (p =.219) for arterial diameter, suggesting satisfactory sonography. There was no significant time (p =.749) or condition × time

interaction (p =.107) effect for positive blood flow, yet a significant condition effect (p =.002), with a greater blood flow in the high-fat compared to the low-fat condition. There was no significant condition (p =.421) or condition × time interaction (p =.723) effect for negative blood flow, but a significant time effect (p <.001) with a significantly greater negative blood flow 30 minutes post-stress compared to baseline and 90 minutes post-stress.

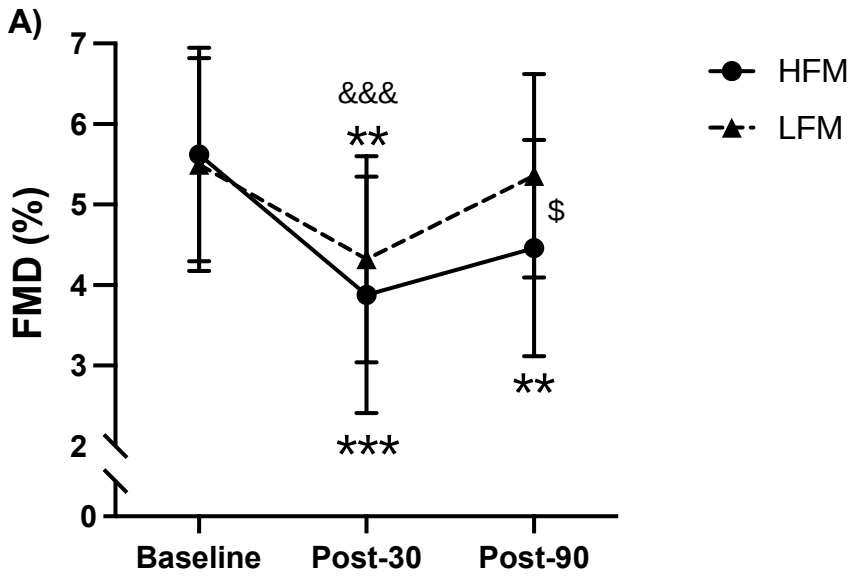

Figure 4.6 Time course of brachial artery FMD (%) [A] during baseline, post-30 and post-90 following either a high-fat or low-fat meal

Data are presented as Mean ± SD. *n = 21*. $ significantly different between conditions, * significantly different compared to baseline, & significantly different compared to post-90. *** / &&& p<.001, ** p<.01, $ p<.05. FMD: Flow-mediated dilatation, HFM: high-fat meal, LFM: low-fat meal.

Table 4.5 Mean ± SD brachial arterial diameter following mental stress

	High-fat meal			Low-fat meal		
Timepoint	Baseline	Post-30	Post-90	Baseline	Post-30	Post-90
Diameter (mm)	3.77 ± 0.63	3.83 ± 0.67	3.84 ± 0.67	3.76 ± 0.67	3.76 ± 0.69	3.75 ± 0.70
Positive blood flow (ml·min⁻1)	98.80 ± 54.98*	107.14 ± 61.39*	105.30 ± 69.20*	86.27 ± 58.49	70.82 ± 40.66	81.86 ± 51.18
Negative blood flow (ml·min⁻1)	-7.84 ± 10.42	-17.04 ± 18.98	-11.51 ± 15.43	-10.90 ± 10.35	-17.74 ± 14.65[&]	-13.10 ± 14.78

*n = 21. *Significantly different to low-fat meal,* [&]Significantly greater than baseline and Post-90.

4.4.8. Sex differences

All analyses were also carried out with sex as a between-subject variable. There were no significant condition × sex interaction effects for FBF (p =.380), FVC (p =.952), HR (p =.665), HRV (p =.947), PEP (p =.856), SBP (p =.746), DBP (p =.826), FMD (p =.710) or TAG (p =.404). There were no significant time × sex interaction effects for FBF (p =.207), FVC (p =.444), HR (p =.612), HRV (p =.193), PEP (p =.846), DBP (p =.065), FMD (p =.893) or TAG (p =.799). However, there was a significant time × sex interaction for SBP (p =.008), whereby males have a significantly lower SBP compared to females at pre-intervention baseline (p =.015), and SBP significantly increases from pre-intervention baseline to rest for males (p =.035) but not for females (p =1.000). Finally, there was no significant condition × time × sex interaction for FBF (p =.665), FVC (p =.930), HR (p =.180), HRV (p =.444), PEP (p =.186), SBP (p =.397), DBP (p =.170), FMD (p =.908) or TAG (p =.357).

4.5. Discussion

The current study investigated the effects of fat consumption and stress on the vasculature in young healthy adults. As expected, and shown previously, we observed peripheral vasodilation and cardiovascular perturbations during mental stress and a decline in brachial FMD 30 minutes following mental stress. To our knowledge, this is the first study to show that consumption of a high-fat meal prevents the recovery of endothelial function 90 minutes following mental stress. Fat consumption did not influence peripheral vasodilation or cardiovascular (HR, PEP, HRV, BP) responses during stress.

As previously shown, mental stress induced an acute increase in FBF (Paine et al., 2013a, Baynham et al., 2021). Similarly, we observed a decline in brachial FMD 30 minutes following mental stress (1.74 % and 1.18 % decline following high and low-fat meals, respectively), in line with our (Baynham et al., 2021) and other previous studies in healthy adults (Broadley et al., 2005, Ghiadoni et al., 2000, Jambrik et al., 2005, Lind et al., 2002, Spieker et al., 2002). NO has been implicated in both the peripheral vasodilation during mental stress and the decline in FMD following mental stress. Sympathetic activation and parasympathetic withdrawal are responsible for increased cardiovascular reactivity during stress (Brindle et al., 2014), and this autonomic activity also contributes to NO-mediated vasodilation during stress (Joyner, 2001, Dietz et al., 1994). Following stress, elevated levels of cortisol and inflammatory markers (Broadley et al., 2005, Steptoe et al., 2007) have been suggested to contribute to post-stress endothelial dysfunction (Plotnick et al., 2017b) through a reduction in NO bioavailability (Marsland et al., 2017, Yamakawa et al., 2009).

No change in resting or stress-induced FBF was reported following the high-fat meal in comparison to the low-fat meal control condition. Only a few studies have investigated the effect of a high-fat meal on resting FBF, with mixed findings reporting attenuated (Shimabukuro et al., 2007), improved (Raitakari et al., 2000) and no change (Gowdak et al., 2010) in peripheral vasodilation. However, differences in fat content, i.e., 30 g fat (Shimabukuro et al., 2007) vs. 60 g fat (Gowdak et al., 2010), calorie count, as well as other nutrients taken together with fat (Raitakari et al., 2000, Steer et al., 2003) make a direct comparison between the studies challenging. Furthermore, the timing of the FBF assessment may also influence these results. For example, the present study measured resting FBF 1 hour 15 minutes post-fat consumption, whilst Shimabukuro et al. (2007) measured FBF 2 hours following a high-fat meal, and other studies' assessments have been at least 3 hours post-fat intake, which is more in line with the fat-induced peak in TAG (Shin et al., 2009, Nappo et al., 2002, Peairs et al., 2011). Therefore, future studies should investigate resting FBF for longer periods following fat consumption, to provide evidence of the mechanisms involved in peripheral vasodilation following fat consumption.

Stress-induced FBF after a high-fat meal was only investigated by one other study, which reported attenuated FBF responses to stress post-fat intake, in contrast with our results (even though we used a comparable intervention) (Gowdak et al., 2010). There are, however, other notable methodological differences between the studies, such as provocativeness of the task, order of meal conditions, and control condition (fasted versus low-fat in the current study). The task used in the current study induced similar and substantial HR responses in both conditions (27 bpm increase in low-fat condition, 28 bpm increase in high-fat condition) in line with previous studies that have used this

protocol (Paine et al., 2013a, Ginty et al., 2013, Veldhuijzen van Zanten et al., 2005). The shorter task applied by Gowdak and colleagues induced a lower HR response, which was significantly lower after the high-fat meal (6 bpm increase) compared to the fasted condition (10 bpm increase). In the present study, the order of conditions was counterbalanced between participants and the conditions were completed on separate days. In contrast, in the previous study, all participants completed the fasted condition prior to the high-fat condition, and both conditions were completed on the same day (Gowdak et al., 2010). Finally, the previous study compared a high-fat condition with a fasted condition, whereas the control condition in the current study was a low-fat meal, meaning both conditions were similar and postprandial with the exception being a difference in fat content. Therefore, it is difficult to determine if the reduced FBF reported by colleagues (Gowdak et al., 2010) following a high-fat meal is due to an order effect of testing, or just a postprandial effect, or fat intake itself.

Fat consumption did not influence the observed decline in FMD 30 minutes post-stress yet did impact the recovery of FMD 90 minutes post-stress. Some studies have shown a < 1 % decrease in FMD following fat consumption (Rendeiro et al., 2016, Newens, 2011) and a more consistent 1 – 3 % decline in FMD following stress (Poitras and Pyke, 2013), yet these findings suggest there is no additive (or interaction) effect of fat and stress on FMD 30 minutes post-stress. However, in the present study, fat consumption did impair the recovery of FMD at 90 minutes following stress, suggesting that consuming fat during stressful periods can prolong impairments in endothelial function in healthy young adults. On the other hand, Poitras et al. (2014) reported no effect of fat consumption on FMD 10 minutes following stress, which is in agreement with our results, as earlier time points (30 minutes post-stress) do not seem to result

in worsened endothelial function. However, direct comparisons between the two studies must be taken with caution as there are significant methodological differences. Poitras and colleagues, subjected participants to four consecutive provocative stress tasks (50 min apart, inducing 20 bpm increases in HR) and no effects on FMD were detected 10 minutes post stress, in both low and high-fat conditions, indicating no impact of stress alone on endothelial function. Indeed, the literature presents more consistent FMD impairments 30 to 90 minutes post-stress (which we targeted in the current study) (Poitras and Pyke, 2013), with one study showing no FMD impairment 15 minutes post-stress (D'Urzo et al., 2019). Furthermore, it is well-established that the autonomic nervous system is stimulated during mental stress (Brindle et al., 2014), and it is possible that sympathetic activation remains elevated at 10 minutes post-stress, making the FMD assessments less reliable. Importantly, the design of the present study allowed us to determine the impact of fat consumption on FMD recovery following stress without the confounder of an activated sympathetic nervous system, which has not been possible with previous study designs. Overall, our data suggests that reduced post-stress endothelial function after fat consumption is only apparent at least 90 minutes post stress, whilst at earlier time points (30 minutes) no fat-stress interaction is detected.

The mechanisms by which fat consumption delays the recovery of FMD following mental stress are not known. TAG and C-reactive protein (CRP) have been evidenced to be increased in circulation 2 – 4 hours post-fat consumption (Shin et al., 2009, Nappo et al., 2002, Peairs et al., 2011), as supported in the present study. This is reflected in our FMD assessments and may explain why intake of fat slows down endothelial function’s recovery 90 minutes post-stress, but not 30 minutes post-stress

(2 hours post-fat intake). The mechanisms driving hypertriglyceridemia-induced endothelial dysfunction are not clear. However, there is evidence that triglyceride-rich lipoprotein particles may cause direct injury to the vascular wall (Plotnick et al., 1997). Alternatively, fat consumption may induce endothelial dysfunction indirectly by increasing oxidative stress, as hypertriglyceridemia has been evidenced to upregulate superoxide anion, a precursor of ROS (Bae et al., 2001). Finally, elevations in triglycerides and CRP following fat consumption have been shown to stimulate vasoconstrictor ET-1 and inflammatory markers (Tsai et al., 2004). All of these mechanisms can subsequently reduce endothelium-derived NO (Man et al., 2020), thus impairing endothelial function, and should be measured in future work. Whilst insulin and TAG start to increase in circulation 30 minutes following fat ingestion (Shin et al., 2009), they are unlikely to have reached their peak during our stress task and FBF assessment (1 hour 15 minutes post-fat consumption), which may explain our null results for FBF. Furthermore, even though postprandial increases in TAG and insulin are likely to modulate FBF, the direction of this response is not well-established (Raitakari et al., 2000, Steer et al., 2003). As FMD was our primary outcome, this informed the choice of timing post-fat consumption and, ensures that we are simultaneously targeting the timeframes in which circulatory TAGs rise and NO declines post-stress. Future studies should be designed to target the FBF response timeframe, allowing the direct assessment of the impact of fat on vascular responses during stress.

In line with previous research, the current study showed no influence of fat consumption on resting cardiovascular parameters (Gouws et al., 2022, Tentolouris et al., 2003). Whilst fat consumption could influence sympathetic activation, there is

evidence that other nutrients and consumption of food in general have a predominant role (Millis et al., 2009, Kaufman et al., 2007). This is supported by the observed postprandial increase in HR at rest, following consumption of both high and low-fat meals. As expected, mental stress induced an immediate change in HR, BP, and measures of sympathetic and parasympathetic activity, which was not impacted by fat consumption. Perhaps this is unsurprising, as fat consumption does not impact resting cardiovascular parameters, so it is also unlikely to modify cardiovascular responses during stress. There is little evidence of the impact of fat on cardiovascular responses during stress, with vast methodological differences (Gowdak et al., 2010), and hence, future research is required in order to make a firm conclusion. Furthermore, while fat consumption does not seem to influence cardiovascular and vasodilatory responses during stress, fat intake may influence resting cardiovascular function following stress. Therefore, future research should similarly assess cardiovascular and vascular changes alongside FMD measurements following stress.

4.5.1. Limitations

One of the limitations in the present study is that the high-fat and low-fat meals were not tailored to individual metabolic rate. This is likely to translate into a higher variability in responses to fat-intake between participants, which can be considered more ecologically valid and further highlights the significance of our results. Furthermore, Jackson et al. (2007)'s review suggests that approximately 50 g fat is sufficient to impact endothelial function, which is comparable to the 56.5 g dose of fat in the present study, previously shown to impair endothelial function (Rendeiro et al., 2016).

The sample used for this study was moderate, yet a robust crossover design was employed, and as effect sizes for non-significant findings are small (interaction effect sizes for FBF, HR, HRV, PEP, and BP were 0.11, 0.08, 0.04, 0.04, and 0.02, respectively), a lack of power was not likely to drive these results. Furthermore, post-hoc power analyses revealed that a sample of 21 participants, power at 90 % and alpha set at 0.05, allowed the detection of a medium size interaction effect (0.33) for our primary outcome measure, brachial FMD (Faul, 2007).

The present study population is estimated to have a relatively healthier habitual diet compared to the UK population. For example, 62 % of participants consumed at least 5 portions of fruit and vegetables (average 5.7 portions/day), compared to 28 % of UK adults (average 3.7 portions/day) (National Health Service, 2020). Similarly, only 19 % of participants exceeded the recommended saturated fat value compared to 75 % of UK adults (Scientific Advisory Committee on Nutrition, 2019). Therefore, the present study sample may represent a healthier population, which highlights additional significance of our observations. It is highly likely that such fat-induced impairments in endothelial function may be further aggravated in a general population with a poorer habitual diet, and particularly in individuals at risk of CVD, such as obese or hypertensive, known to have disturbed vascular responses to stress (Hamer et al., 2007). Therefore, future research should target these populations. Furthermore, it would be interesting to understand how aspects of baseline characteristics, such as diet, fitness level, blood pressure and TAG concentration might influence responses to stress following a high-fat meal, yet a larger sample is required to address this. Therefore, future research should explore what characteristics may put people at higher risk from consuming fat during stress.

4.6. Conclusion

This study demonstrates the detrimental impact of a high-fat meal and stress on endothelial function. Whilst fat had no effect on vascular and cardiovascular responses during stress, the prolonged impairment in endothelial function following stress is significant. Given that a 1 % impairment in FMD has been correlated with a 13 % increase in CVD risk (Inaba et al., 2010), future work should investigate how long such fat and stress-induced impairments in endothelial function last. This might be particularly critical if the combination of stress and fat ingestion becomes chronic, preventing the endothelium's chance to fully recover. Given the documented change in eating behaviour during periods of heightened stress in some individuals, such as increased consumption of high-fat foods, our data can have important implications for future dietary recommendations to protect the vascular system during periods of enhanced vulnerability (such as those rendered by stress).

5. Fat Consumption Attenuates Cortical Oxygenation during Mental Stress in Young Healthy Adults

5.1. Abstract

Mental stress has been associated with cardiovascular events and stroke, and has also been linked with poorer brain function, likely due to its impact on cerebral vasculature. During periods of stress, individuals often increase their consumption of unhealthy foods, especially high-fat foods. Both high-fat intake and mental stress are known to impair endothelial function, yet few studies have investigated the effects of fat consumption on cerebrovascular outcomes during periods of mental stress. Therefore, this study examined whether a high-fat breakfast prior to a mental stress task would alter cortical oxygenation and carotid blood flow in young healthy adults. In a randomised, counterbalanced, cross-over, postprandial intervention study, 21 healthy males and females ingested a high-fat (56.5 g fat) or a low-fat (11.4 g fat) breakfast 1.5 h before an 8-min mental stress task. Common carotid artery (CCA) diameter and blood flow were assessed at pre-meal baseline, 1 h 15 min post-meal at rest, and 10, 30, and 90 min following stress. Pre-frontal cortex (PFC) tissue oxygenation (near-infrared spectroscopy, NIRS) and cardiovascular activity were assessed post-meal at rest and during stress. Mental stress increased heart rate, systolic and diastolic blood pressure, and PFC tissue oxygenation. Importantly, the high-fat breakfast reduced the stress-induced increase in PFC tissue oxygenation, despite no differences in cardiovascular responses between high- and low-fat meals. Fat and stress had no effect on resting CCA blood flow, whilst CCA diameter increased following consumption of both meals. This is the first study to show that fat consumption may impair PFC perfusion during episodes of stress in young healthy adults. Given the prevalence of consuming high-fat foods during stressful periods, these findings have important implications for future research.

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5.2. Introduction

Episodes of acute stress have been shown to trigger cardiovascular events (Bergovec et al., 1992, Carroll et al., 2002, Leor and Kloner, 1996), as well as stroke (Prasad et al., 2020), potentially via stress-induced impairments in vascular function (Burg et al., 2009). Cardiovascular health can also directly impact brain health, with well-established associations between lower cardiovascular diseases (CVD) risk and reduced rates of dementia and cognitive decline in older age (Samieri et al., 2018, Gardener et al., 2016). Similarly, chronic stress affects brain function and cerebrovascular responsiveness, with stress altering the functional connectivity of the pre-frontal cortex (PFC) and impairing attention control in healthy adults (Liston et al., 2009), both known to be modulated by vascular health (Raz et al., 2007).

Acute laboratory mental stress has been evidenced to increase cerebral blood velocity (Shoemaker et al., 2019), as well as increase oxyhaemoglobin and decrease deoxyhaemoglobin concentration in the PFC (Tanida et al., 2007, Nagasawa et al., 2020), both outcome measures indicative of elevated cerebral blood flow (CBF). Increased nitric oxide (NO) bioavailability and systemic increases in blood pressure and cardiac output have all been implicated as potential mechanisms for the increased cerebral perfusion induced during stress (Nagasawa et al., 2020, Bonvento et al., 1994, Iadecola and Zhang, 1994). Importantly, CBF during mental stress is impaired in populations at risk of CVD, such as people with hypertension (Naqvi and Hyuhn,

2009). Therefore, it is likely that vascular dysfunction attenuates the cerebrovascular response to stress, which may have clinically significant consequences, such as increased CVD risk (Perdomo et al., 2020).

The impact of acute stress on vascular function is often measured in the fasted state, yet during periods of stress, individuals are more likely to overeat and consume unhealthy foods, i.e., fat (Hill et al., 2021). Interestingly, fat consumption has been shown to impair endothelial function (Jackson et al., 2007), and endothelial dysfunction has been associated with poorer peripheral vascular responses during stress (Sherwood et al., 1999).

However, few studies have investigated how fat consumption can influence cerebrovascular function. Initial rodent-based research presented cognitive impairments following a chronic high-fat diet (Winocur and Greenwood, 2005). Furthermore, a two-year population-based study showed that increased total fat consumption associated with a greater incidence of dementia in older adults (Kalmijn, 2000). As far as we are aware, only two studies to date have investigated the acute ingestion of fat on cerebrovascular outcomes in humans. One reported no change in cerebral perfusion or conductance (Patik et al., 2018), whereas the other presented decreased CBF to the hypothalamus following fat consumption (Frank et al., 2012). Although the mechanisms underlying fat-induced changes in cerebrovascular function are unknown, post-fat impairments in endothelial function (Zimmerman et al., 2021) as well as increases in inflammation and oxidative stress have been well-documented (Freeman et al., 2014). These markers are also suggested to play a role in stress-induced changes in vascular function (Poitras and Pyke, 2013).

Given the high prevalence of consumption of high-fat foods during stressful periods (Zellner et al., 2006) and the clinical significance of a healthy cerebrovascular response during stress, in the present study we investigated the impact of fat consumption on cerebrovascular responses during mental stress in healthy young adults. More specifically, we assessed changes in PFC cerebral haemodynamics during a laboratory-based mental stress task following a high- and low-fat meal. We also assessed upstream macrovasculature, by measuring common carotid artery (CCA) vasodilation and blood flow up to 90 min following stress, which is in line with previously evidenced stress-induced and fat-induced impairments in endothelial function. We further quantified changes in mood following high- and low-fat meals and following stress. We hypothesised that a high-fat meal would impair cerebrovascular responses to stress.

5.3. Materials and Methods

5.3.1. Participants

Healthy, young (age range inclusion: 18 – 45 years) participants (*n* = 21, 11 male, 10 female), were recruited through email and poster advertisements, and all gave written informed consent prior to participation in the study. Females were tested during the early-follicular phase of the menstrual cycle to control for the effect of menstrual hormones. Inclusion criteria were non-smokers, no history of disease, no allergies or intolerances, and no use of dietary supplements or long-term medication. This study was approved by the University of Birmingham Ethics Committee (ERN17_1755D).

5.3.2. Procedure

The present study was a cross-over intervention study, with two laboratory visits at least a week apart for males, and approximately a month apart for females. The order of dietary conditions was randomised and counterbalanced. Participants visited the lab at 08:00 h and were asked to refrain from food 12 h before, and from alcohol, vigorous exercise, and caffeine 24 h before each testing session. We also requested that participants followed a similar diet for 24 h prior to each visit. Pre-intervention peripheral vascular measurements were assessed (data reported elsewhere: (Baynham et al., 2023b)) as well as common carotid artery (CCA) diameter and blood flow, prior to consumption of a high-fat or low-fat meal. After 1 h 15 min, prefrontal cortical haemodynamics were assessed using near-infrared spectroscopy (NIRS) during an 8-min rest (Rest) and during an 8-min mental stress task (Stress) (Figure 5.1). Cardiovascular activity was also recorded throughout rest and stress. Immediately following stress, CCA diameter and blood flow were assessed (Post-10). CCA diameter and blood flow were also measured 30 min (Post-30) and 90 min (Post-90) following stress. Each session lasted 5 h, and participants were debriefed following completion of both visits.

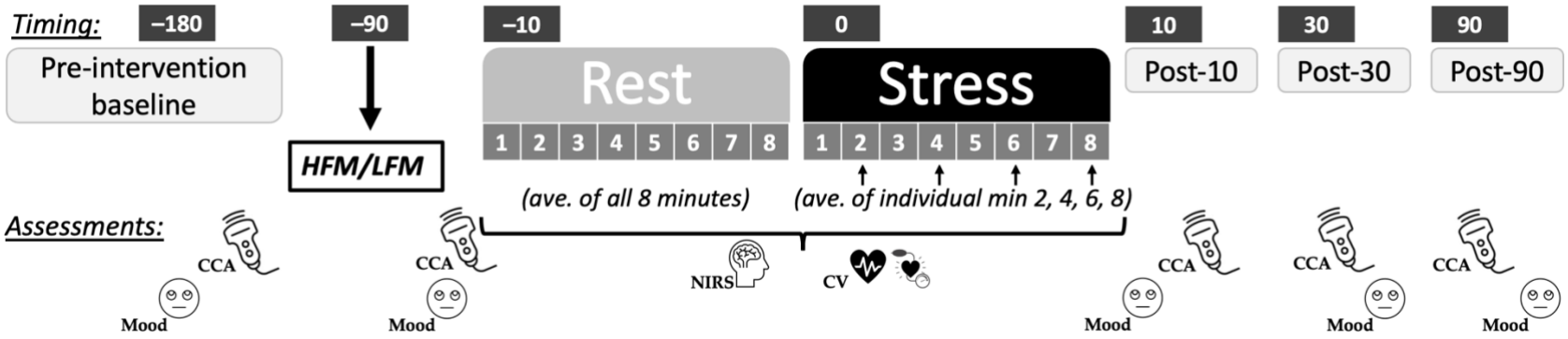

Figure 5.1 Protocol diagram

5.3.3. Meal Interventions

Both meals were prepared just before consumption, and fresh ingredients were bought within 24 h of each session. The calorie-matched meals consisted of a high-fat meal (HFM, 56.5 g fat) and a low-fat meal (LFM, 11.4 g fat). Nutrients were closely matched, apart from carbohydrate quantity (Table 5.1). Each meal was consumed within 20 min (excluding 7 participants who consumed approx. 90 % of the meals), and no adverse side effects were reported.

Table 5.1 Nutrient composition of the high-fat and low-fat meals

Meal Type	High-Fat Meal [1]	Low-Fat Meal [2]
Energy (Kcal)	891.00	886.00
Fat (g)	56.50	11.40
Saturated fat (g)	35.10	5.55
Carbohydrate (g)	65.00	160.10
Sugars (g)	20.2	19.40
Fibre (g)	2.40	5.90
Protein (g)	29.85	33.30
Salt (g)	2.00	2.53

[1] This meal consists of 2 butter croissants with 10 g salted butter, 1.5 slices of cheese, and 250 mL whole milk. [2] This meal consists of 4 slices of white bread with 30 g Philadelphia light spread, 90 g SO organic cornflakes, and 250 mL semi-skimmed milk.

5.3.4. Mental Stress Task

The 8-min paced-auditory-serial-addition-task (PASAT) was used to induce mental stress, shown to have good test–retest reliability and to perturb the cardiovascular system (Paine et al., 2013a). The task requires participants to add two sequentially presented, single-digit numbers, adding the number presented to the previous number

they heard. The time interval between numbers was reduced throughout the task. Elements of social evaluation, punishment, time pressure, and competition (detailed in (Baynham et al., 2023b) were included, shown to enhance the provocativeness of the task (Veldhuijzen van Zanten et al., 2002). Immediately following the task, participants were asked to verbally rate how difficult, stressful, competitive, and enjoyable they found the task, and to what extent they were trying to perform well, scored on a 7-point scale ranging from 0 ‘not at all’ to 6 ‘extremely’. Following both visits, participants were informed about the deception of the task.

5.3.5. Cardiovascular Activity

Systolic (SBP) and diastolic (DBP) blood pressure were measured using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). A small cuff was placed around the intermediate phalanx of the middle finger, and continuous data were recorded via a Power1401 (CED) connected to a computer programmed in Spike2.

An ambulatory monitor (VU-AMS) and 7 Ag/AgCl spot electrodes (Invisatrace, ConMed Corporation; Largo, FL, USA) recorded electrocardiographic and impedance cardiographic signals continuously, in accordance with published guidelines (de Geus et al., 1995, Sherwood et al., 1990). Sixty-second ensemble averages were used to determine heart rate (HR, bpm), heart rate variability (HRV, ms), pre-ejection period (PEP, ms), and stroke volume (SV, mL) as measures of sympathetic and parasympathetic activity. Cardiac output (CO, L/min) was calculated as (HR × SV)/1000.

5.3.6. Prefrontal Cortical Haemodynamics

Near-infrared spectroscopy (NIRS, NIRO-200NX, Hamamatsu Photonics KK, Shizuoka, Japan) was used to assess prefrontal cortical haemodynamics. The NIRS device measures changes in chromophore concentrations of oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb), providing depth-resolved measures of tissue oxygen saturation (total oxygenation index, TOI) and tissue haemoglobin content (relative value of total haemoglobin normalised to the initial value, nTHI). Probes were positioned over the left pre-frontal site and secured to the head with a black headband. Probes were enclosed in light-shielding rubber housing that maintained emitter-to-detector optode spacing (4 cm), and signals were acquired at a sample interval of 0.2 s (5 Hz). NIRS was assessed during 8 min of rest and 8 min of stress. Measures of TOI, nTHI, O_2Hb, and HHb were averaged to provide 1 value for each minute of rest and stress.

5.3.7. Common Carotid Artery Diameter and Blood Flow

Duplex ultrasound was used to assess common carotid artery (CCA) diameter and blood flow. A 15–4 Mhz (15L4 Smart MarK™; Terason, Burlington, MA, USA) transducer was attached to a Terason Duplex Ultrasound System (Usmart 3300 NexGen Ultrasound; Terason). This was combined with wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Via Moruzzi, Pisa, Italy), which allows for continuous measurement of diameter and blood velocity. Following 10 min of supine rest, the participant was asked to turn their head and neck slightly to the left side. Then, a 2-min recording of the right CCA was obtained. All file images were analysed by a trained researcher, blinded to condition and measurement details.

Analysis allows estimation of resting arterial diameter and calculation of arterial blood flow based on a time-average across 2 min of the recording.

5.3.8. Mood Questionnaire

Mood was assessed with a short form of the Profile of Mood States (POMS) questionnaire (McNair et al., 1981), calculating 6 constructs: tension–anxiety, anger–hostility, vigour–activity, fatigue–inertia, confusion–bewilderment, and depression–dejection. Participants were asked to rate on a 5-point scale (1 = not at all, 5 = extremely), how they felt at that precise moment. Total mood disturbance (TMD) was calculated (as recommended in the POMS manual) by summing all negative items (tension, anger, fatigue, confusion, and depression) and subtracting the positive (vigour) score (Grove and Prapavessis, 1992). Overall mood scores ranged from 10 to 46, with a higher score indicating a more negative emotional state (i.e., greater mood disturbance). TMD is a global estimate of affective state which has been examined in across psychological studies using the POMS (Grove and Prapavessis, 1992). POMS questionnaires were completed at pre-intervention baseline (Baseline), post-intervention rest (Rest), immediately following stress (Stress), and 30 and 90 min post-stress (Post-30 and Post-90).

5.3.9. Data Reduction and Statistical Analysis

NIRS and cardiovascular measures were averaged per minute of assessment for the Rest and Stress periods. For the NIRS variables, the eight rest values were then averaged to one resting baseline value, and reactivity scores during stress were calculated as Stress minus Rest, for minutes 2, 4, 6, and 8 of stress (corresponding to Stress 1, Stress 2, Stress 3, and Stress 4, respectively).

All data were statistically analysed using IBM SPSS software (version 25). Task perceptions and PASAT scores were compared between visits using a one-way repeated measures ANOVA. Cardiovascular variables were analysed using a two-way repeated measures ANOVA with condition (HFM, LFM) and time (Rest, Stress 1, Stress 2, Stress 3, Stress 4) as within-subject factors. NIRS variables at rest and during stress (8 min averaged) were compared using separate one-sample t-tests for both conditions. This was the most appropriate statistical approach given that the resting values were 0, so there is no variability around the mean. We then further analysed the NIRS variables using a two-way repeated measures ANOVA with condition (HFM, LFM) and time (Stress 1, Stress 2, Stress 3, Stress 4) as within-subject factors. CCA diameter and blood flow were analysed using a 2-condition (HFM, LFM) by 5-time (Baseline, Rest, Post-10, Post-30, Post-90) repeated measures ANOVA. TMD was similarly analysed using a 2-condition (HFM, LFM) by 5-time (Baseline, Rest, Stress, Post-30, Post-90) repeated measures ANOVA. Where appropriate, pairwise comparisons using Bonferroni correction were conducted as post-hoc analyses. All values reported in text, tables, and graphs are mean ± standard deviation. Occasional missing data are reflected in the reported 'n' values, and include n – 1 due to VU-AMS malfunction, n – 2 due to finapress malfunction, and n – 2 due to NIRS malfunction. All statistical tests were also carried out excluding 7 participants who did not complete both meals; however, as the results were similar to the analyses with the full sample, all participants were included to maximise power. For all analyses, significance was set at $p < 0.05$.

5.4. Results

5.4.1. Participant Characteristics

Participants (*n* = 21) ranged from 20 to 30 years old (22.1 ± 2.7 years old), had a healthy BMI (23.6 ± 3.1 kg/m^2), and identified as either white European ethnicity (*n* = 19) or Asian ethnicity (*n* = 2). Participants self-reported to be physically active and have a healthy habitual diet (daily energy: 1576.5 ± 418.9 Kcal, fat: 59.4 ± 18.9 g, saturated fat: 21.3 ± 6.5 g, carbohydrate: 185.5 ± 57.5 g, sugars: 87.3 ± 42.5 g, fibre: 14.1 ± 5.7 g, protein: 74.3 ± 25.1 g, fruit & vegetables: 5.7 ± 3.3 portions (Baynham et al., 2023b). Resting cardiovascular activity is displayed in Table 5.2. There were no significant differences in BP, HRV, PEP, and CO between conditions at rest (all $p > 0.261$), although there was a significant difference in post-intervention/pre-stress resting HR between conditions ($p = 0.027$). However, there was no significant difference HR between conditions at the previous pre-intervention timepoint (data shown in (Baynham et al., 2023b)).

Table 5.2 Mean ± SD Resting participant characteristics in the high-fat and low-fat conditions

	High-Fat Meal	Low-Fat Meal
SBP (mmHg)	127 ± 18	123 ± 14
DBP (mmHg)	52 ± 11	50 ± 8
HR (bpm)	67 ± 9	64 ± 8 *
HRV (ms)	75 ± 50	77 ± 41
PEP (ms)	99 ± 23	99 ± 18
CO (L/min)	7 ± 2	6 ± 2

n = 19 (BP)/20 (HR, HRV, PEP, CO). * $p < 0.05$. SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, HRV: heart rate variability, PEP: pre-ejection period, CO: cardiac output.

5.4.2. Mental Stress Task Ratings

Two-condition (HFM, LFM) ANOVAs revealed no significant difference in PASAT score between conditions (n = 21, p =.544), and participants perceived the task as equally difficult, stressful, competitive, and enjoyable, and tried to perform well to the same extent (all p >.576) after both high-fat and low-fat meals (Table 5.3).

Table 5.3 Mean ± SD Task performance and ratings in each meal condition

Task Ratings	High-Fat Meal	Low-Fat Meal
PASAT Score	141 ± 34	138 ± 35
Perceived difficulty	4.8 ± 0.6	4.7 ± 0.7
Perceived stressfulness	4.9 ± 0.9	4.7 ± 0.7
Perceived competitiveness	4.3 ± 1.2	3.9 ± 1.4
Perceived enjoyment	2.0 ± 1.2	1.5 ± 1.1
Perception of trying to perform well	5.0 ± 0.9	5.1 ± 1.0

n = 21. *Note:* Task ratings scored from 0–6 and PASAT score /228.

5.4.3. Cardiovascular Responses during Mental Stress

Separate 2-condition (HFM, LFM) × 5-time (Rest, Stress 1, Stress 2, Stress 3, Stress 4) ANOVAs revealed an overall time effect for HR (n = 20, p <.001), HRV (n = 20, p <.001), PEP (n = 20, p <.001), CO (n = 20, p <.001), SBP (n = 19, p <.001), and DBP (n = 19, p <.001) (Figure 5.2). Post-hoc analyses are displayed on Figure 5.2 (data reported as the change during mental stress relative to rest). In summary, HR, CO, SBP and DBP significantly increased during stress and HRV and PEP significantly decreased during stress. There were no significant condition or condition × time interaction effects for HR, HRV, PEP, CO, SBP, and DBP (all p >.207).

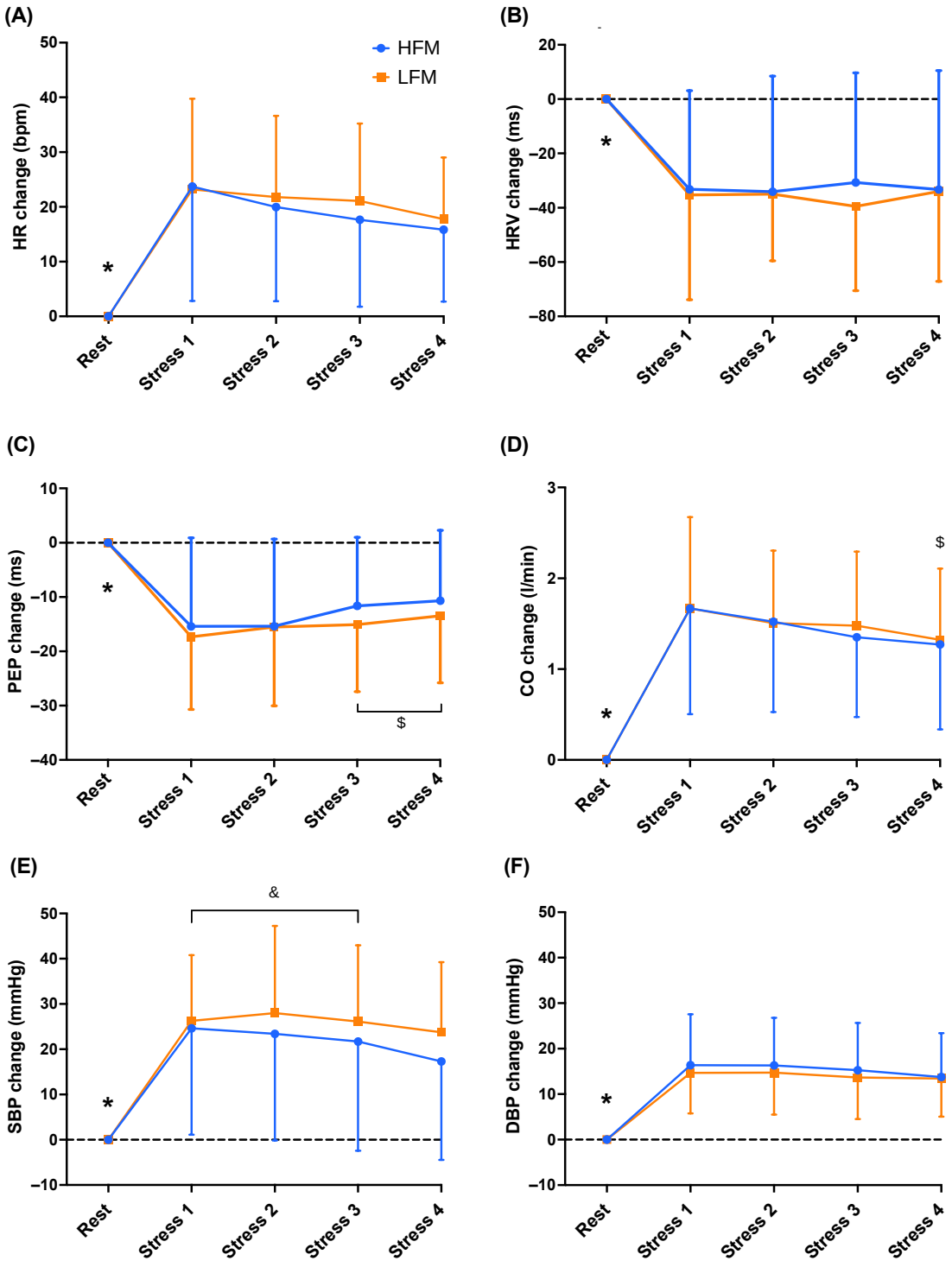

Figure 5.2 Time course of cardiovascular responses (HR (A), HRV (B), PEP (C), CO (D), SBP (E), DBP (F)), during rest and stress following either an HFM or LFM

Data are presented as reactivity mean ± standard deviation. *n* = 20 (A–D)/19 (E,F). * Significantly different compared to Stress 1, 2, 3, and 4, $ significantly different compared to Stress 1, & significantly different compared to Stress 4. HR: heart rate, HRV: heart rate variability, PEP: pre-ejection period, CO: cardiac output, SBP: systolic blood pressure, DBP: diastolic blood pressure, HFM: high-fat meal, LFM: low-fat meal.

5.4.4. Prefrontal Cortical Haemodynamics during Mental Stress

One sample *t*-tests revealed that TOI was significantly greater during stress compared to rest in the LFM condition (p = 0.005) but not the HFM condition (Figure 5.3). There were no significant differences in nTHI during stress compared to rest in both conditions.

Separate 2-condition (HFM, LFM) × 4-time (Stress 1, Stress 2, Stress 3, Stress 4) ANOVAs revealed an overall condition effect (n = 19, p =.019) for TOI (Figure 5.3). Post-hoc analyses revealed that TOI was higher in the LFM condition compared to the HFM condition. However, there were no significant time or condition × time interaction effects for TOI (both p >.099). There were no significant time, condition, or condition × time interaction effects for nTHI (all p >.061).

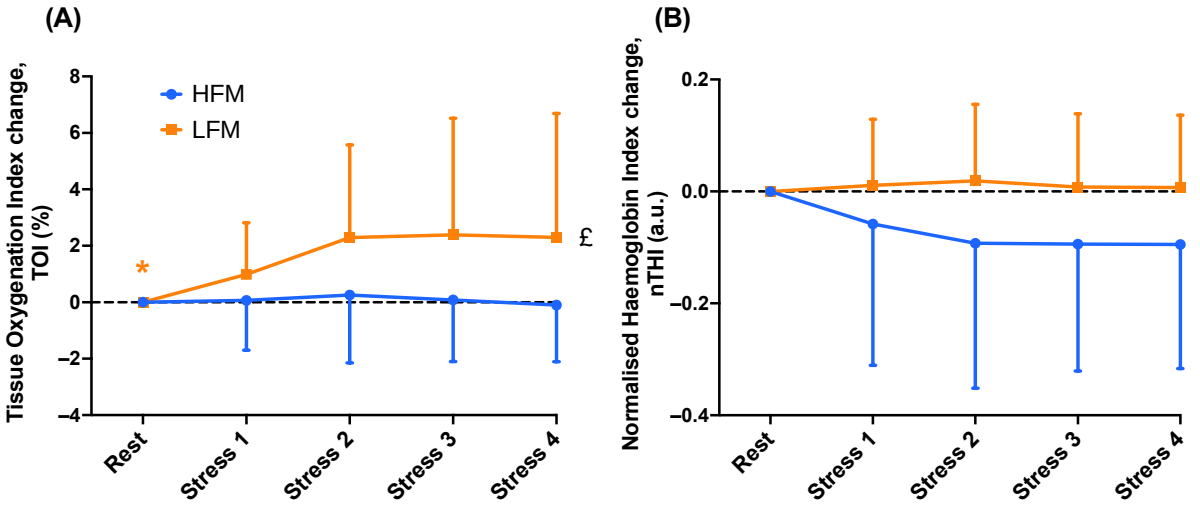

Figure 5.3 Time course of prefrontal cortical haemodynamics (TOI (A) & nTHI (B)) during rest and stress following either an HFM or LFM

Data are presented as reactivity mean ± standard deviation. n = 19. * Significantly different compared to stress in the LFM condition (*t*-test), £ significantly higher following LFM compared to HFM. TOI: tissue oxygenation index, nTHI: normalised haemoglobin index, HFM: high-fat meal, LFM: low-fat meal.

One sample *t*-tests revealed that O_2Hb and HHb were significantly different (O_2Hb increased and HHb decreased) during stress compared to rest in both conditions (both p <.001) (Figure 5.4).

A 2 × 4 ANOVA revealed an overall condition effect (n = 19, p =.048) for O_2Hb (Figure 5.4). Post-hoc analyses revealed that O_2Hb was higher in the LFM condition compared to the HFM condition. There were no significant time nor time × condition interaction effects for O_2Hb (all p >.088). A time 2 × 4 ANOVA revealed an overall time effect (n = 19, p =.002) for HHb. Post-hoc analyses revealed that HHb was lower during Stress 2 and Stress 3 compared to Stress 1. There were no condition nor time × condition interaction effects for HHb (all p >.217).

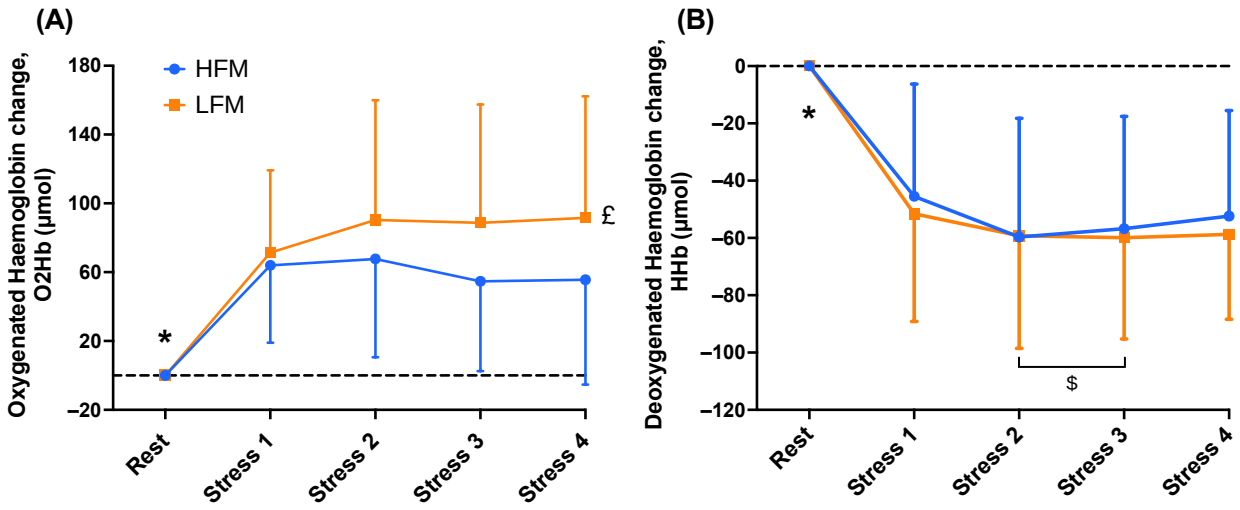

Figure 5.4 Time course of prefrontal cortical haemodynamics (O2Hb (A) & HHb (B)) during rest and stress following either an HFM or LFM

Data are presented as reactivity mean ± standard deviation. n = 19. * Significantly different compared to stress (t-test), £ significantly higher following LFM compared to HFM, $ significantly different compared to Stress 1. O_2Hb: oxygenated haemoglobin change, HHb: deoxygenated haemoglobin change, HFM: high-fat meal, LFM: low-fat meal.

5.4.5. Common Carotid Arterial Diameter and Blood Flow Following Mental Stress

CCA diameter and blood flow are reported in Table 5.4. There were no significant differences in CCA diameter (p =.561), anterograde blood flow (p =.698), and retrograde blood flow (p =.370) between conditions at pre-intervention baseline. A 2-condition (HFM, LFM) × 5-time (Baseline, Rest, Post-10, Post-30, Post-90) ANOVA revealed a significant time effect for CCA diameter (p <.001). Post-hoc analyses showed that CCA diameter was significantly lower at baseline compared to post-meal rest (p =.047), 10 min (p =.047), 30 min (p =.002), and 90 min post-stress (p <.001), and CCA diameter at 90 min post-stress was significantly higher than post-meal rest (p =.026). Furthermore, there was a significant condition × time interaction effect for CCA diameter (p =.033). Further exploration of this interaction effect revealed that CCA diameter was significantly higher 90 min post-stress in the high-fat condition compared to the low-fat condition (p =.026). There was no significant condition (p =.333) effect for CCA diameter. Separate 2-condition × 5-time ANOVAs also revealed no significant time (p =.535), condition (p =.357), or condition × time interaction (p =.924) effect for anterograde blood flow, nor time (p =.096), condition (p =.809), or condition × time interaction (p =.457) effect for retrograde blood flow (Table 5.4).

Table 5.4 Mean ± SD common carotid arterial diameter and blood flow following mental stress

	High-Fat Meal					Low-Fat Meal				
Timepoint	Baseline	Rest	Post-10	Post-30	Post-90	Baseline	Rest	Post-10	Post-30	Post-90
Diameter (mm)	6.69 ± 0.58	6.76 ± 0.56$^{\&}$	6.75 ± 0.59$^{\&}$	6.79 ± 0.58*	6.87 ± 0.56*$^{£}$	6.67 ± 0.56	6.75 ± 0.55 *	6.76 ± 0.51*	6.76 ± 0.51*	6.76 ± 0.53*$^{£}$
Anterograde blood flow (cm^3/min)	695.34 ± 205.10	717.03 ± 195.22	707.69 ± 193.96	709.37 ± 199.11	699.49 ± 140.94	683.40 ± 142.39	712.70 ± 165.50	680.56 ± 182.32	699.44 ± 186.51	668.28 ± 149.80
Retrograde blood flow (cm^3/min)	−0.72 ± 0.94	−1.83 ± 1.97	−1.06 ± 1.77	−2.28 ± 3.42	−2.63 ± 3.27	−1.53 ± 4.36	−1.18 ± 1.52	−1.06 ± 1.40	−2.64 ± 5.45	−1.51 ± 2.16

n = 21. * Significantly different compared to baseline, $^{\&}$ significantly different compared to post-90, $^{£}$ significantly different between conditions.

5.4.6. Mood following High and Low-Fat Meal Consumption and Mental Stress

Total mood disturbance (TMD) is presented in Figure 5.5. There was a significant condition (p =.013), time (p =.004), and condition × time interaction effect (p =.011) for TMD. The time effect revealed that TMD was significantly lower at 90 min post-stress compared to rest (p =.014) and stress (p =.030). Furthermore, the condition effect revealed that TMD was overall greater in the high-fat condition compared to the low-fat condition (p =.013). Finally, as shown in Figure 5.5, the condition × time interaction effect revealed a significantly higher TMD in the high-fat condition compared to the low-fat condition at post-intervention rest (p =.003) and immediately following stress (p =.041). Thus, there was greater mood disturbance following the high-fat meal at rest and stress compared to the low-fat meal.

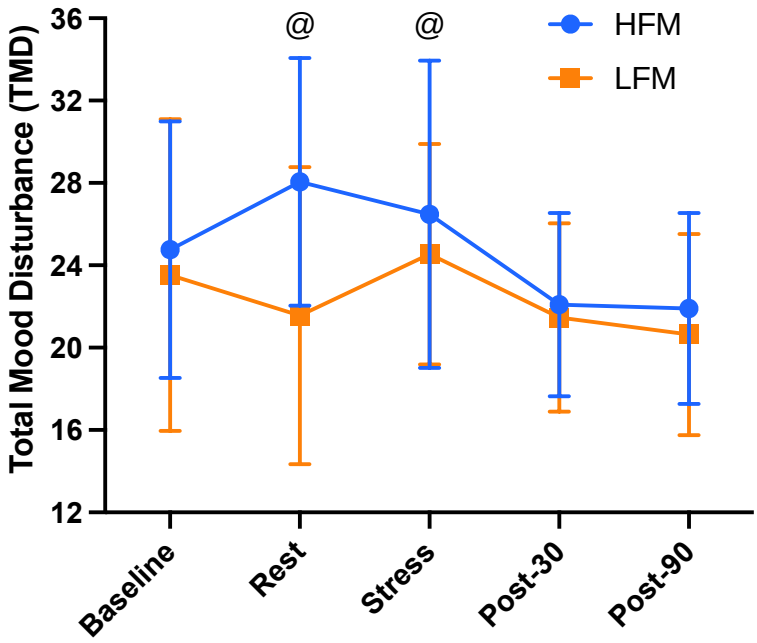

Figure 5.5 Time course of total mood disturbance at baseline, rest, immediately post-stress, and 30- and 90- min post-stress, following either an HFM or LFM

Data are presented as mean ± standard deviation. TMD = (Tension + Anger + Fatigue + Depression + Confusion) − Vigour. n = 18. @ Significant difference between HFM and LFM at these time points. TMD: total mood disturbance, HFM: high-fat meal, LFM: low-fat meal.

5.5. Discussion

The current study showed that mental stress induced increases in HR, CO, SBP, and DBP, decreases in HRV and PEP, and increases in PFC tissue oxygenation (as indexed via changes in TOI and O_2Hb volume). Following fat consumption (HFM condition), stress-induced increases in PFC tissue oxygenation were attenuated, yet there were no differences in the cardiovascular responses to stress. These cardio/cerebrovascular changes were observed despite no significant differences in stress task perceptions or performance between conditions, indicating a consistent stress experience between visits. We further observed no effect of fat consumption or stress on resting CCA blood flow, whilst CCA diameter increased following consumption of both meals. Consumption of fat influenced mood (TMD) at rest and immediately post-stress, suggesting that fat consumption may negatively affect mood. Taken together, these findings indicate that fat consumption alters cerebral haemodynamic activity while completing a mentally stressful task, potentially via impaired cerebral perfusion to the PFC as a result of fat-induced alterations in CBF regulation.

Our observation that mental stress increases PFC tissue perfusion (by virtue of increased TOI and O_2Hb, and decreased HHb) is in line with previous findings that have reported elevated CBF during such stress (Shoemaker et al., 2019, Nagasawa et al., 2020). The increase in CBF is likely to be mediated in part by the systemic increase in CO shown during mental stress, driven by stress-induced elevations in HR (Figure 5.2). CO is a key independent factor influencing CBF (Willie et al., 2014b), with changes in CO shown to be correlated with CBF at rest and during exercise (Ogoh et

al., 2005). In addition, the observed stress-induced increases in BP (SBP and DBP increased by ~20 mmHg) would also contribute to elevated CBF, as even with BP-induced adjustments to cerebrovascular resistance via cerebral autoregulation, CBF will be affected by the large magnitude of observed BP changes (Brassard et al., 2021). Moreover, our findings are consistent with those of Brindle et al. (2018), where the same stress task resulted in similar changes in BP and increased the TCD-based measures of CBF (i.e., increased middle cerebral blood velocity). Another mechanism by which cerebral perfusion could increase during this stress task is via neurovascular coupling, due to the increased neural activation related to the cognitive demand of the mental arithmetic task. Indeed, Shoemaker and colleagues (2019) showed increased CBF (TCD-based measures of middle cerebral blood velocity) during the cognitive tasks they used, and this occurred independently of other key regulators of CBF (i.e., BP and arterial carbon dioxide content). Similarly, a positive correlation has been evidenced between stress perception and CBF (magnetic resonance imaging, MRI—arterial spin labelling, ASL) (Wang et al., 2005), which may contribute to the increase in perfusion via neurovascular coupling, given that both physiological and self-reported data showed the task to be very stressful and difficult, and participants reported to be fully engaged with the task in this study.

Little is known about how fatty acids affect cerebral oxygenation. To our knowledge, this is the first study to show that fat consumption attenuated the increase in PFC tissue oxygenation during stress, indicating that CBF was relatively lower and therefore more oxygen was extracted from the haemoglobin to meet the metabolic demand of the tissue during the task (assuming brain metabolism was similar for the diet conditions). A previous study has also presented a decreased CBF to the hypothalamus following

fat consumption at rest (Frank et al., 2012). Given the association between reduced cerebral oxygenation and impaired cognitive performance (Williams et al., 2019), the lower tissue oxygenation we observed here (2 ± 4 %; Figure 5.3A) may have significant implications for brain function. In the present study, there were no differences in CCA resting blood flow (velocity and diameter) approximately 1 h following fat consumption, but it is possible that more subtle regional changes downstream in the cerebrovasculature could have occurred which we did not assess. Furthermore, another study, with comparable fat quantity to the present study, found no change in CBF during rebreathing-induced hypercapnia following fat consumption (Patik et al., 2018), potentially suggesting some specificity of the fat effect in the context of mental stress. However, differences in methodology for CBF assessments, brain area investigated (i.e., TCD-based cerebral blood velocity (Patik et al., 2018) vs. fMRI of hypothalamic and insular cortex using ASL (Frank et al., 2012)), and differences in fat source might also contribute to some of the differences reported. The mechanisms underlying the fat-induced attenuation of cortical blood perfusion during stress are not known. One possibility is that fat consumption affects cerebral metabolism (exchange of primary molecules of oxygen, glucose, and lactate across arterial and venous circulations in the brain) during stress (Smith and Ainslie, 2017). As neural activity increases, e.g., at the onset of mental stress (Wang et al., 2016), dendrites rapidly consume oxygen, reducing P_{O2} and oxyhaemoglobin concentration (Gordon et al., 2008). The resulting shifts in brain metabolism enhance glycolysis in astrocytes and induce a release of lactate, which subsequently causes vasodilation to increase oxygen delivery (Gordon et al., 2008, Smith and Ainslie, 2017). Therefore, whilst speculative, fat consumption may reduce the metabolic efficiency of the brain by

attenuating this shift in metabolism and hence, reducing perfusion during stress. Whilst evidence is limited to support this idea, evidence that obesity and a high-fat diet can alter metabolic-related cerebral signalling and induce neuroinflammation, thus disrupting cognitive function, has been reviewed (Miller and Spencer, 2014). Further research is needed to explore this mechanism, for example using broadband NIRS measurements of cytochrome-c-oxidase (CCO) to investigate brain metabolism following fat consumption. Another possible mechanism is that fat intake, and specifically hyperlipidaemia, influences cerebral autoregulation (Ayata et al., 2013). However, if fat consumption did impair cerebral autoregulation, it would be expected that CBF would increase more to the same stress-induced increase in BP; however, we observed the opposite effect with respect to changes in PFC tissue oxygenation. nTHI responses seem to be attenuated during mental stress following the high-fat meal, albeit non-significantly. Given that nTHI is an index of tissue blood flow (via measures of total haemoglobin volume), these data suggest that CBF responses to stress are reduced under high-fat conditions and that cerebral autoregulation impairment does not play a significant role in the observed responses. Whilst the mechanisms by which fatty acids affect cerebral oxygenation are unclear, the literature on muscle physiology clearly shows that acute fatty acid intake can blunt leg blood flow responses to NG-monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor (Steinberg et al., 1997), providing evidence that fatty acid elevation impairs NO-mediated vasodilation in the leg microvasculature. Furthermore, animal models show that acute fat intake induces insulin resistance and subsequent impairments in capillary recruitment and muscle glucose uptake (Liu et al., 2009, Clerk et al., 2002). The extent

to which some of these mechanisms translate into the brain microvasculature is unclear and needs to be investigated further.

Interestingly, although fat consumption alters cerebral haemodynamics during mental stress, from 10 to 90 min following stress, no differences in resting carotid arterial blood flow between diets were detectable. It should be noted that stress-induced and fat-induced declines in peripheral vascular function have been well established during the period of 30 – 90 min post stress (Inaba et al., 2010, Poitras and Pyke, 2013), and was the reason we targeted this timeframe for our post-stress assessments. Given that elevated BP and CO are shown to influence CBF (Ogoh et al., 2005), perhaps once these have returned to baseline (~10 min following stress), there is no longer a detectable effect on CBF. Furthermore, we are assessing the upstream macrovasculature (common carotid artery), which supplies the whole brain (as well as some extracranial tissue via the external carotid artery that originates from the CCA), and not specifically the PFC, so more subtle and specific changes might have been missed.

Finally, we observed that CCA diameter significantly increased following consumption of both meals and was significantly greater after the high-fat meal compared to the low-fat meal only at 90 min post stress. This is possibly driven by cholecystokinin (CCK), a peptide hormone that increases postprandially to stimulate digestion, and has been shown to induce cerebral vasodilation (Sánchez–Fernández et al., 2003). More specifically, the release of CCK in response to a meal has been shown to trigger local postprandial hyperaemia in the gut and evoke vasodilation in the cerebral vasculature (Lovick, 2009). CCK has also been shown to stimulate neuronal NO synthase and NO

release, via intracellular calcium (Ruiz-Gayo et al., 2006), which may also induce vasodilation. Furthermore, there is some evidence that CCK levels are higher following a high-fat meal compared to a high-carbohydrate meal (Gibbons et al., 2016), which may explain the increase in CCA diameter in the high-fat meal condition in the present study. However, as there was no change in CCA blood flow, future research should continue this investigation, utilising assessments of the internal carotid artery, to assess the impact of fat consumption on resting CBF.

Fat consumption had a significant impact on mood in the present study, shown by a greater mood disturbance at rest and immediately following stress in the high-fat condition compared to the low-fat condition. When exploring the individual constructs that are used to calculate total mood disturbance, it was particularly fatigue which was significantly higher following the high-fat meal compared to the low-fat meal, which is in line with previous research (Wells et al., 1997). Whilst the relationship between fat consumption and mood outcomes is currently unclear (Tzenios, 2023), previous evidence suggests that high-fat feeding leads to negative emotional states and even increased stress sensitivity in rodent models (Sharma et al., 2012). Furthermore, it is widely recognised that the PFC plays a central role in emotion regulation via efferent projections to limbic areas (responsible for emotional responses) (Del Arco and Mora, 2009). As such, there might be a link between the observed decline in PFC oxygenation during stress and the decline in mood that follows, although this needs to be further addressed in future studies. Therefore, whilst individuals may seek comfort through consumption of high-fat foods when stressed, such food choices may further worsen mood, increase fatigue, and affect an individual's ability to cope with stress, possibly via disturbances in PFC oxygenation.

5.5.1. Limitations

One of the potential limitations of the current study was that the meals were not tailored to individual metabolic rate. Yet, previous studies have shown that a similar fat content (50 g) is sufficient to impact vascular function, and the current study was in line with a similar study showing fat consumption impairs endothelial function (Rendeiro et al., 2016). Secondly, our study has a moderate sample size; nevertheless, a crossover design was employed, and effect sizes for non-significant findings were found to be small, suggesting that a lack of power is not likely to drive these results. Furthermore, as these analyses are secondary, no a priori power calculations were undertaken. Yet, based on the effect size of the condition effect revealed in TOI (0.27), with a sample of 21 participants and alpha set at 0.05, we were able to detect a power of 82%. Importantly, this is the first study to investigate the impact of fat consumption on cerebrovascular responses in a sample that includes females, which is more ecologically valid. Finally, it would have been ideal to assess changes in blood flow in the upstream carotid artery during stress to have a more complete picture of cerebral regulation, but this would be unreliable due to significant movement and positioning of the participant. We also noted that it could significantly interfere with the completion of the stress task itself. Future studies should use combined approaches to assess macro- and microvasculature significantly, as well as explore regional differences across the brain (Burley et al., 2022), for example, by using techniques such as transcranial doppler and ASL-MRI, in addition to NIRS and ultrasound. Importantly, using specific technical approaches such as broadband NIRS and functional MRI would allow for a simultaneous assessment of vascular and neuronal metabolic

responses during stress, which may shed light on the mechanisms by which fat reduces cortical blood perfusion during stress.

5.6. Conclusions

This is the first study to explore the relationship between fat consumption and cerebral dynamics during mental stress, providing, for the first time, evidence that fat consumption impairs PFC perfusion during stress. Experiencing stress is tightly associated with consuming high-fat foods (Hill et al., 2021). This, combined with the high prevalence of stress and obesity in our societies, and further associations with cognitive decline later in life, makes it an important area of research to inform our dietary choices during periods of enhanced stress.

6. Cocoa flavanols can rescue the stress-induced decline in endothelial function after a high-fat breakfast, but do not improve cortical oxygenation during stress

6.1. Abstract

Background: A single episode of mental stress can induce acute impairments in endothelial function, increasing the risk of cardiovascular events. Food choices often worsen during stressful periods, which can influence the consequences of stress on vascular health. For example, fat consumption can disrupt the recovery of endothelial function following mental stress. Furthermore, flavanols (plant-derived polyphenolic compounds) can improve endothelial function post-stress, yet this has only been shown in a fasted state. Therefore, this study examined whether flavanols consumed in combination with fat can mitigate the negative impact of fat on stress-induced impairments in endothelial function.

Methods: In a randomised, counterbalanced, cross-over, postprandial intervention study, 23 healthy males and females ingested a high-fat meal (56.5 g fat) with high-flavanol cocoa (150 mg (-)-epicatechin) or low-flavanol cocoa (< 6 mg (-)-epicatechin) 1.5 hours before an 8-minute mental stress task (Paced-Auditory-Serial-Addition-Task, PASAT). Forearm blood flow (FBF), blood pressure (BP), and cardiovascular activity were assessed pre-meal at baseline, and post-meal at rest and during stress. Pre-frontal cortical oxygenation (NIRS) was assessed at post-meal rest and during stress. Endothelial function, measured by brachial flow-mediated dilatation (FMD), and common carotid artery (CCA) blood flow were assessed at pre-meal baseline and 30- and 90-minutes post-stress. Mood was assessed at baseline, rest, stress, and 30- and 90-minutes post-stress.

Results: Mental stress induced similar increases in peripheral vasodilation, BP, cardiovascular activity, and pre-frontal cortical oxygenation, and similar disruptions to

mood, in both conditions. FMD was impaired at 30- and 90-minutes post-stress in the low-flavanol cocoa condition, showing a stress-induced and fat-induced decline in FMD. High-flavanol cocoa attenuated the impairment in FMD at 30 minutes post-stress and improved FMD at 90 minutes post-stress. CCA diameter increased and CCA retrograde blood flow decreased post-stress, with no impact of the flavanol intervention.

Conclusion: Flavanols attenuated the stress and fat-induced decline in endothelial function yet did not influence pre-frontal cortical oxygenation or carotid artery blood flow in response to stress. These findings have important implications for dietary interventions which could include flavanols to protect the vasculature from stress and stress-induced changes in food choices.

6.2. Introduction

Stress is increasingly prevalent, with 17.1 million working days lost to work-related stress in the last year (Health and Safety Executive, 2023) and currently, the most substantial increase in anxiety is being experienced by young adults (18 – 25 years olds) (Goodwin et al., 2020). Episodes of acute mental stress have been implicated as a trigger for myocardial infarction and sudden cardiac death (Carroll et al., 2002, Bergovec et al., 1992, Leor and Kloner, 1996). Acute mental stress can also trigger stroke (Prasad et al., 2020), and there is a well-established relationship between poor cardiovascular health and future dementia as well as cognitive dysfunction (Samieri et al., 2018, Gardener et al., 2016). Transient impairments in vascular function have been implicated as a mechanism linking stress to poor cardiovascular health. For example, individuals who experience stress-induced myocardial ischaemia also have attenuated peripheral vasodilatory responses during stress (Burg et al., 2009), and increased vascular resistance (Goldberg et al., 1996, Jain et al., 1998).

Importantly, mental stress continues to impact the vasculature following a stressful event, as shown by transient declines in endothelial function (as measured by brachial flow-mediated dilatation; FMD) from 15 to 90 minutes following stress in young, healthy adults (Poitras and Pyke, 2013, Ghiadoni et al., 2000, Lind et al., 2002, Spieker et al., 2002). This is of clinical significance, given that a 1 % reduction in FMD corresponds to a 13 % increase in CVD risk (Inaba et al., 2010). The impact of stress on the cerebral vasculature is less understood (Shoemaker et al., 2019), but impairments in FMD are also associated with increased risk of vascular events, including stroke (Santos-García et al., 2011). Mechanisms underpinning stress-induced impairments in vascular

function may include reduced NO bioavailability (Toda and Nakanishi-Toda, 2011a), driven by increases in cortico-releasing hormone (CRH), cortisol, inflammatory cytokines (Poitras and Pyke, 2013), and oxidative stress markers (Wadley et al., 2014).

Interestingly, stress can also negatively influence health through changes in behaviour (Hill et al., 2021, O'Connor et al., 2021). During stressful periods young adults are likely to overeat and consume more unhealthy foods (i.e., high-fat) and fewer fruits and vegetables (Newman et al., 2006, Roberts et al., 2014b, Oliver and Wardle, 1999, Zellner et al., 2006, Gardiner et al., 2021). For example, 38 % of adults report to have overeaten or eaten unhealthy foods in the previous month due to stress, and half of these adults report this shift in food choices at least weekly (Amerian Psychological Association, 2013). Numerous mechanisms underpinning the stress-eating relationship have been suggested, including increased cortisol reactivity (Pool et al., 2015) and maladaptive effects on brain regions responsible for decision making and emotion regulation (McEwen and Gianaros, 2010). Importantly, these shifts in eating behaviour are likely contributing to increased weight gain, as stress has been identified as an independent risk factor for obesity (Moore and Cunningham, 2012, Wardle et al., 2011). Weight gain may be particularly accelerated as fat oxidation slows down during stressful periods (Kiecolt-Glaser et al., 2015), and body weight plays a role in stress-induced eating, with a higher incidence of overeating during stressful periods in overweight individuals (Cotter and Kelly, 2018). Notably, obesity is a risk factor in the development of CVD (Powell-Wiley et al., 2021). Therefore, stress can not only directly impact vascular function, but also indirectly contribute to poorer vascular health, through unhealthier food choices.

We have recently shown that saturated fat consumption impairs the recovery of endothelial function following mental stress in young healthy adults, with FMD remaining significantly impaired (reduction of 1.15 % FMD) in the high-fat condition in comparison to the low-fat condition 1.5 hours post stress (Baynham et al., 2023b). We have also demonstrated that saturated fat consumption attenuates cerebral oxygenation in the prefrontal cortex during mental stress (Baynham et al., 2023a). Both fat consumption and stress exposure have been shown to stimulate the vasoconstrictor endothelin-1 (ET-1), reactive oxygen species (ROS) and inflammatory markers (Tsai et al., 2004, Bae et al., 2001, Steinberg et al., 1997), which are known to reduce endothelium-derived NO (Man et al., 2020) and likely underpin the stress and fat-induced reductions in vascular function. As such, unhealthy food choices (such as foods high in saturated fats) during stressful periods can exacerbate the negative impact of stress on vascular health. Therefore, it is important to find dietary strategies that may be able to counteract the negative impact of stress and fat in the vasculature.

We have previously shown that an intervention rich in flavonoids, a group of small molecules present in most fruits and vegetables, can be protective for the vasculature in the context of a stressful episode. Specifically, we demonstrated that cocoa flavanols improved vasodilatory responses during stress and attenuated the impairment in brachial FMD following stress in young healthy adults in a fasted state (Baynham et al., 2021). Similarly, previous research has shown cocoa flavanols to improve brachial FMD within 1 – 3 hours of intake (Monahan et al., 2011, Rodriguez-Mateos et al., 2015, Sansone et al., 2017, Schroeter et al., 2006). Cocoa flavanols exert a protective vascular effect by increasing NO bioavailability and reducing ET-1 (Schroeter et al., 2006, Moreno-Ulloa et al., 2014, Ramirez-Sanchez et al., 2010, Loke et al., 2008), and

are associated with lower CVD-related mortality (Bondonno et al., 2019, Cassidy et al., 2016, Geleijnse et al., 2002, Goetz et al., 2016). Furthermore, cocoa flavanols have been shown to improve cerebral oxygenation responses to hypercapnia (Gratton et al., 2020) and hypoxia (Bloomfield et al., 2023), but their effect on the brain during stress, and in combination with a high-fat meal, remains unknown. As such, adding a flavonoid-rich food to a high-fat snack during stress might be an effective strategy to, at least partially, reduce the negative impact of poor food choices (if these cannot be avoided) on the human vasculature.

Therefore, the current study aimed to investigate whether high-flavanol cocoa (HFC), consumed in combination with a high-fat meal (HFM), can mitigate the negative impact of fat on stress-induced impairments in endothelial function, as measured by brachial FMD. Furthermore, we aimed to investigate whether HFC can restore cortical oxygenation during mental stress following fat consumption. We hypothesised that HFC will attenuate the stress-induced decline in brachial FMD and improve cortical oxygenation during stress following fat consumption.

6.3. Methods

6.3.1. Participants

Twenty-three participants (11 male, 12 female) were recruited via email and poster advertisements. Participants were between 18 and 45 years old. Exclusion criteria were: (i) smokers, (ii) consumption of > 21 units alcohol per week, (iii) acute illness/infection, (iv) history of cardiovascular, respiratory, metabolic, liver, inflammatory diseases, or blood-clotting disorders, (v) allergies or food intolerances, (vi) weight reducing dietary regiment or dietary supplements, and (vii) long-term

medication or antibiotics in the previous 3 months. Participants were awarded course credit marks when applicable. Ethical approval was obtained from the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN17_1755E), and all participants gave written informed consent prior to participation in the study.

6.3.2. Study design

The study design was a randomised, counterbalanced, cross-over, postprandial intervention study (Figure 6.1). Participants visited the laboratory twice, at least a week apart for males and approximately one month apart for females. Females were tested during the same phase of the menstrual cycle (early follicular, days 1 – 5 of menstruation) to control for the influence of menstrual hormones (Thomas et al., 2015, Thijssen et al., 2011). Participants were asked to refrain from food for 12 hours and from alcohol, caffeine, flavonoid-rich foods, and vigorous exercise 24 hours before each testing session. Each session commenced at approximately 8AM, and firstly, compliance with pre-visit requirements were checked and mood was assessed. Then, habitual dietary intake was recorded (visit 1 only). Following this, participants rested in a supine position for 20 minutes before pre-intervention (baseline) measurements were taken: i) common carotid artery (CCA) blood flow, ii) brachial flow-mediated dilatation [FMD], iii) forearm blood flow [FBF], iv) cardiovascular activity (beat-to-beat blood pressure [BP], heart rate [HR], heart rate variability [HRV] and R-wave to pulse interval [RPI]). Following these assessments, participants consumed a high-fat meal (HFM) with either a high-flavanol cocoa (HFC) intervention or a low-flavanol cocoa (LFC) intervention. Participants then rested for 1.5 hours during which they completed a

mood questionnaire and lifestyle questionnaires (data not reported, session 1) and had the option to complete their own work or watch a nature documentary. Subsequently, FBF, cardiovascular activity and prefrontal cortex (PFC) tissue oxygenation (NIRS) were measured during an 8-minute rest (rest) and during an 8-minute mental stress – Paced-Auditory-Serial-Addition-Task (PASAT) (stress). During each 8-minute assessment, FBF was measured during minutes 2, 4, 6, and 8. BP, HR, RPI, HRV and PFC oxygenation were analysed during all 8 minutes. CCA blood flow, brachial FMD and mood were measured 30 minutes and 90 minutes following stress. Both sessions lasted 5 hours and participants were debriefed following completion of both visits (Figure 6.2).

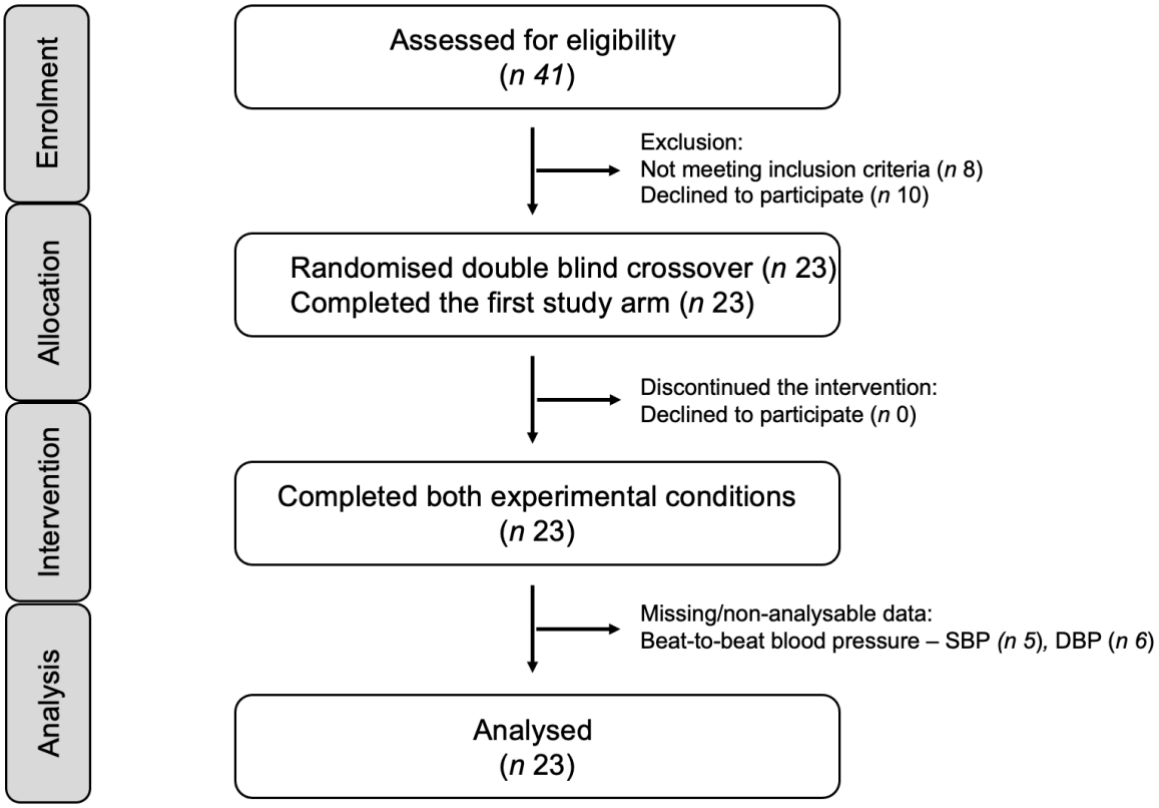

Figure 6.1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram for postprandial intervention study

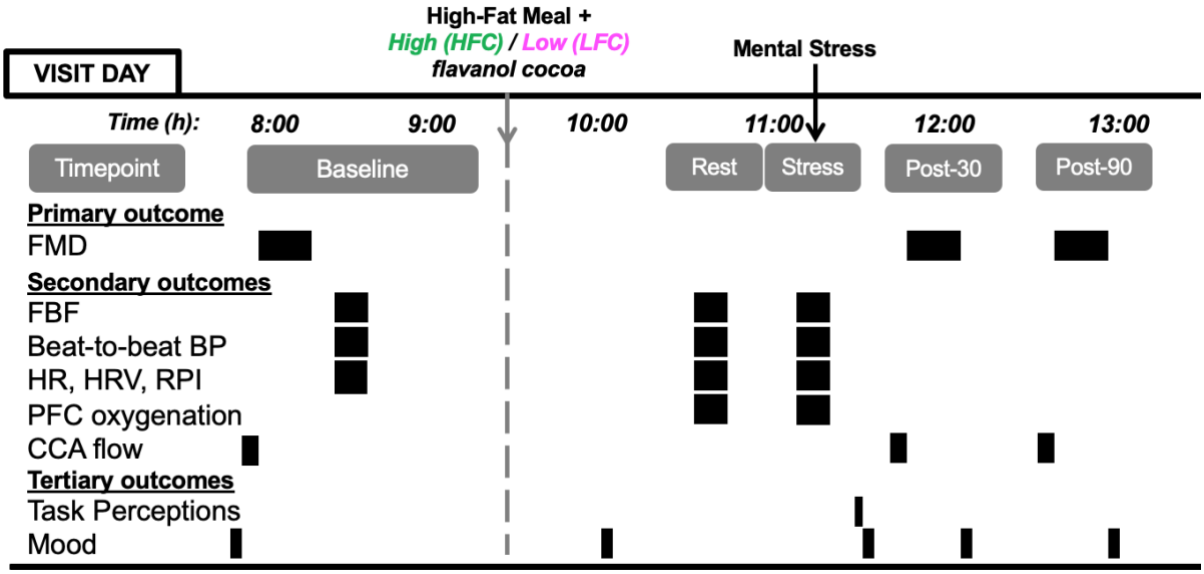

Figure 6.2 Experimental study design

HFC: high-flavanol cocoa, LFC: low-flavanol cocoa, FMD: flow-mediated dilatation, FBF: forearm blood flow, BP: blood pressure, HR: heart rate, HRV: heart rate variability, RPI: R-wave to pulse interval, PFC: pre-frontal cortex, CCA: common carotid artery.

6.3.3. Habitual dietary intake

Habitual dietary intake was assessed using the validated European Prospective Investigation into Diet and Cancer (EPIC) Norfolk Food Frequency Questionnaire (FFQ) (Bingham et al., 2001). The questionnaire consists of 131 different food items for participants to select the frequency of consumption on a 9-point scale (never or less than once per month, 1 – 3 per month, once a week, 2 – 4 per week, 5 – 6 per week, once a day, 2 – 3 per day, 4 – 5 per day, and 6 + per day) to estimate usual dietary intake over the previous 12 months. The FFQ EPIC Tool for Analysis (FETA) was used to calculate nutrient data (Mulligan et al., 2014). In order to calculate flavonoid intake, the FLAVIOLA food composition database was inputted into the FETA software, which allows the estimation of flavonoids and its subclasses (Vogiatzoglou et al., 2015a). The following nutrients are reported in this study: energy (kcal), fat (g), saturated fat (g),

carbohydrate (g), sugars (g), fibre (g), protein (g), total flavonoids (mg), and portions of fruit and vegetables (calculated as 1 portion corresponding to 80 g, NHS guidelines), to give a general view of habitual dietary intake.

6.3.4. High-fat meal intervention

The HFM was prepared just before consumption, and all fresh ingredients were bought within 24 hours of each testing session. The HFM contained 56.5 g fat (Table 6.1), as used previously (Baynham et al., 2023b). Participants were asked to consume the meal within 20 minutes. 4 participants did not finish the meal, but no adverse side effects were reported.

Table 6.1 Nutrient composition of the high-fat meal

Meal type	High-Fat Meal (HFM)
Nutrient composition:	
Energy (Kcal)	891.00
Fat (g)	**56.50**
Saturated fat (g)	**35.10**
Carbohydrate (g)	65.00
Sugars (g)	20.20
Fibre (g)	2.40
Protein (g)	29.85
Salt (g)	2.00

The HFM consisted of 2 butter croissants with 10 g salted butter, 1.5 slices of cheese and 250 ml whole milk.

6.3.5. High-and low-flavanol interventions

Cocoa flavanol beverages were prepared by dissolving 12 g cocoa powder into 250 mL of whole milk (from the HFM). The cocoa powders are commercially available (Barry Callebaut, Zurich, Switzerland): the low-flavanol powder was a fat-reduced alkalized cocoa powder (commercial name: 10/12 DDP Royal Dutch) delivering < 6.0 mg (-)-epicatechin and 5.6 mg of total flavanols per serving; and the high-flavanol cocoa powder was a non-alkalized fat-reduced powder ('Natural Acticoa'), delivering 150.0 mg (-)-epicatechin powder and 695.0 mg total flavanols per serving, as used in previous research (Gratton et al., 2020, Baynham et al., 2021) (Table 6.2). Both interventions were matched for all other micro- and macro-nutrients, including caffeine and theobromine. Cocoa powder levels for flavanol monomers, procyanidin and methylxanthines (caffeine, theobromine) were measured by high-performance liquid chromatography (HPLC) as described previously (Alsolmei et al., 2019, Robbins et al., 2012). Total levels of polyphenols were also assessed by a Folin-Ciocalteu reagent calorimetric assay as described previously (Miller et al., 2008). The dose of flavanol monomers used in the present study is in line with previous studies, shown to be safe and effective in modifying human endothelial function in young healthy adults (Heiss et al., 2003, Sansone et al., 2017, Schroeter et al., 2006). The cocoa powder sachets were labelled with an alphanumeric identifier, and were stored at −20 ^{O}C. Intervention beverages were identical in texture, consistency and taste, and were presented in an opaque container with a black opaque straw to ensure double-blindness. All participants finished both intervention beverages. The unblinding of the interventions was performed only after all data analyses were completed.

Table 6.2 Composition of cocoa interventions (12 g per dose) containing high and low flavanol content

	High-flavanol cocoa (HFC)	Low-flavanol cocoa (LFC)
Total polyphenols (mg)	1246.80	260.04
Total flavanols (mg)	695.00	5.60
(–)-Epicatechin (mg)	150.00	< 6.00
(+)-Catechin (mg)	85.44	< 6.00
Procyanidins (mg)	459.60	ND
Theobromine (mg)	262.80	278.40
Caffeine (mg)	27.60	22.20
Fat (g)	1.68	1.32
Carbohydrates (g)	2.70	1.24
Protein (g)	2.69	2.66
Fibre (g)	1.82	4.02
Energy (Kcal)	41.40	36.60

6.3.6. Mental stress task

The mental stress task used was the 8-minute PASAT, shown to have good test-retest reliability and to induce a physiological response (Paine et al., 2013a, Ginty et al., 2013, Veldhuijzen van Zanten et al., 2005). The PASAT requires participants to add two sequentially presented single-digit numbers (1-9), adding the number presented to the previous number they heard. The delivery of the numbers became quicker, with time intervals reducing every 2 minutes; from a 2.8 second interval to 2.4 seconds, 2.0 seconds, and finally 1.6 seconds. Participants were filmed and asked to watch themselves on a screen, which they were told would be evaluated by 2 independent body language assessors. An experimenter marked the participants' responses, whilst

sounding a loud aversive buzzer at standard intervals once every 10 answers: either following an incorrect response or at the end of the 10-number block. The participants were told they were in direct competition with other participants and lost points for each incorrect answer. These elements of social evaluation, punishment, and competition have been used previously (Baynham et al., 2021) and have been shown to enhance the provocativeness of the task (Veldhuijzen van Zanten et al., 2002). Immediately following the PASAT, an experimenter asked the participant to verbally rate how difficult, stressful, competitive, and enjoyable they found the task, and to what extent they were trying to perform well, scored on a 7-point scale ranging from 0 ‘not at all’ to 6 ‘extremely’. Following both visits, participants were informed about the task deception.

6.3.7. Mood ratings

In order to reduce participant burden, instead of using the Profile of Mood States (POMS) as in Chapter 5 which includes 44 items, mood was investigated using items that assessed current positive affect, negative affect, energy, and fatigue. The positive affect scale included items that represent activated (happy, cheerful) and deactivated (calm) pleasure. The negative affect scale included items that represent activated (anxious, stressed) and deactivated (depressed, angry) displeasure, as detailed previously (Liao et al., 2017). Physical feeling states were represented by the assessment of energy and fatigue. These nine constructs (happy, stressed, energetic, cheerful, anxious, fatigued, depressed, calm, and angry), were rated on a 5-point scale (1 = not at all, 5 = extremely), and correspond to how participants felt at that moment.

Therefore, improved feeling states refer to higher scores for positive affect and energy, and lower scores for negative affect and fatigue.

6.3.8. Cardiovascular activity

6.3.8.1. Electrocardiograph

Indices of cardiodynamic activity were measured using an electrocardiogram, recorded continuously at 1000Hz with a Morgan 509 Cardiac monitor and three disposable pregelled Ag/AgCl spot electrodes (Invisatrace, ConMed). The electrodes were positioned in a modified chest position; after the skin was prepared with nuprep and alcoholic wipes, two active electrodes were placed on the right collar bone and left ribs beneath the heart, while the ground electrode was placed on the left collar bone. An earlobe clip measured peripheral pulse using infrared photoplethysmography (1020, UFI). The ECG and pulse data was stored on a computer programmed in Spike2 (CED) and collected via a Power1401 (CED). R-wave and pulse signal artifacts were visually identified and removed. Heart rate (HR, beats per minute) was calculated from the cardiac inter-beat interval, determined from successive R-waves. Sixty seconds ensemble averages were calculated for these physiological data. R-wave to pulse interval (RPI, ms) was calculated from the ECG R-wave to the foot of the systolic upstroke of the ear pulse. The foot of the systolic upstroke was determined by the point when the slope of the pulse upstroke was 25 % of its maximum (Lane et al., 1983). The RPI has been shown to be correlated with the cardiac pre-ejection period (de Boer et al., 2007, Newlin, 1981), an index of sympathetic activity of the heart (Newlin and Levenson, 1979). Finally, heart rate variability (HRV, ms) was calculated with the root

mean square of successive differences of RR-intervals, as a measure of parasympathetic activity.

6.3.8.2. Beat-to-beat blood pressure

Beat-to-beat arterial BP was measured using a Finometer (Finapres Medical Systems; Amsterdam, The Netherlands), with a cuff around the intermediate phalanx of the middle finger. Continuous data was recorded via a Power1401 (CED, Cambridge, UK) connected to a computer programmed in Spike2. Data was analysed and averaged for each minute of assessment. Analyses were undertaken offline whereby each file was visually inspected, and systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were obtained.

6.3.9. Forearm blood flow

FBF was measured using venous occlusion plethysmography. A mercury-in-silastic strain gauge was connected to a plethysmograph (ECG, Hokanson; Jacksonville, WA, USA), producing an output voltage with frequency 0-25 Hz. The plethysmograph signal was digitised at 100 Hz with 16-bit resolution, via a Power1401 (CED) connected to a computer programmed in Spike2, as previously described by Paine et al. (2013). One congestion cuff was placed around the wrist (TMC7, Hokanson), and inflated for 1 minute to supra-systolic blood pressure (> 220 mmHg). Another congestion cuff was placed around the brachial region of the upper arm (SC12, Hokanson), and inflated for 5 seconds to above venous pressure (40 mmHg), every 15 seconds providing 3 blood flow measurements each minute. Blood flow analysis and calibration were undertaken offline using Spike2 (CED). Each increase in limb circumference is identified as a slope, which were averaged to yield a mean blood flow per minute (Paine et al., 2013a).

Forearm vascular conductance (FVC) was calculated by dividing FBF by MAP per minute of assessment.

6.3.10. Prefrontal cortical haemodynamics

NIRS (NIRO-200NX, Hamamatsu Photonics KK, Japan) was used to assess prefrontal cortical haemodynamics. The NIRS device measures changes in chromophore concentrations of oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb), providing depth-resolved measures of tissue oxygen saturation (total oxygenation index, TOI) and tissue haemoglobin content (relative value of total haemoglobin normalised to the initial value, nTHI) (Davies et al., 2015). Probes were positioned over the left and right pre-frontal sites and secured to the head with a black headband. Probes were enclosed in light-shielding rubber housing that maintained emitter-to-detector optode spacing (4 cm), and signals were acquired at sample interval 0.2 s (5 Hz). NIRS was assessed during 8 minutes of rest and 8 minutes of stress. Measures of TOI, nTHI, O_2Hb and HHb were averaged to provide 1 value for each minute of rest and stress. Minutes 2, 4, 6 and 8 of stress are reported, in line with the minutes by which peripheral vasodilation (FBF) was assessed.

6.3.11. Flow-mediated dilatation

FMD was used to assess endothelial function of the brachial artery. A 15–4 Mhz (15L4 Smart MarK™; Terason, MA, USA) transducer was attached to a Terason Duplex Doppler System (Usmart 3300 NexGen Ultrasound; Terason). This has a wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Pisa, Italy), which allows for continuous measurement of diameter and blood velocity throughout the FMD assessment. Following 20 minutes of supine rest, the brachial artery was

imaged longitudinally, 5 – 10 cm proximal to the antecubital fossa. A brachial cuff was placed around the forearm and, following a 1-minute baseline, this was inflated to 220 mmHg for 5 minutes, to cause ischaemia. Subsequently, the rapid cuff deflation caused reactive hyperaemia, and the image was recorded continuously for 5 minutes post-pressure release. This is in accordance with established guidelines (Thijssen et al., 2010). All file images were analysed by a trained researcher, blinded to condition and measurement details. Peak diameter was defined as the largest diameter obtained after occlusion is released. The FMD response was calculated as the relative diastolic diameter change between baseline and peak diameter. Resting arterial diameter was also estimated based on a time-average across the first minute of recording. All measurements were undertaken by the same trained researcher, who demonstrates sufficient reproducibility in brachial FMD (coefficient of variation: intra-day 5.49 %, inter-day 10.87 %). Allometrically scaled FMD was calculated in accordance with published guidelines (Atkinson and Batterham, 2013). The slope of the regression between the logarithmically transformed baseline diameter and peak diameter was 0.94.

6.3.12. Common carotid artery diameter and blood flow

Duplex ultrasound was used to assess CCA diameter and blood flow. A 15–4 Mhz (15L4 Smart MarK^{TM}; Terason, MA, USA) transducer was attached to a Terason Duplex Ultrasound System (Usmart 3300 NexGen Ultrasound; Terason). This was combined with wall-tracking and automatic edge-detection software (Cardiovascular Suite, Quipu; Pisa, Italy), which allows for continuous measurement of diameter and blood velocity (Thomas et al., 2015). Following 10 minutes of supine rest, the

participant was asked to turn their head and neck slightly to the left side. Then, a two-minute recording of the right CCA was obtained. All file images were analysed by a trained researcher, blinded to condition and measurement details. Analysis allows estimation of resting arterial diameter and calculation of arterial blood flow based on a time-average across two minutes of the recording (Thomas et al., 2015). All measurements were undertaken by the same trained researcher, who demonstrates sufficient reproducibility of CCA diameter (intra-day variability: 1.93 %, inter-day variability: 2.37 %) and shear rate (intra-day variability: 11.79 %, inter-day variability: 12.27 %).

6.3.13. Statistical analysis

All statistical analyses were conducted using IBM SPSS software (version 29). The cardiovascular and FBF measurements during pre-intervention baseline, rest, and stress were averaged separately to provide a mean pre-intervention baseline, rest, and stress value for each outcome. Pre-intervention baseline measures (FMD, FBF, HR, SBP, DBP), task perceptions and PASAT scores were compared using a 2 condition (HFM+HFC, HFM+LFC) repeated measures analysis of variance (ANOVA). Separate 2 condition (HFM+HFC, HFM+LFC) by 5 time (baseline, rest, stress, post-30, post-90) repeated measures ANOVAs were used to assess mood ratings. A series of 2 condition (HFM+HFC, HFM+LFC) by 3 time (baseline, rest, stress) repeated measures ANOVAs were conducted to analyse the cardiovascular and FBF variables. A mixed effects model was used for SBP, DBP, and FVC analysis to account for missing data due to finapress malfunction. NIRS variables at rest and during stress (eight minutes averaged) were compared using separate one-sample t-tests for both conditions,

which is most appropriate given that the resting values were 0. NIRS variables were further analysed using a two-way repeated measures ANOVA with condition (HFM+HFC, HFM+LFC) and time (stress 2, stress 4, stress 6, stress 8) as within-subject factors. FMD (including resting arterial diameter) and CCA variables were analysed using a 2 condition (HFM+HFC, HFM+LFC) by 3 time (baseline, post-30, post-90) repeated measures ANOVA. Where appropriate, pairwise comparisons using Bonferroni correction were conducted to investigate significant effects in more detail. A Linear Mixed Model was used to analyse allometrically scaled FMD. Given the lack of disparity in SD following allometric correction, 95 % confidence intervals have been reported in text for this allometrically scaled FMD. All values reported in text, tables, and graphs are mean ± SD. All analyses were also conducted with sex as a between-subject variable. As there were no significant condition × sex, time × sex, or condition × time × sex interaction effects, these results are not reported. 4 participants did not finish the meal. All statistical tests were repeated excluding these 4 participants. The results were broadly similar to the analyses with the full sample; therefore, it was decided to include all participants to maximise power. For all analyses, significance was set at $\alpha < .05$. Sample size was estimated based on previous data from our laboratory on flavanol-induced changes in brachial FMD (Baynham et al., 2021), denoting a sample size of 11 participants was required to detect an interaction effect of the cocoa intervention on FMD post-stress, with power at 99 % and alpha set at .05 (Faul, 2007). This sample size should also be sufficient to detect the effect of cocoa flavanols on cortical oxygenation, as shown with *n = 18* by Gratton et al. (2020).

6.4. Results

6.4.1. Participant characteristics

Participant characteristics are presented in Table 6.3. Participants were aged 19 to 35 years old, with a healthy body mass index (BMI) and identified as white European ethnicity (*n = 20*), Asian ethnicity (*n = 1*) or black African ethnicity (*n = 2*). Pre-intervention baseline FMD, FBF, HR, BP, brachial and CCA diameter were similar in both conditions (Table 6.3).

Table 6.3 Mean ± SD participant pre-intervention baseline characteristics in HFM + LFC and HFM + HFC conditions

Participant characteristics	HFM + LFC	HFM + HFC	*p value**
n	23 (M:11, F:12)		/
Age (years)	21.57 ± 4.11		/
BMI (kg/m^2)	22.31 ± 2.58		/
FMD (%)	6.93 ± 0.87	6.55 ± 2.73	*.065*
FBF (mm/100ml/min)	2.63 ± 0.87	2.58 ± 0.75	*.823*
HR (bpm)	58.29 ± 8.57	57.45 ± 9.78	*.533*
SBP (mmHg)	113.38 ± 15.40	110.53 ± 10.78	*.371*
DBP (mmHg)	52.17 ± 6.86	50.25 ± 4.82	*.419*
Brachial Diameter	3.60 ± 0.54	3.61 ± 0.55	*.561*
CCA Diameter	6.54 ± 0.39	6.52 ± 0.38	*.656*

HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa, BMI: body mass index, FMD: flow-mediated dilatation, FBF: forearm blood flow, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, CCA: common carotid artery. *P value from ANOVAs.

6.4.2. Habitual dietary intake

Table 6.4 displays participant's estimated daily intake of key nutrients, as well as the percentage of participants exceeding or not meeting daily recommendations, as

suggested by the National Health Service (NHS). The average daily intake of fat was 66.76 ± 19.99 g (43.48 % exceeding the recommended daily intake) and of saturated fat was 25.18 ± 7.72 g (47.83 % exceeding the recommended daily intake). 75 % of females exceeded the saturated fat recommendation (20 g), whilst only 18 % of males exceeded their saturated fat recommendation (30 g). The average intake of fruit and vegetables was 4.76 ± 2.55 portions per day, with females consuming over 1 extra portion a day compared to males. 65 % of all participants did not meet the suggested recommendation of 5 portions a day. 100 % of participants did not meet the suggested recommendations for fibre intake and exceeded the recommended sugar intake. The average daily intake of flavonoids was 239.55 ± 195.88 mg, which is greater than the estimated average of flavonoid intake in UK adults (approx. 195 mg/day) (Vogiatzoglou et al., 2015b), yet the variability in this measure should be acknowledged.

Table 6.4 Mean ± SD estimated daily intake of key nutrients

Nutrients	Sample average	% of participants over/under recommended daily intake
Energy (Kcal)	1726.10 ± 494.46	N/A
Fat (g)	66.76 ± 19.99	43.48 % over
Saturated fat (g)	25.18 ± 7.72	47.83 % over
Carbohydrate (g)	195.14 ± 67.86	N/A
Sugars (g)	90.01 ± 39.19	100.00 % over
Fibre (g)	12.84 ± 5.88	100.00 % under
Protein (g)	84.11 ± 30.82	N/A
Total flavonoids (mg)	239.55 ± 195.88	N/A
Portions of fruit & vegetables*	4.76 ± 2.55	65.22 % under

Recommendations – *fat*: < 70g/day, *saturated fat*: < 30g/day (male) / < 20g/day (female), *sugar*: < 30g/day, *fibre*: > 30g/day, *fruit & vegetables*: > 5 portions/day. *1 portion = 80g. (NHS).

6.4.3. Mental stress task ratings

Separate two condition (HFM+HFC, HFM+LFC) ANOVAs revealed no significant difference in task performance (PASAT score) or task perceptions between flavanol conditions. Participants perceived the task as similarly difficult, stressful, competitive, enjoyable, and tried to perform well to the same extent after both conditions (Table 6.5).

Table 6.5 Mean ± SD task performance (PASAT) and ratings

Task Ratings	HFM + LFC	HFM + HFC	**p value*
PASAT Score (/228)	133 ± 39	134 ± 30	*.869*
Perceived difficulty (0-6)	4.83 ± 0.65	4.78 ± 0.80	*.814*
Perceived stressfulness (0-6)	4.96 ± 0.77	5.04 ± 0.83	*.628*
Perceived competitiveness (0-6)	4.48 ± 0.85	4.35 ± 1.07	*.601*
Perceived enjoyment (0-6)	1.95 ± 1.51	1.65 ± 1.43	*.186*
Perception of trying to perform well (0-6)	5.30 ± 0.70	5.35 ± 0.83	*.770*

n = 23. Note: maximum score for PASAT is 228, task ratings are scored from 0 – 6. PASAT: paced auditory serial addition task, HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa. *P value from ANOVAs.

6.4.4. Mood ratings

Two condition (HFM+HFC, HFM+LFC) × 5 time (baseline, rest, stress, post-30, post-90) ANOVAs revealed a significant decrease in happiness, calmness, and cheerful (*p's <.001*) and increase in stress, tension and anger (*p's <.001*) immediately after stress compared to all other time points. Participants also reported to feel calmer at 30- and 90-minutes post-stress compared to baseline. There was a significant time effect for tiredness (*p =.005*) and energy (*p <.001*), whereby participants felt more

energetic and less tired at rest compared to baseline, and less energetic 30 minutes post-stress compared to rest and stress, and less energetic and more tired 90 minutes post-stress compared to rest and stress. There was a significant time (*p =.018*) and condition × time interaction effect (*p =.013*) for sadness, but post-hoc analyses revealed no significances between time points. Post-hoc analyses for main effects are reported in Table 6.6. In summary, mood ratings changed over time but were not impacted by the flavanol intervention.

Table 6.6 Mean ± SD Mood ratings across each study visit

	HFM + LFC					**HFM + HFC**				
	Base-line	Rest	Stress	Post-30	Post-90	Base-line	Rest	Stress	Post-30	Post-90
Happy	3.57 ± 0.73	3.74 ± 0.62	2.26 ± 0.81 *	3.39 ± 0.66	3.57 ± 0.66	3.48 ± 0.67	3.57 ± 0.66	2.26 ± 0.86 *	3.48 ± 0.67	3.61 ± 0.58
Stressed	1.57 ± 0.84	1.26 ± 0.45	4.09 ± 0.56 *	1.22 ± 0.42	1.22 ± 0.42	1.48 ± 0.51	1.26 ± 0.45	3.91 ± 0.85 *	1.35 ± 0.57	1.30 ± 0.56
Energetic	2.35 ± 1.03	3.04 ± 0.93 #	2.87 ± 1.14	2.26 ± 0.86 @	2.17 ± 0.83 @	2.43 ± 0.73	3.00 ± 1.04 #	3.00 ± 1.00	2.17 ± 0.94 @	2.22 ± 0.80 @
Cheerful	3.17 ± 0.83	3.48 ± 0.90	2.39 ± 1.03 *	3.17 ± 0.72	3.35 ± 0.71	3.26 ± 0.75	3.43 ± 0.79	2.17 ± 1.07 *	3.22 ± 0.74	3.39 ± 0.78
Tense	1.52 ± 0.73	1.35 ± 0.57	3.74 ± 0.86 *	1.39 ± 0.78	1.26 ± 0.54	1.43 ± 0.73	1.22 ± 0.42	3.74 ± 0.86 *	1.17 ± 0.39	1.22 ± 0.42
Tired	2.91 ± 0.90	2.35 ± 0.83 #	2.22 ± 1.09	2.70 ± 1.15	2.91 ± 0.85 @	2.87 ± 1.06	2.39 ± 0.99 #	2.30 ± 1.02	2.87 ± 1.14	3.17 ± 0.94 @
Sad	1.30 ± 0.56	1.13 ± 0.34	1.22 ± 0.42	1.13 ± 0.34	1.04 ± 0.21	1.09 ± 0.29	1.00 ± 0.00	1.48 ± 0.85	1.00 ± 0.00	1.04 ± 0.21
Calm	3.35 ± 0.83	3.57 ± 0.99	1.83 ± 0.78 *	3.91 ± 0.85 #	3.87 ± 0.87 #	3.26 ± 0.69	3.65 ± 0.78	1.65 ± 0.78 *	3.70 ± 0.88 #	3.70 ± 0.97 #
Angry	1.04 ± 0.21	1.00 ± 0.00	2.30 ± 0.93 *	1.04 ± 0.21	1.00 ± 0.00	1.04 ± 0.21	1.00 ± 0.00	2.43 ± 0.90 *	1.00 ± 0.00	1.00 ± 0.00

n = 23. * Significantly different compared to all other time points, # Significantly different to baseline, @ Significantly different to rest and stress, p<.05. Mood rating scored from 1 – 5. HFM: high-fat meal, LFC: low-flavanol cocoa, HFC: high-flavanol cocoa.

6.4.5. Cardiovascular activity during acute mental stress

Separate 2 condition (HFM+HFC, HFM+LFC) × 3 time (baseline, rest, stress) ANOVAs revealed an overall time effect for HR, RPI, HRV, SBP and DBP (all *p's <.001*) (Figure 6.3). Post-hoc analyses revealed that HR was significantly higher during rest and stress compared to baseline *(p's <.001*) and significantly higher during stress compared to rest (*p <.001*). RPI and HRV were significantly lower during rest and stress compared to baseline (all *p's <.001*), and lower during stress compared to rest (*p's <.001*). SBP was significantly higher during rest and stress compared to baseline *(p's <.001*) and significantly higher during stress compared to rest (*p <.001*). DBP was significantly higher during stress compared to baseline (*p <.001*) and rest (*p <.001*), with no difference between baseline and rest (*p =.497*).

There were no significant condition or condition × time interaction effects for HR (condition: *p =.160*, interaction: *p =.210*), RPI (condition: *p =.051*, interaction: *p =.050*), HRV (condition: *p =.968*, interaction: *p =.192*), SBP (condition: *p =.854*, interaction: *p =.204*) or DBP (condition: *p =.367*, interaction: *p =.224*). Thus, cardiovascular activity differed during rest and stress, but this was not affected by the flavanol intervention.

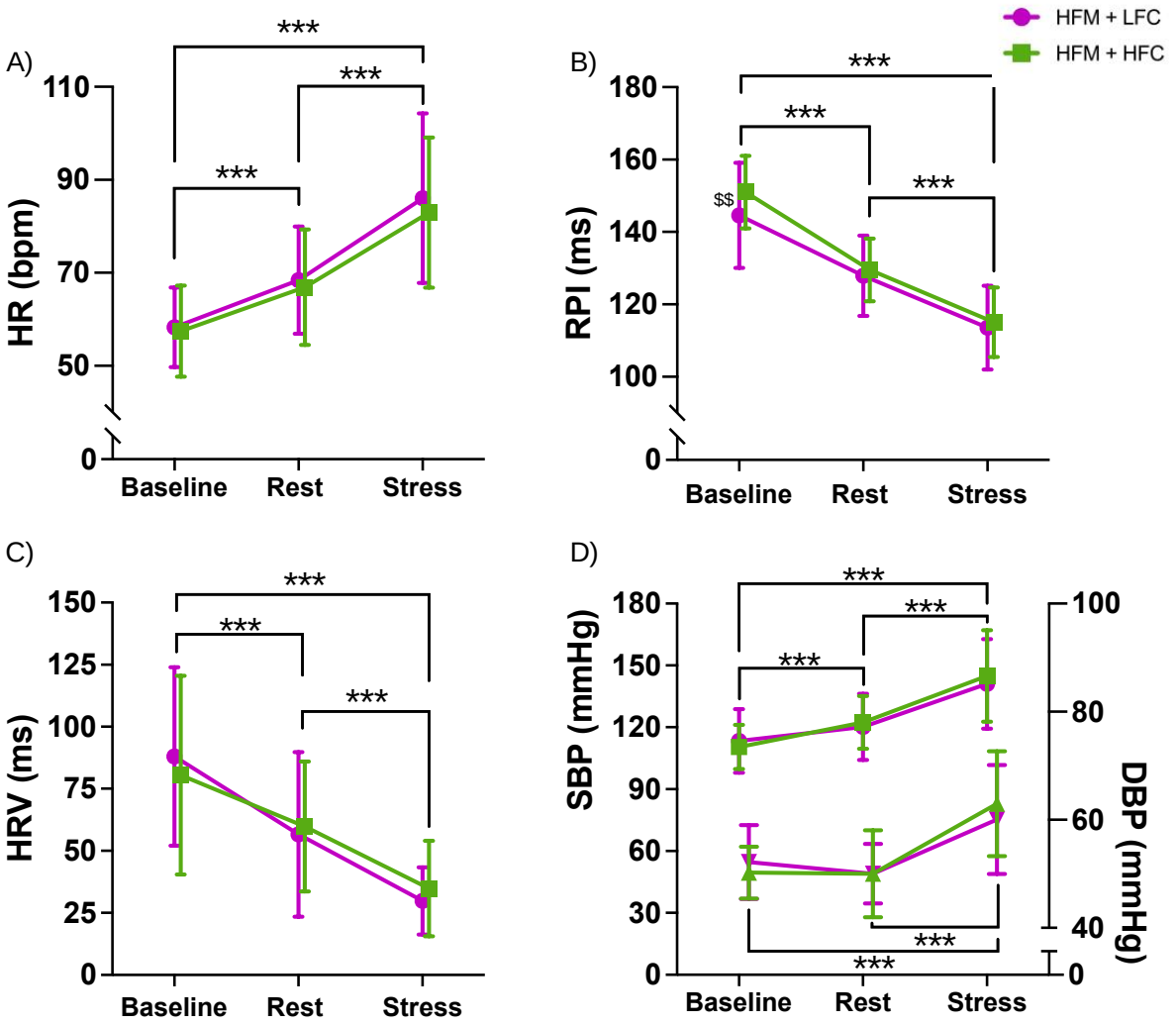

Figure 6.3 Cardiovascular activity (HR [A], RPI [B], HRV [C], SBP/DBP [D]) during baseline, rest and stress following a high-fat meal (HFM) with either high-flavanol cocoa (HFC) or low-flavanol cocoa (LFC)

Data are presented as Mean ± SD. *n = 23.* * significant difference between values, $ significant difference between conditions at this time point. *** p<.001, $$ p<.01. HR: heart rate, HRV: heart rate variability, RPI: R-wave to pulse interval, SBP: systolic blood pressure, DBP: diastolic blood pressure, HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa.

6.4.6. Forearm blood flow during acute mental stress

A 2 condition × 3 time ANOVA revealed an overall time effect for FBF and FVC (*p's <.001*) (Figure 6.4). Post-hoc analyses revealed that FBF and FVC were significantly higher during stress compared to both baseline (*p's <.001*) and rest (*p's <.001*), and FBF and FVC were significantly higher at rest compared to baseline (FBF: *p =.002,* FVC: *p =.001*). There were no condition nor condition × time interaction effects for FBF (condition: *p =.486*, interaction: *p =.576*) or FVC (condition: *p =.351*, interaction: *p =.439*).

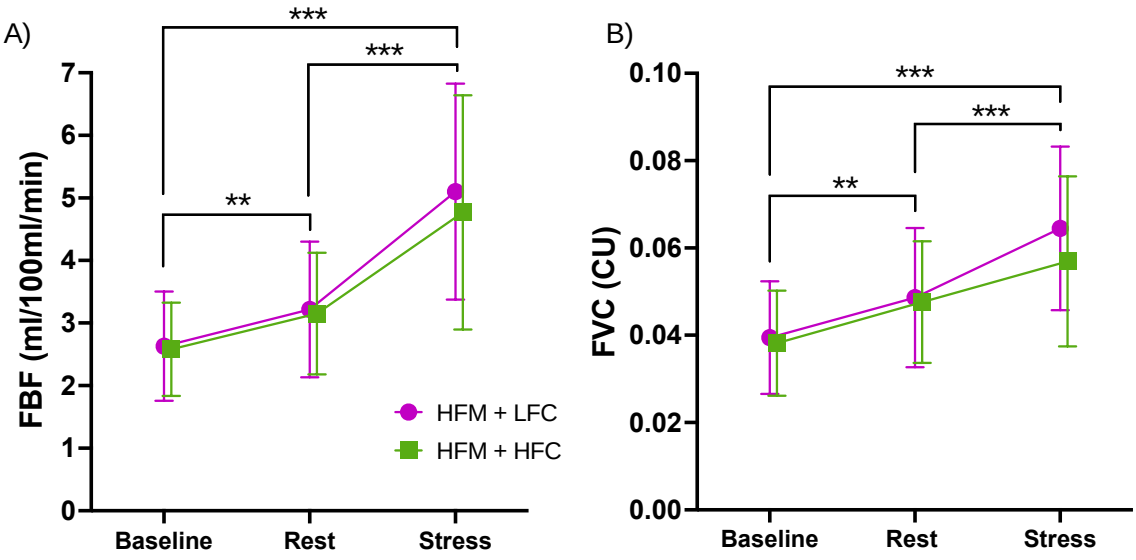

Figure 6.4 Time course of forearm blood flow (FBF [A] & FVC [B]) during baseline, rest and stress following a high-fat meal (HFM) with high-flavanol cocoa (HFC) or low-flavanol cocoa (LFC)

Data are presented as Mean ± SD. *n = 23* * significant difference between time points. ***p<.001, **p<.01. FBF: forearm blood flow, FVC: forearm vascular conductance, HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa.

6.4.7. Cerebral oxygenation during acute mental stress

One sample *t*-tests revealed that left and right PFC TOI were significantly greater during stress compared to rest in both conditions (left: HFC *p <.001*, LFC *p =.001*; right:

HFC *p* <*.001*, LFC *p* =*.002*). One sample *t*-tests revealed that left nTHI was significantly higher during stress compared to rest in the HFC condition (*p* =*.024*) but not the LFC condition (*p* =*.736*). There were no significant differences in right nTHI during stress compared to rest in both conditions (HFC: *p* =*.938*, LFC: *p* =*.384*). One sample *t*-tests revealed that left and right O_2Hb and HHb were significantly different during stress compared to rest in both conditions (all *p* < .001) (Figure 6.5). In summary, stress significantly increased left and right PFC TOI, left and right PFC O_2Hb, and decreased left and right PFC HHb, in both conditions. Stress also increased left nTHI in the HFC condition only.

Separate 2-condition (HFC, LFC) × 4-time (stress 2, stress 4, stress 6, stress 8) ANOVAs revealed an overall time effect for right TOI (*p* =*.008*), right HHb (*p* =*.015*) and left HHb (*p* =*.005*). There were no significant time effects for left TOI (*p* =*.119),* left nTHI *(p* =*.218)*, right nTHI *(p* =*.058),* left O_2Hb (*p* =*.666)* or right O_2Hb (*p* =*.256)*. There were no significant condition (L-TOI: *p* =*.254,* R-TOI: *p* =*.229,* L-nTHI: *p* =*.346,* R-nTHI: *p* =*.451,* L-O_2Hb: *p* =*.619*, R-O_2Hb: *p* =*.255*, L-HHb: *p* =*.314,* R-HHb: *p* =*.674*) effects for any outcome measure. There was a significant condition × time interaction for right O_2Hb (*p* =*.018*) but no other condition × time interaction (L-TOI: *p* =*.324,* R-TOI: *p* =*.064,* L-nTHI: *p* =*.283,* R-nTHI: *p* =*.517,* L-O_2Hb: *p* =*.118*, R-O_2Hb: *p* =*.055*, L-HHb: *p* =*.083,* R-HHb: *p* =*.400*) effects. In summary, right and left PFC HHb significantly decreased during stress (time), right PFC TOI significantly increased during stress (time), and there was a different pattern of right PFC O_2Hb response between HFC and LFC during stress (interaction). Post-hoc analyses for the time effects are reflected in Figure 6.5.

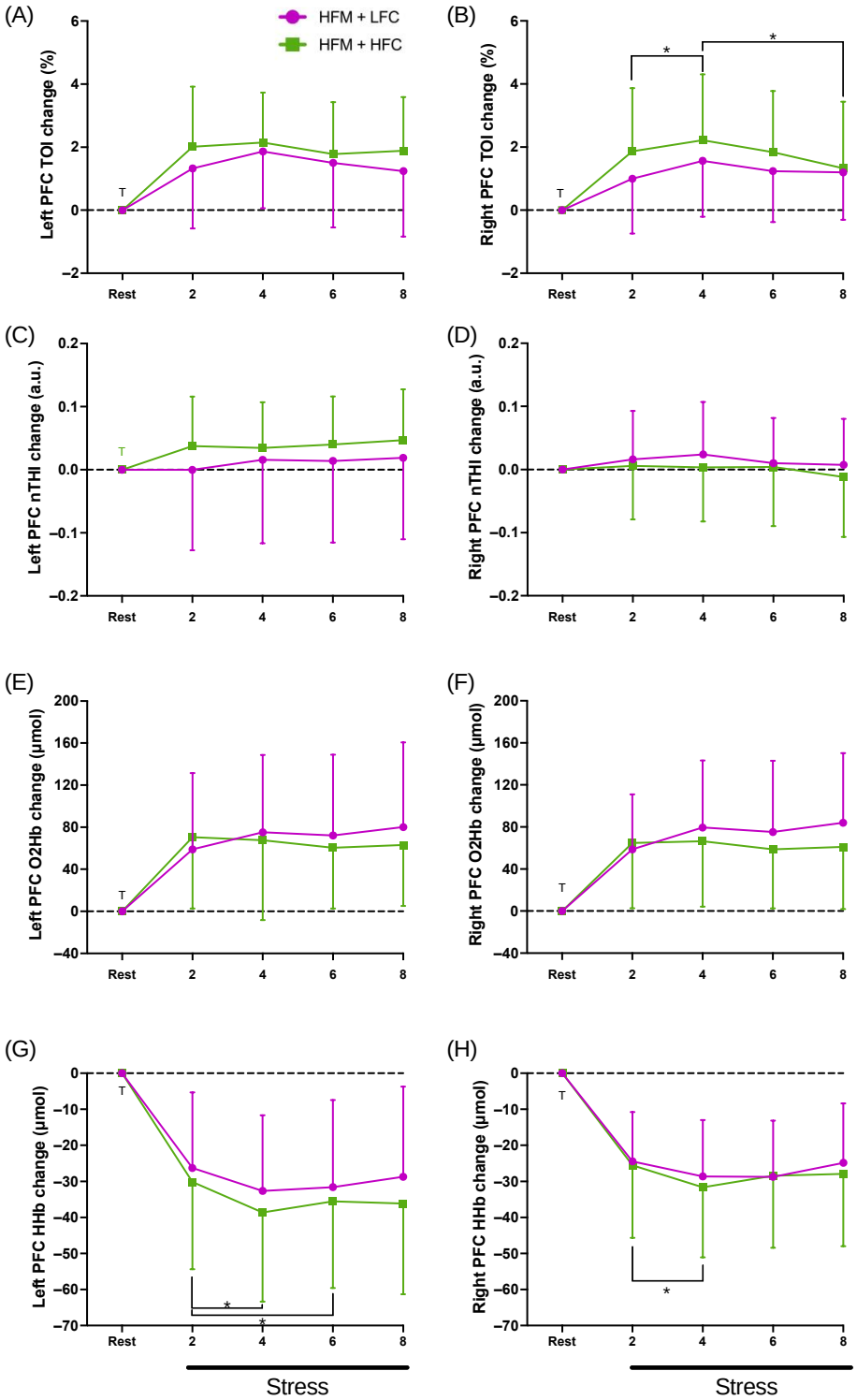

Figure 6.5 Time course of prefrontal cortical haemodynamics (L-TOI (A), R-TOI (B), L-nTHI (C), R-nTHI (D), L-O2Hb (E), R-O2Hb (F), L-HHb (G), R-HHb (H) during rest and stress following a HFM and either HFC or LFC

Data are presented as reactivity mean ± SD. *n = 23.* ^{T} Significant difference between rest and stress (t-test), * significant difference between values. TOI: tissue oxygenation index, nTHI: normalised haemoglobin index, O_2Hb: oxygenated haemoglobin change, HHb: deoxygenated haemoglobin change, HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa.

6.4.8. Flow-mediated dilatation following acute mental stress

Brachial FMD following mental stress is reported in Figure 6.6. A 2 condition × 3 time ANOVA revealed a significant condition effect for brachial FMD (*p =.002*). Post-hoc analyses showed that FMD was significantly higher in the HFM + HFC condition compared to the HFM + LFC condition. Furthermore, there was a significant condition × time interaction effect for brachial FMD (*p <.001*). Further exploration of this interaction effect revealed that FMD was significantly higher 30 minutes (*p =.006*) and 90 minutes (*p <.001*) post-stress in the HFC condition compared to the LFC condition. Examination of the time effects in both conditions separately, showed that in the HFC condition, there was no significant difference in FMD at 30 minutes post-stress compared to baseline (*p =.383*), yet FMD significantly increased at 90 minutes post-stress compared to baseline (*p =.004*). However, in the LFC condition FMD was significantly lower at 30 minutes post stress (*p =.003*) and 90 minutes post-stress (*p =.017*) compared to baseline. In summary, in the LFC condition, FMD was significantly impaired at 30 and 90 minutes post-stress, yet in the HFC condition, FMD was unchanged at 30 minutes post-stress and increased at 90 minutes post-stress. There was no significant time effect for brachial FMD (*p =.373*).

Allometrically scaled FMD is also reported in Figure 6.6. A 2 × 3 Linear Mixed Model revealed a significant condition (*p <.001*) and condition × time interaction (*p <.001)* effect but no significant time effect (*p =.325*). Post-hoc analyses showed that FMD was significantly higher in the HFM + HFC condition compared to the HFM + LFC condition. Examination of the time effects in both conditions separately, showed that in the HFC condition, there was no significant difference in FMD at 30 minutes post-stress [95%CI:

6.08, 8.44] compared to baseline [95%CI: 5.34, 7.68] (*p =.105*). Yet, FMD was significantly higher at 90 minutes post-stress [95%CI: 7.14, 9.53] compared to baseline (*p <.001*) and 30 minutes post-stress (*p =.023*). In the LFC condition, FMD was significantly lower at 30 minutes post stress [95%CI: 4.50, 6.82] compared to baseline [95%CI: 5.65, 8.00] (*p =.014*). There was no significant difference in FMD between 90 minutes post-stress [95%CI: 4.71, 7.04] and baseline (*p =.050*), and between 30 minutes post-stress and 90 minutes post-stress (*p =.616*). In summary, in the LFC condition FMD was significantly impaired at 30 minutes post-stress, yet in the HFC condition FMD was unchanged at 30 minutes post-stress but significantly increased at 90 minutes post-stress.

Brachial arterial diameter, anterograde blood flow and retrograde blood flow are reported in Table 6.7. There was a significant time effect for arterial diameter (*p =.002*), with a significantly higher diameter at 30 minutes (*p =.008*) and 90 minutes (*p =.010*) post-stress compared to baseline. There was also a significant condition × time interaction for arterial diameter (*p =.019*), whereby diameter is significantly greater in the LFC condition compared to the HFC condition at 90 minutes post-stress (*p =.011*). Similarly, in the LFC condition diameter significantly increased following stress, yet was unchanged following stress in the HFC condition. There was no condition effect for brachial artery diameter (*p =.091*). There was no significant condition (*p =.830*), time (*p =.633*) or condition × time interaction (*p =.824*) effect for anterograde blood flow. There was a significant time effect for retrograde blood flow (*p <.001*), whereby retrograde blood flow was significantly greater at 30 minutes (*p <.001*) and 90 minutes (*p =.006*) post-stress compared to baseline, and was significantly greater at 30 minutes

post-stress compared to 90 minutes post-stress (*p =.002*). There was no condition (*p =.635*) or condition × time interaction (*p =.968*) effect for retrograde blood flow.

Brachial blood pressure is presented in Table 6.7. A 2 × 3 ANOVA revealed a significant time effect for SBP (*p <.001)* and DBP (*p =.002)*, whereby SBP is higher and DBP is lower at 30- and 90-minutes post-stress compared to baseline. There were no significant condition (SBP: *p =.273*, DBP: *p =.211*) or condition × time interaction (SBP: *p =.152*, DBP: *p =.582*) effects for blood pressure.

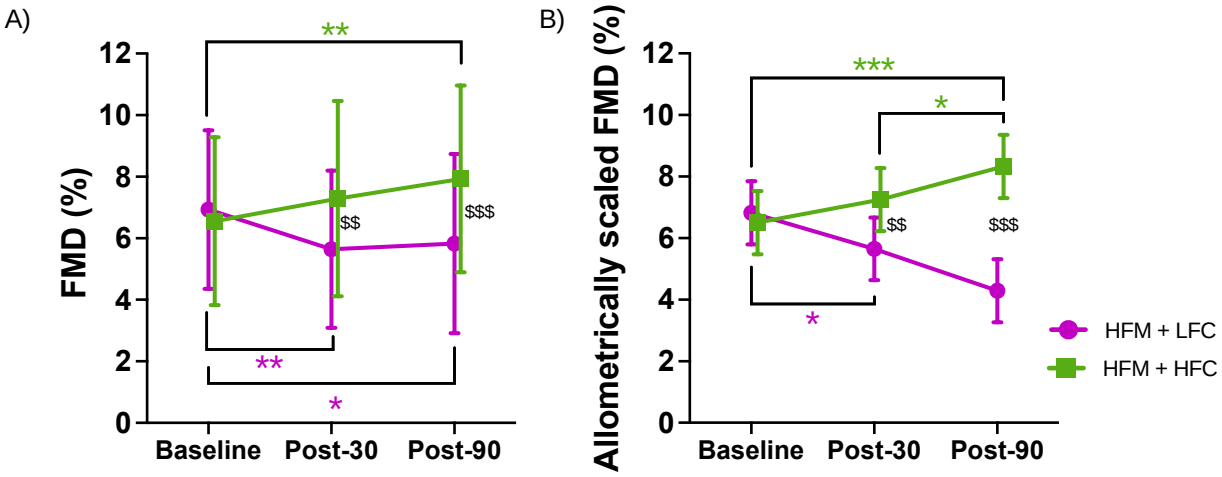

Figure 6.6 Time course of brachial artery FMD (%) [A] and allometrically scaled FMD (%) [B] during baseline, post-30 and post-90 following a high-fat meal (HFM) and either high-flavanol cocoa (HFC) or low-flavanol cocoa (LFC)

Data are presented as Mean ± SD. *n = 23*. $ significantly different between conditions, * significantly different between time-points ***/$$$ p<.001, **/$$ p<.01, *p<.05. FMD: Flow-mediated dilatation, HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa.

Table 6.7 Mean ± SD brachial arterial diameter following mental stress

	HFM + HFC			HFM + LFC		
Timepoint	Baseline	Post-30	Post-90	Baseline	Post-30	Post-90
Brachial SBP (mmHg)	116.81 ± 8.72	121.00 ± 9.97**	120.14± 6.98**	118.54 ± 9.90	120.83 ± 10.61**	122.36 ± 10.81**
Brachial DBP (mmHg)	64.97 ± 5.44	63.03 ± 5.01**	62.82 ± 5.99*	67.04 ± 6.50	63.77 ± 4.51**	63.86 ± 6.02*
Brachial Diameter (mm)	3.61 ± 0.55	3.63 ± 0.53**	3.63 ± 0.51*	3.60 ± 0.54	3.68 ± 0.55**	3.72 ± 0.55*$
Brachial anterograde blood flow (ml·min⁻1)	91.06 ± 42.25	94.23 ± 61.20	95.62 ± 55.46	87.29 ± 56.81	95.59 ± 57.47	94.11 ± 48.80
Brachial retrograde blood flow (ml·min⁻1)	–7.66 ± 9.23	–17.15 ± 14.79***	–12.17 ± 10.30**/^^	–7.26 ± 7.22	–16.11 ± 9.65***	–11.67 ± 8.23**/^^

n = 23. * significantly different to baseline, $ significantly different to HFM + HFC. ^ Significantly different to post-30. *** p<.001, **/^^ p<.01, */$ p<.05. HFM: high-fat meal, HFC: high-flavanol cocoa, LFC: low-flavanol cocoa. SBP: systolic blood pressure, DBP: diastolic blood pressure.

6.4.9. Common carotid artery blood flow following acute mental stress

CCA diameter and blood flow are reported in Table 6.8. A 2 condition × 3 time ANOVA revealed a significant time effect in CCA diameter (*p <.001*), with a significantly greater diameter at 30 and 90 minutes post-stress compared to baseline (*p's <.001*), and a significantly greater diameter at 90 minutes post-stress compared to 30 minutes post-stress (*p <.001*). There was no significant condition (*p =.056)* or condition × time interaction (*p =.163*) for CCA diameter. There was no significant condition (*p =.655*), time (*p =.740*) or condition × time interaction (*p =.153*) for CCA anterograde blood flow. There was a significant time effect for CCA retrograde blood flow (*p <.001*), whereby retrograde blood flow is significantly less at 30- and 90-minutes post stress compared to baseline (*p's <.001*). There was no significant condition (*p =.779*) or condition × time interaction (*p =.324*) effect for retrograde blood flow.

Table 6.8 Mean ± SD CCA diameter following mental stress

	HFM + HFC			HFM + LFC		
Timepoint	Baseline	Post-30	Post-90	Baseline	Post-30	Post-90
CCA diameter (mm)	6.52 ± 0.38	6.66 ± 0.39***	6.74 ± 0.36***/^^^	6.54 ± 0.39	6.74 ± 0.37***	6.85 ± 0.41***/^^^
CCA anterograde flow (ml·min⁻1)	578.33 ± 155.19	585.32 ± 165.72	534.97± 195.97	546.13 ± 149.58	552.74 ± 134.96	567.59 ± 131.13
CCA retrograde flow (ml·min⁻1)	–5.95 ± 4.76	–1.44 ± 1.56***	–2.10 ± 3.52***	–4.92 ± 4.81	–3.10 ± 4.48***	–2.11 ± 3.15***

n = 23. * significantly different to baseline, ^ Significantly different to post-30, ***/^^^ p<.001. CCA: common carotid artery.

6.5. Discussion

This study aimed to investigate whether cocoa flavanols can be used as a dietary strategy to protect endothelial function and improve brain oxygenation in the context of mental stress following a high-fat breakfast. To our knowledge, this is the first study to show that high-flavanol cocoa can attenuate the stress-induced decline in brachial FMD following a high-fat meal. On the other hand, flavanol intake did not improve cortical oxygenation during stress. Both brachial and carotid artery diameter increased following stress and fat intake, with a greater increase in the brachial artery following low-flavanol cocoa compared to high-flavanol cocoa. Retrograde blood flow increased post-stress and fat intake in the brachial artery and decreased in the carotid artery, yet these were unaffected by the flavanol intervention. As predicted and shown previously, mental stress induced changes in HR, HRV, RPI, BP and FBF, but these were not affected by flavanol intake. Stress significantly impacted mood with participants reporting to feel less happy, cheerful, and calm, and more stressed, tense, and angry

during stress compared to the other time points, but no differences between conditions. Similarly, participants reported to feel more energetic and less tired at rest (post-meal) compared to baseline (pre-meal), yet to feel less energetic and more tired post-stress compared to rest. Importantly, perceptions of the stress task and task performance were not significantly different between conditions, suggesting a consistent stress experience across interventions.

The stress-induced decline in brachial FMD at 30 minutes post-stress (1.29 %) is in line with previous work, showing a 1 – 3 % impairment in endothelial function following stress in healthy adults (Broadley et al., 2005, Ghiadoni et al., 2000, Jambrik et al., 2005, Lind et al., 2002, Spieker et al., 2002). Furthermore, the fat-induced delay in endothelial recovery at 90 minutes post-stress (FMD remains 1.11 % lower than baseline) is in line with our previous work showing FMD to remain impaired by 1.16 % 90 minutes following stress, when a high-fat meal had been consumed (Baynham et al., 2023b). The mechanisms by which saturated fat consumption prolong the impairment in FMD following stress are not known. However, a reduction in circulating levels of NO species following a high-fat meal has been observed (Rendeiro et al., 2016). Similarly, triglycerides and C-reactive protein have been evidenced to increase in circulation 2-4 hours post-fat consumption (Shin et al., 2009, Nappo et al., 2002, Peairs et al., 2011), and these are known to stimulate ET-1, inflammatory markers (Tsai et al., 2004) and oxidative stress (Bae et al., 2001), all attenuating NO bioavailability (Man et al., 2020) and thus impairing endothelial function.

Cocoa flavanols were effective at preventing the decline in endothelial function post-stress, following fat consumption, with brachial FMD being significantly higher following

high-flavanol cocoa compared to low-flavanol cocoa at both 30- and 90-minutes post-stress. Given the significant difference in brachial artery diameter at 90 minutes post-stress between conditions, allometrically scaled FMD was also estimated, confirming the significant differences in FMD between high versus low flavanol interventions even when diameter changes are corrected for. More specifically, cocoa flavanols attenuated the 1.29 % decline in FMD at 30 minutes post-stress and improved FMD by 1.37 % at 90 minutes post-stress (compared to the 1.16 % decline below baseline following low-flavanol cocoa). We have previously reported these differences at 30 - 90 minutes post-stress in participants in a fasted state: the high-flavanol cocoa attenuated the stress-induced decline in brachial FMD by approx. 1.36 % (Baynham et al., 2021). In agreement with our data, previous research has shown that both cocoa flavanols (Westphal and Luley, 2011) and citrus flavanones (Rendeiro et al., 2016) attenuate fat-induced impairments in FMD, yet do not completely eliminate the negative effect of fat. However, this is the first study to investigate fat intake prior to mental stress, and to show that cocoa flavanols can mitigate the combined impact of fat consumption and stress. The mechanisms by which flavanol ingestion improve vascular function are thought to be NO-related (Schroeter et al., 2006), with evidence of (-)-epicatechin enhancing eNOS activation through activation of signalling pathways such as P13K, Akt and PKA. Flavanols have also been shown to downregulate the bioavailability of ET-1 (Loke et al., 2008, Gomez-Guzman et al., 2012), reduce IL-6 production and ROS (Wang et al., 2020). These mechanisms likely drive the improvements in FMD following stress and fat consumption, as flavanols do not seem to influence the bioavailability of free fatty acids or triglycerides (Westphal and Luley, 2011). In summary, our data on peripheral vascular function, suggest that when food

choices during stress target high-fat foods, the addition of a high-flavanol food can be effective at preventing the negative compounded effect of stress and fat on endothelial function.

The present study showed a greater brachial artery retrograde blood flow at 30- and 90-minutes post-stress, with the greatest retrograde flow at 30 minutes post-stress, which is in line with the largest stress-induced reduction in brachial FMD observed (Ghiadoni et al., 2000). This is in agreement with our previous work, showing increases in retrograde blood flow post-stress (Baynham et al., 2023b). An increased retrograde blood flow response to stress has been shown previously (Rocha et al., 2023b), and likely results in elevated ET-1, expression of adhesion molecules, ROS-producing enzymes and decreased NO production (Thijssen et al., 2009). In line with that, increased retrograde shear rate has been associated with a reduction in endothelial function (Thijssen et al., 2009), which is what we observe in the current study. Importantly, the flavanol intervention does not seem to affect retrograde flow, suggesting that dietary flavanols improve FMD independently of retrograde blood flow.

In regard to the cerebral vasculature, we have observed an increase in PFC tissue oxygenation during stress, which is in line with previous research evidencing elevated CBF during similar arithmetic tasks (Shoemaker et al., 2019, Nagasawa et al., 2020, Baynham et al., 2023a). Stress-induced increases in cardiac output (driven by increased HR, Figure 6.3a and BP (Figure 6.3d/3e) have been shown to contribute to elevated cerebral perfusion (Willie et al., 2014a, Ogoh et al., 2005, Brindle et al., 2018) as well as neurovascular coupling mechanisms driven by increases in neural activation during the cognitively demanding mental arithmetic task (Wang et al., 2005). Critically,

we have shown recently that fat consumption during mental stress reduces PFC tissue oxygenation (Baynham et al., 2023a). Although, little is known about how fat may affect cerebral oxygenation, disruptions to cerebral metabolism and/or cerebral autoregulation are possible mechanisms (Ayata et al., 2013, Smith and Ainslie, 2017). We hypothesised that cocoa flavanols would counteract the fat-induced reduction in PFC tissue oxygenation during stress. However, contrary to their effect in the periphery, cocoa flavanols did not improve cortical oxygenation during mental stress. Previous research has shown cocoa flavanols to improve cerebral oxygenation during hypercapnia (Gratton et al., 2020), yet their effect on the brain in the context of mental stress and fat consumption is unclear. Timing of ingestion may influence the effect of flavanols, as Gratton and colleagues assessed cerebral oxygenation 2 hours following flavanol consumption, yet in the present study, we investigated oxygenation 1.5 hours post-flavanol ingestion, and may also encounter a delay in flavanol absorption due to the concomitant intake of a fatty meal. It is also plausible that the effect of flavanol consumption on the brain during stress is smaller than the periphery, possibly due to the greater need to tightly control and regulate brain blood flow (which can increase maximally by < onefold) compared to peripheral blood flow (which can increase 5 – 10 fold) (Koep et al., 2022). Retrospective power calculations reveal that using the observed medium effect size in TOI (0.26, $\eta_p^2 = 0.065$) and with power set to 0.9 and alpha at 0.05, 40 participants are required to appropriately power our study to detect changes in cortical oxygenation due to flavanol intake. Therefore, future research should investigate the impact of flavanol and fat intake on the cerebrovasculature in larger samples and, investigate whether these effects are replicable in other areas of the brain, for example by utilising fMRI, in addition to NIRS and ultrasound. Overall,

the present study suggests that the potential protective effect of flavonoids during mental stress seems to be smaller in the brain than the periphery.

Our previous work has also shown that alongside reduced PFC tissue oxygenation during mental stress, fat intake also induced a disturbance in mood (Baynham et al., 2023a). Importantly, high-flavanol cocoa did not influence mood parameters in the present study. Whilst there is some evidence to suggest polyphenols can reduce negative mood and improve positive mood (Lamport and Williams, 2021), this might not be the case in the context of saturated fat intake and mental stress.

In agreement with observations during stress, there were no differences in carotid artery blood flow and diameter between high and low-flavanol cocoa interventions. The postprandial increase in carotid artery diameter is in line with our previous findings (Baynham et al., 2023a). We also detected a postprandial increase in brachial artery diameter at these time points (Table 6.7), in line with previous studies (Raitakari et al., 2000, Gokce, 1998). There is evidence that fat consumption induces peripheral vasodilation (FBF), likely mediated by changes in insulin and triglycerides (Raitakari et al., 2000). However, in the present study, flavanol consumption seems to mask this effect, shown by a reduced brachial artery diameter following high-flavanol cocoa compared to low-flavanol cocoa at 90 minutes post-stress ($p = .011$). Interestingly, this condition effect at 90 minutes post-stress does not quite reach significance in the carotid artery ($p = .056$). This is possibly due to being underpowered given the large effect size ($\eta^2p = .156$, $f^2 = 0.43$). Given the disparity in arterial diameter and retrograde blood flow across the brachial and carotid artery, future research should continue this

investigation and utilise assessments of the internal carotid artery, to directly assess the impact of fat and flavanol consumption on CBF and the peripheral vasculature.

In line with previous research, the present study showed an increase in cardiovascular, BP and peripheral vasodilatory responses during stress (Baynham et al., 2021, Paine et al., 2013a). There was no difference in the FBF response to stress between conditions. Whilst previous evidence has shown an increase in FBF following cocoa flavanols at rest (Heiss et al., 2015) and during stress (Baynham et al., 2021), these participants ingested flavanols in a fasted state. As previously mentioned, fat consumption may delay the absorption of flavanol metabolites, and thus, the NO-induced increase in vasodilation evidenced following flavonoid intake (Dietz et al., 1994), may not have reached full effect during the stress task (only 1.5 hours post fat and flavanol consumption). Similarly, flavanol consumption had no effect on cardiovascular responses during stress, in agreement with our previous results in a fasted state (Baynham et al., 2021).

The habitual diet of the participants in the current study appears to be similar to the diet of the UK population. For example, whilst 100 % of participants exceeded the daily recommended sugar value compared to 61 % of British citizens (Rauber et al., 2019), 43 % exceeded the recommended saturated fat value compared to 75 % of UK adults (Scientific Advisory Committee on Nutrition, 2019). Furthermore, 35 % of participants consumed at least 5 portions of fruit and vegetables, in line with the UK average of 28 %. However, the present sample consumed on average 1 extra portion per day compared to UK adults (4.8 portions /day vs. 3.7 portions /day) (National Health Service, 2020), yet their flavonoid intake (239 mg /day) was more in line with

participants with lower flavonoid intake (mean quintile 1: 174 mg /day, mean quintile 2: 321 mg / day) in a large cross-sectional study (Bondonno et al., 2021). Therefore, our findings have relevance for the general population. However, it is likely that fat-induced decreases and flavonoid-induced protection of endothelial function, may be aggravated/beneficial in populations with poorer habitual diet. For example, a longitudinal study found 1 year of flavanol consumption only improved memory in participants in the lowest tertile of habitual diet quality (Brickman et al., 2023). Therefore, future research should target these populations.

6.5.1. Limitations

One limitation of the present study is that the high-fat meal was not tailored to individual metabolic rate. However, tailoring fat consumption to metabolic rate is not very relevant to everyday life, and intervening with one consistent dose of fat likely results in higher variability in responses between participants. Similarly, it has been established that 50 g of fat is sufficient to impact endothelial function (Jackson et al., 2007), which is in line with the dose used in the current study. Secondly, previous evidence has shown that polyphenol microbial-derived metabolites can be detected in the blood for up to 48 hours (Rodriguez-Mateos et al., 2014) yet we only restricted polyphenol intake for 24 hours prior to each visit. However, as there is a large reduction in urinary polyphenol metabolites from 24 to 48 hours, most metabolites are likely to be excreted within the first 24 hours (Borges et al., 2018). Finally, our sample size was moderate. However, a robust crossover design was employed and post-hoc power analyses revealed that a sample of 23 participants, power at 90 % and alpha at 0.05, allowed the detection of a medium size interaction effect (0.31) for our primary outcome measure brachial FMD

(Faul, 2007). Nevertheless, more participants are likely required to detect the effect of flavanol-rich cocoa on cortical oxygenation (NIRS), and so future research should continue this investigation in a larger sample. Similarly, a more in-depth investigation of mechanisms of action underlying these responses should be a focus of future work.

6.6. Conclusion

In summary, this study demonstrates that flavonoid-rich foods have the potential to acutely protect endothelial function against poor food choices, such as high-fat snacks, during episodes of stress in young healthy adults. It further suggests that such protection does not extend to the cerebral vasculature. However, our data indicates that the size of the flavanol effect in the brain is smaller (effect size = .26), hence a larger sample is needed to clarify the protective effects in the brain during stress. Given the prognostic value of FMD for future risk of CVD (Inaba et al., 2010), these findings are clinically relevant (7.93 % vs. 5.83 % brachial FMD at 90 minutes post-stress, following high-flavanol and low-flavanol cocoa, respectively), particularly given the documented prevalence of stress and the trend towards increased consumption of high-fat foods during periods of heightened stress. This work has relevance for application in everyday diet, as the administered dose of flavanols could be achieved through consumption of, for instance, 2 cups of green tea, 5.5 tbsp of unprocessed cocoa or 300 g of berries (Bhagwat et al., 2013). As such, our data have important implications for future dietary recommendations to protect the vasculature during stressful periods.

7. The relationship between stress and dietary choices: A daily diary study

7.1. Abstract

Introduction: Mental stress can worsen physical and psychological health, partly driven by a deterioration in dietary choices during periods of stress. Crucially, food choices made during stressful periods may protect or exacerbate the impact of stress on health. There is limited research exploring the complex relationship between stress/psychological wellbeing and nutrient intake in a free-living environment. Therefore, this study aimed to investigate the within-and between-person associations between daily stress-related psychological wellbeing and dietary nutrient intake.

Methods: Stress-related psychological wellbeing (stress, anxiety, depression, positivity, energy, fatigue) and dietary intake (fat, saturated fat, sugar, fibre, flavonoids) were collected using a daily diary in 67 young healthy participants over 7 days. Multi-level linear mixed models examined within-and between-person associations between daily stress-related psychological wellbeing and nutrient intake.

Results: Fifty-nine participants were included in analyses. Symptoms of stress, anxiety, depression, or energy did not predict fat, saturated fat, or sugar consumption. However, a lower within-person positivity associated with saturated fat and sugar consumption, and higher between-person fatigue associated with fat intake. Higher perceptions of between-person anxiety associated with fibre intake and higher within-person stress associated with flavonoid intake. No other psychological assessments associated with fibre or flavonoids. Further analyses did not show the source of flavonoids driving the relationship between stress and flavonoid intake.

Discussion: This study revealed that it is unclear how stress is associated with some dietary choices in a free-living environment. However, given the association between stress and flavonoids, flavonoid-rich foods could be used as a coping mechanism to protect health during stress. Furthermore, given the associations between positivity and fatigue, and consumption of fat and sugar, future work should discover ways to break the cycle of consuming unhealthy nutrients when experiencing poorer psychological wellbeing.

7.2. Introduction

Stress is extremely prevalent, being the leading cause of work-related sickness, and costing UK employers up to £7.9 billion annually (GOV.UK, 2019). Stress can negatively impact both physical and mental health. For example, stress has been associated with increased incidence of myocardial infarction and ischaemia (Bergovec et al., 1992, Carroll et al., 2002, Strike and Steptoe, 2003), disruptions to brain function and cognition (Yaribeygi et al., 2017), increased incidence of depression (Hammen et al., 2009) and other psychological disorders (Schneiderman et al., 2005).

Stress has also been associated with obesity (Moore and Cunningham, 2012, Wardle et al., 2011). Obesity is a prominent risk factor for cardiovascular-related diseases (Powell-Wiley et al., 2021) and the fourth biggest risk factor for all-cause mortality (World Health Organisation, 2022). During stressful periods fat oxidation slows down (Kiecolt-Glaser et al., 2015), resulting in weight gain. Furthermore, stress indirectly increases the risk for obesity through changes in behaviour (Hill et al., 2021, O'Connor et al., 2021). Notably, 46 % of adults perceive themselves to overeat or eat unhealthily during stress (Mental Health Foundation, 2018a), with overweight individuals typically being more vulnerable to stress-induced overeating (Cotter and Kelly, 2018). Therefore, stress can directly and indirectly increase the risk for obesity, and subsequently worsen long-term health.

The relationship between stress and diet is complex and a number of studies have aimed to investigate how food choices change during stress. For example, a laboratory-based study showed that young adults opted for high-fat and high-sugar foods over fruit following exposure to stress (Zellner et al., 2006). Additionally, greater

calorie consumption was observed in high cortisol compared to low cortisol reactors to psychological stress (Epel et al., 2001). Whilst laboratory-based studies can elucidate such mechanisms linking stress and eating behaviour, many of these settings are not conducive to eating freely as food choices will be influenced by the food provided. In agreement with the randomised-controlled trials, cross-sectional and prospective studies have reported higher perceived stress to be linked with increased consumption of unhealthy snacks and reduced consumption of fruits and vegetables (Newman et al., 2006, Roberts et al., 2014b, Oliver and Wardle, 1999). These studies further suggest that sex differences could mediate this relationship (Papier et al., 2015). In addition, correlational studies have largely assessed stress as the frequency of major life events or daily hassles (Araiza and Lobel, 2018), rather than the perceived stress. Therefore, these methodologies likely overlook the complex fluctuations typical of stress and the individual differences involved in eating behaviour (Hill et al., 2021). For example, eating alone vs with people influences food intake (Ruddock et al., 2019), revealing the effect of social context and social norms on eating behaviour (Robinson et al., 2014). Whilst not specifically investigating the context in which stress and eating behaviours take place, there is a need to investigate the relationship between stress and dietary choices in a more ecologically valid setting where contextual factors may vary and influence both perceived stress and food choices.

Assessing daily diaries is a methodology used to examine participant behaviour and experiences in real-time, in their free-living environment (Shiffman et al., 2008). Furthermore, daily diaries enable the consideration of both between-person (deviation from average group level) and within-person (deviation from individuals own average) associations across the same period (Dunton, 2017). Given that stress exposure and

eating behaviour likely fluctuate differently within individuals, within-person analyses can reveal the extent of this fluctuation, and thus, present underlying trait-level patterns (Dunton, 2017). Few studies have investigated both between and within-day associations between stress and diet, with most health behaviour research focussing on differences between people (interindividual variation) instead of differences within people (intraindividual variation) (Dunton, 2017). Furthermore, previous research using similar methodological approaches designs to investigate the relationship between stress and diet, have again assessed number of daily hassles (O'Connor et al., 2008, Newman et al., 2006), which does not shed light on individual's perceptions, and therefore perceived stressfulness, of these daily hassles. Furthermore, previous work has focussed on eating behaviour and regulation (Reichenberger et al., 2021, Smith et al., 2022a), and assessed diet as snacking (Zenk et al., 2014), the act and frequency of eating (Ruf et al., 2023) or asked participants to select foods they are currently craving from a list of pre-determined foods (Mason et al., 2019), which overlooks specific nutrients which may be over or under consumed during periods of stress.

Importantly, the nutritional composition of foods consumed during stressful periods may protect or exacerbate the impact of stress on health. For example, we have recently shown that consumption of a high-fat breakfast (high-fat foods are often opted for when stressed) impaired vascular recovery following stress (Baynham et al., 2023b) and attenuated cerebral oxygenation during stress (Baynham et al., 2023a). However, we have also demonstrated an acute dose of a flavonoid-rich food to mitigate the decline in vascular function following stress (Baynham et al., 2021). Therefore, it is important to understand the association between stress (and psychological wellbeing) and nutritional choices in a free-living environment, which can then help to understand

how best to intervene with diet to protect health from stress. To our knowledge, this is the first study to use within- and between-person analyses to investigate associations between perceived stress and the nutritional composition of diet.

Given that the intention of this work was to not intervene with specific exposure to stress, the included assessments also encompass psychological wellbeing, which relate to stress, and provide a more comprehensive examination of overall mood states, and how they may impact dietary consumption. We aimed to investigate the associations between stress, and psychological wellbeing, and intake of both macro/micronutrients and food bioactives, specifically 1) fat (total and saturated) and sugar consumption, 2) fibre (as an index of fruit and vegetable consumption) and, 3) flavonoids. We hypothesised that during stressful days, or days with poorer psychological wellbeing, participants would increase their consumption of fat and sugar, and decrease their consumption of fibre and flavonoids.

7.3. Methods

7.3.1. Study design

The present study used a 7-day daily diary design and was approved by the University of Birmingham Ethics Committee (ERN17_1755C). Informed consent was obtained online from all participants before enrolment in the study. The data was collected during the COVID-19 pandemic.

7.3.2. Participants

67 young, healthy adults were recruited via email advertisements. Inclusion criteria were i) aged 18-45 years, ii) able to read English, and iii) no current medically diagnosed mental health condition.

7.3.3. Procedures

Data collection occurred February 2021 – August 2021 using an online questionnaire platform (SmartSurvey). If eligible and consenting, participants were distributed with a set of questionnaires to obtain demographic information and other psychological assessments (data not reported). Following completion of these questionnaire's, data collection commenced for 7 days, including 5 weekdays and 2 weekend days, starting on a Monday where possible (Figure 7.1). Participants were briefed about how to complete daily food diaries (distributed approx. 8:00 am) and a daily questionnaire (distributed approx. 9:00 – 9:30 pm), which included psychological wellbeing assessments and instructions to upload the food diary recorded that day. At the end of the data collection period, data was downloaded to a research computer, and the surveys were closed. The data was then organised and analysed.

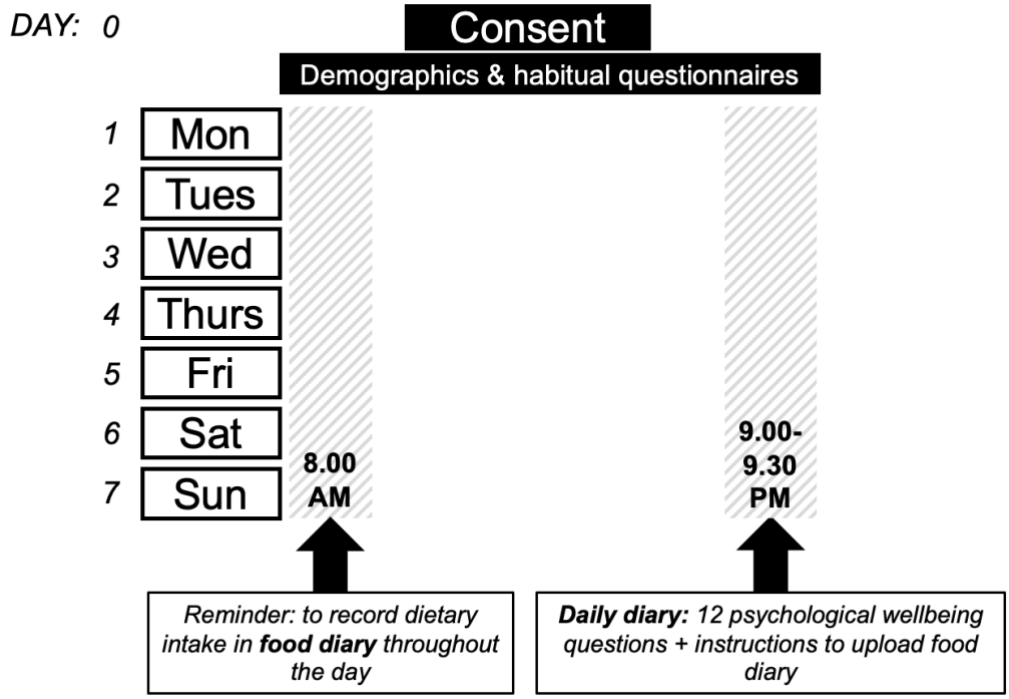

Figure 7.1 Study protocol

Day 0: online questionnaire pack to obtain demographic and habitual information. Day 1 – 7: daily diaries to obtain daily psychological wellbeing and dietary intake.

7.3.4. Outcome measures

7.3.4.1. Demographic information

Demographic information was obtained during the initial questionnaire pack, and included measures of age, gender, ethnicity, nationality, height, and weight (and general health, engagement in extracurricular activities, long-term conditions/diseases, alcohol consumption and smoking status; data not reported). Terms ‘male’ and ‘female’ used in this paper refer to self-identified gender, and 100 % of participants identified as male or female. COVID-19 lockdown status was also reported by the participant and assessed retrospectively using the UK Government’s official lockdown dates and timestamps.

7.3.4.2. Daily dietary intake

Daily dietary intake was assessed using a 24-h recall, following standard procedures (Asamane et al., 2020). Participants were reminded to complete a daily food diary at 8 am, with a reminder to upload their diary sent with the evening survey, via SmartSurvey. Instructions were distributed, including examples of detail, use of weighing scales and space for detailed recipes for home-cooked meals. Participants were asked to eat and drink as they normally would and record everything they ate and drank across the day. Brand names and cooking methods were also recorded, as well as nutrient supplement usage. Following the first day of data collection, participant's food diaries were checked for accuracy and feedback was given as necessary. Following the 7-day data collection, data was processed into a dietary software analysis package, Dietplan 7.0 (Forestfield Software Ltd., Horsham, West Sussex, UK). Dietplan 7.0 was appropriate as it utilises UK-specific databases such as McCance and Widdowson, as well as relevant ethnic minority food databases. The USDA database was used for assessment of polyphenol (flavonoid) intake. In instances that a food item could not be found in the software databases, these were added to the database using food manufacturing websites and other food composition tables. All nutrients and food bioactives were exported, with fat, saturated fat, sugar, fibre, and flavonoids included in data analysis. Daily fruit and veg, tea, and red wine consumption were manually calculated and included as a subsequent sensitivity analysis.

7.3.4.3. Daily psychological wellbeing

One survey was sent each day (via text message or email, depending on participant preference) and took less than 2 minutes to complete. The survey consisted of 12 questions examining how stressed, anxious, positive, depressed, fatigued, and energetic the participant felt (included in analysis) and cheerful, angry, happy, calm, and how well they felt they coped with stress, and how on top of things they felt across the day (data not reported). This was scored on a scale of 1 to 5, with 1 being 'not at all' and 5 being 'extremely'. There was also a 'prefer not to answer' option. Each item was scored individually.

7.3.5. Data analysis

59 participants (30 female) were included in the analysis, following removal of 8 participants (exclusion reasons: $n = 2 > 50$ years, $n = 1$ current mental health condition, $n = 1$ not completing data collection period, $n = 4$ not starting data collection period). All analyses were conducted using IBM SPSS Statistics for Windows (version 29.0). Intra-class coefficients (ICC) are reported for daily dependent variables. One-way ANOVAs assessed gender differences across demographics and all daily study variables.

Multi-level linear mixed models were tested to examine within-and between-person associations between daily psychological wellbeing and dietary intake. In these models, stress, anxiety, positivity, depression, energy, and fatigue were added as separate predictors with each nutrient/food bioactive (fat, saturated fat, sugar, fibre, and flavonoids) as an outcome.

In all models, within-person predictors were person-centred, providing 'daily' fluctuations. At level 2, the average of daily assessments over the 7 days were entered as between-person (grand sample centred), providing 'typical' (or average across the data collection period) levels. Age, sex, BMI, and COVID-19 status were also entered at level 2 as covariates in all models. For all models the intercepts were random, and the slopes were fixed. Multilevel models' β ± SE are reported. For all analyses, significance was set at $\alpha < .05$. Interaction analyses was included for every model, investigating the interaction between each predictor and habitual stress (PSS, data not reported). However, no meaningful interactions were found and are therefore not reported.

To calculate the amount of variance in each dependent variable explained by within- and between-person predictors, a series of models were analysed. The pseudo-R squared models presented in the results investigated the amount of variance in the dependent variable explained by within-person predictors (R_1^2) and between-person predictors (R_2^2), relative to the empty model (model with covariates only) (Hox et al., 2018).

We also conducted sensitivity analysis to explore models with fruit and veg, tea, and red wine consumption. In these models, stress, anxiety, positivity, depression, energy, and fatigue were added as separate predictors with each nutrient (fruit and veg, tea, and red wine) as an outcome.

7.4. Results

7.4.1. Overview of results

Included participants (*n = 59*) completed 412 (99.8 %) of 413 (over 7 days) of daily questions assessing psychological wellbeing and 410 (99.3 %) of 413 daily food diaries (with 3 participants completing only 6 out of 7 days of their food diaries). The skewness scores for the dependent variables of psychological wellbeing (stress: 0.70, anxiety: 0.73, positivity: –0.24, depression: 1.77, energy: –0.13 and fatigue: 0.40) and diet (fat: 0.72, saturated fat: 1.40, sugar: 1.41, fibre: 0.98, flavonoids: 2.40) were mostly within an acceptable range (skewness $\pm$ 2) (Gravetter and Wallnau, 2016).

Participant characteristics, psychological wellbeing, and dietary intake are displayed in Table 7.1. Participants were young, with a normal weight BMI and reported to be relatively healthy. Participants were mostly British (81.3 %) and of white ethnicity (88.1 %). Daily psychological wellbeing and dietary intake are also reported in Table 7.1.

Table 7.1 Descriptive statistics and ICCs for study variables

Demographics	Mean ± SD	ICC	Min	Max
Age (years)	24.24 ± 3.57	-	18.00	36.00
BMI (kg/m^2)	23.35 ± 2.62	-	19.86	32.02
Health rating /5	3.39 ± 1.03	-	1	5
Gender (n (%))	Female: 30 (51%)			
	Male: 29 (49%)			
Psychological wellbeing				
Stress /5	2.08 ± 1.04	.278	1	5
Anxiety /5	2.07 ± 0.98	.361	1	5
Positivity /5	3.50 ± 0.95	.426	1	5
Depression /5	1.46 ± 0.71	.293	1	5
Energy /5	3.11 ± 0.96	.363	1	5
Fatigue /5	2.65 ± 1.01	.223	1	5
Daily dietary intake (/day)				
Fat (g)	82.56 ± 38.28	.315	11.12	225.10
Saturated fat (g)	30.37 ± 17.97	.221	1.71	123.48
Sugar (g)	84.12 ± 54.23	.491	3.90	391.24
Fibre (g)	22.04 ± 10.65	.458	1.00	59.78
Flavonoids (mg)	175.57 ± 267.02	.756	0	1516.19

n = 59. BMI: body mass index, SD: standard deviation, ICC: intraclass correlation coefficient, Min: minimum, Max: maximum.

7.4.2. Gender differences

Gender differences are reported in Table 7.2. Females were younger (p =.003), had a lower BMI (p <.001) and reported a higher health rating (p =.026). Females reported lower levels of daily stress (p <.001) and higher levels of daily energy (p =.015)

compared to males. Females reported to consume more flavonoids (p =.010) per day compared to males.

Table 7.2 Gender differences in study variables

Demographics	Male (Mean ± SD)	Female (Mean ± SD)	Difference*	η_p^2
Age (years)	24.76 ± 3.65	23.73 ± 3.43	**p =.003**	.021
BMI (kg/m^2)	24.07 ± 2.76	22.65 ± 2.28	**p <.001**	.073
Health rating /5	3.28 ± 095	3.50 ± 1.09	**p =.026**	.012
Psychological wellbeing				
Stress /5	2.27 ± 1.06	1.90 ± 0.98	**p <.001**	.033
Anxiety /5	2.00 ± 0.94	2.13 ± 1.02	p =.153	.005
Positivity /5	3.47 ± 0.99	3.53 ± 0.91	p =.552	.001
Depression /5	1.46 ± 0.73	1.45 ± 0.70	p =.826	.000
Energy /5	3.00 ± 0.95	3.22 ± 0.96	**p =.015**	.014
Fatigue /5	2.67 ± 1.03	2.63 ± 0.99	p =.677	.000
Daily dietary intake (/day)				
Fat (g)	83.46 ± 39.12	81.70 ± 37.54	p =.643	.001
Saturated fat (g)	30.92 ± 17.26	29.84 ± 18.65	p =.540	.001
Sugar (g)	80.23 ± 58.63	87.83 ± 49.55	p =.156	.005
Fibre (g)	22.74 ± 12.39	21.36 ± 8.61	p =.195	.004
Flavonoids (mg)	140.79 ± 255.80	208.70 ± 273.80	**p =.010**	.016

BMI: body mass index, SD: standard deviation, η_p^2: partial eta squared. * P value from ANOVAs.

7.4.3. Psychological wellbeing predicting dietary intake

Table 7.3 provides multilevel models with psychological wellbeing (perceptions of stress, anxiety, positivity, depression, energy, and fatigue) predicting daily dietary

consumption (fat, saturated fat, sugar, fibre, and flavonoids). All psychological wellbeing scores are scored on a scale of 1 to 5, and so a 1 unit increase in stress for example shifts perceptions of feeling stressed from ‘not at all’ to ‘a little’ or from ‘quite a lot’ to ‘extremely’.

7.4.3.1. The impact of covariates on nutrient consumption

All models included covariates age, gender, BMI, and COVID-19 status. BMI significantly predicted fibre intake in the stress (–0.79 (0.35), p =.029), anxiety (–0.77 (0.35), p =.031), positivity (–0.74 (0.35), p =.042), depression (–0.77 (0.36), p =.037), energy (–0.74 (0.35), p =.041), and fatigue (–0.82 (0.36), p =.025) model, but did not significantly predict any other dietary outcomes. Age, gender, and COVID-19 status did not significantly predict any nutrient intake.

7.4.3.2. Low positivity associated with saturated fat and sugar intake, and higher fatigue associated with fat intake

Within-person positivity significantly predicted saturated fat and sugar consumption, whereby a 1 unit decrease in positivity compared to their own average predicted 2.38 ± 1.19 g more saturated fat (p =.046) and 6.41 ± 2.96 g more sugar (p =.031) consumed. However, within-person positivity did not predict fat consumption and between-person positivity did not predict fat, saturated fat, or sugar consumption. Between-person fatigue significantly predicted fat consumption, whereby a 1 unit increase in fatigue compared to the group average predicted 11.32 ± 5.44 g more fat consumed (p =.042). Between-person fatigue did not predict saturated fat or sugar consumption, and within-person fatigue did not predict fat, saturated fat, or sugar

consumption. Within-person or between-person stress, anxiety, depression, or energy did not predict fat, saturated fat, or sugar consumption.

7.4.3.3. Higher levels of anxiety associated with fibre intake

Between-person anxiety significantly predicted fibre consumption, whereby a 1 unit increase in anxiety compared to the group average predicted 2.76 ± 1.32 g more fibre consumed (p =.041). However, within-person anxiety did not predict fibre consumption. Within-person or between-person stress, positivity, depression, energy, or fatigue did not predict fibre consumption.

7.4.3.4. Higher levels of stress associated with flavonoid intake

Within-person stress significantly predicted flavonoid consumption, whereby a 1 unit increase in perceptions of stress compared to your own average predicted 18.93 ± 8.33 mg more flavonoids consumed (p =.024). Yet, between-person stress did not predict flavonoid consumption. Within-person or between-person anxiety, positivity, depression, energy, or fatigue did not predict flavonoid consumption.

7.4.3.5. Variance

The amount of variance explained by within-person fluctuations (R_1^2) was generally higher than the amount of variance explained by between-person differences (R_2^2) in each of the outcome measures (Table 7.3). For example, between-person fluctuations in psychological wellbeing (stress, anxiety, positivity, depression, energy, and fatigue) accounted for 0 – 0.89 % of the variation in fat consumption, whereas within-person fluctuations in psychological wellbeing accounted for up to 9.52 % of the variation in fat consumption.

Table 7.3 Multilevel modelling coefficients of psychological wellbeing predicting diet

	Fat β (SE)	Sat fat β (SE)	Sugar β (SE)	Fibre β (SE)	Flavonoids β (SE)
Fixed effects					
Intercept	**79.32 (5.89) – 80.55 (5.71)*** **	**29.65 (2.63) – 30.02 (2.59)*** **	**79.93 (10.19) – 80.73 (10.37)*** **	**19.71 (1.62) – 20.30 (1.61)*** **	**141.70 (58.24) – 149.18 (58.72)***
Level 1 predictors					
Stress	–0.82 (1.90)	–0.17 (0.97)	–2.12 (2.40)	0.05 (0.49)	**18.93 (8.33)***
Anxiety	0.08 (2.13)	0.60 (1.08)	0.21 (2.70)	–0.56 (0.54)	–5.28 (9.41)
Positivity	–4.01 (2.34)	**–2.38 (1.19)***	**–6.41 (2.96)***	0.78 (0.60)	–1.82 (10.40)
Depression	–0.71 (2.90)	0.43 (1.47)	4.98 (3.67)	–0.82 (0.74)	–8.11 (12.82)
Energy	0.74 (2.23)	1.24 (1.13)	5.02 (2.82)	–0.34 (0.57)	–13.21 (9.85)
Fatigue	–1.86 (1.88)	–1.02 (0.287)	–2.65 (2.38)	–0.46 (0.48)	14.51 (8.27)
Level 2 predictors					
Stress	3.46 (5.25)	0.66 (0.78)	4.10 (9.22)	2.57 (1.44)	57.72 (51.97)
Anxiety	1.70 (4.88)	–0.65 (2.15)	–2.51 (8.55)	**2.76 (1.32)***	–9.28 (48.62)
Positivity	2.66 (4.73)	1.36 (2.08)	10.90 (8.17)	–1.75 (1.31)	27.00 (47.08)
Depression	1.32 (7.30)	–0.11 (3.23)	–3.80 (12.78)	2.57 (2.02)	56.94 (68.05)
Energy	–2.24 (5.23)	0.27 (2.31)	8.75 (9.10)	–2.14 (1.44)	–24.64 (52.06)
Fatigue	**11.32 (5.44)***	2.86 (2.46)	3.88 (9.86)	2.86 (1.54)	5.42 (56.08)

Pseudo R^2					
R_1^2 (%)	0.06 – 9.53	–0.20 – 3.25	0.10 – 3.35	3.57 – 8.98	–0.03 – 2.21
R_2^2 (%)	0.00 – 0.89	0.01 – 1.21	0.00 – 1.41	0.03 – 0.53	0.01 – 1.55
Model fit					
AIC	3794.13 – 3799.20	3260.87 – 3265.18	4009.66 – 4015.97	2698.40 – 2701.15	5020.83 – 5026.83
-2 LL	3776.13 – 3781.20	3242.87 – 3247.18	3991.66 – 3997.97	2680.40 – 2683.15	5002.83 – 5008.83

n = 59. SE: standard error, AIC: Akaike's information criterion, LL: log likelihood. * p <.05, ** p <.01, *** p <.001. *Note*: all models were run separately for each predictor (within and between-person), so intercept, pseudo R^2 and model fit indices are given as a range. R_1^2 – variance explained at level 1 (within-person) relative to the variance explained in the covariates only model, R_2^2 – variance explained at level 2 (between-person) relative to the variance explained in the covariates only model.

7.4.4. Sensitivity analysis: Identifying food sources of flavonoids that mediate the perceptions of stress

Contrary to our hypothesis, higher levels of stress associated with flavonoid intake. Flavonoids are present in a variety of different food sources and given this contradictory finding and the potential for flavonoids to improve cardiovascular health, further analysis was undertaken to identify food sources of flavonoids that may mediate the positive association between within-person stress and flavonoid consumption.

In the subsequent analyses, fruit and veg, tea, and red wine consumption were investigated. The skewness scores for these dependent variables were the following: fruit and veg: 0.94, tea: 1.85 and red wine: 6.34. Mean dietary intake and gender differences in these food sources are displayed in Table 7.4. Females reported to consume more tea (p <.001) per day compared to males.

Table 7.4 Descriptive statistics, intraclass correlation coefficients and gender differences for sources of flavonoids

	Dietary Intake				Gender Differences			
	Mean ± SD	ICC	Min	Max	Male (M ± SD)	Female (M ± SD)	Difference*	η_p^2
Fruit and veg (g)	364.32 ± 261.77	.388	0	1223.50	348.35 ± 282.73	379.53 ± 239.81	p =.228	.004
Tea (ml)	139.56 ± 241.68	.741	0	1200.00	93.15 ± 230.99	183.76 ± 243.88	**p <.001**	.035
Red wine (ml)	13.20 ± 73.56	.139	0	750.00	18.75 ± 88.81	7.92 ± 54.96	p =.136	.005

n = 59. BMI: body mass index, veg: vegetables, SD: standard deviation, ICC: intraclass correlation coefficient, Min: minimum, Max: maximum, η_p^2: partial eta squared. * P value based off ANOVA.

7.4.5. Psychological wellbeing predicting dietary sources of flavonoids

Table 7.5 depicts multilevel models with psychological wellbeing (perceptions of stress, anxiety, positivity, depression, energy, and fatigue) predicting food sources of flavonoids (fruit and veg, tea, red wine) that mediate perceptions of stress.

7.4.5.1. Age and gender are associated with food sources of flavonoids

Age significantly predicted fruit and veg consumption in all models (16.17 (7.36) – 18.71 (7.52), p's <.036) and tea consumption in the positivity (19.66 (9.31), p =.039), energy (19.47 (9.60), p =.047), and fatigue (20.02 (9.39), p =.038) model. Therefore, increased age associates with increased fruit and veg and tea intake. Gender significantly predicted tea intake in the anxiety (121.68 (59.88), p =.047) model only, whereby being female predicted more tea consumption compared to males.

7.4.5.2. Sources of flavonoids do not mediate the relationship with stress

Within-person or between-person stress did not predict fruit and veg, tea, or red wine intake. Similarly, within-person and between-person anxiety, positivity, depression, energy, and fatigue did not predict any food sources of flavonoids.

7.4.5.3. Variance

As before, the amount of variance explained by within-person fluctuations (R_1^2) was generally higher than the amount of variance explained by between-person differences (R_2^2) (Table 7.5). For example, within-person fluctuations in psychological wellbeing accounted for up to 6.39 % of the variation in fruit and veg intake, whereas between-person fluctuations accounted for up to 0.21 % of the variation in fruit and veg intake.

Table 7.5 Multilevel modelling coefficients of psychological wellbeing predicting food sources of flavonoids

	Fruit and veg β (SE)	Tea β (SE)	Red wine β (SE)
Fixed effects			
Intercept	**320.39 (40.55) – 330.39 (40.58)*** **	83.15 (51.39) – 92.27 (51.85)	**20.59 (9.15) – 21.77 (9.02)***
Level 1 predictors			
Stress	8.02 (12.55)	12.72 (7.73)	0.75 (4.22)
Anxiety	–3.92 (14.08)	–5.50 (8.70)	–3.07 (4.73)
Positivity	–4.03 (15.55)	–9.95 (9.60)	6.97 (5.21)
Depression	–15.78 (19.17)	–3.32 (11.86)	–1.74 (6.45)
Energy	–9.17 (14.76)	–9.36 (9.11)	2.07 (4.96)
Fatigue	4.93 (12.42)	9.13 (7.66)	1.93 (4.17)
Level 2 predictors			
Stress	41.06 (36.19)	3.63 (46.15)	–1.31 (8.12)
Anxiety	47.52 (33.27)	–52.94 (42.13)	1.04 (7.57)
Positivity	–21.02 (32.77)	54.20 (40.84)	1.02 (7.34)
Depression	33.37 (50.49)	8.60 (63.84)	7.12 (11.30)
Energy	–40.19 (35.97)	23.42 (45.71)	–8.29 (8.06)
Fatigue	65.38 (38.17)	–57.30 (48.67)	12.32 (8.62)
Pseudo R^2			
R_1^2 (%)	0.92 – 6.39	–0.06 – 3.19	–0.34 – 7.16
R_2^2 (%)	0.02 – 0.21	0.02 – 0.82	0.01 – 0.53
Model fit			
AIC	5248.50 – 5251.05	4958.47 – 4961.18	4369.13 – 4371.29
-2 LL	5230.50 – 5233.05	4940.47 – 4943.18	4351.13 – 4353.29

n = 59. SE: standard error, veg: vegetables, AIC: Akaike's information criterion, LL: log likelihood. * p <.05, ** p <.01, *** p <.001. *Note*: all models were run separately for each predictor (within and between-person), so intercept, pseudo R^2 and model fit indices are given as a range. R_1^2 variance explained at level 1 (within-person) relative to the variance explained in the covariates only model, R_2^2 – variance explained at level 2 (between-person) relative to the variance explained in the covariates only model.

7.5. Discussion

The present study investigated the associations between stress and psychological wellbeing and dietary intake. Contrary to our hypothesis, perceptions of stress were not associated with the consumption of fat, saturated fat, and sugar. However, positivity and fatigue did associate with these macronutrients, with feeling less positive relating to higher consumption of saturated fat and sugar, and feeling more fatigued associating with increased fat consumption. Furthermore, stress was not related to fibre consumption (as an index of fruit and vegetable intake), but positively associated with flavonoid intake. Further analysis of the association between stress and sources of flavonoids did not indicate fruit and vegetables, tea, or red wine to drive this association. Our findings revealed that it is unclear how stress is associated with some dietary choices in a free-living environment, but the findings related to fat and sugar intake were not consistent with previous cross-sectional and laboratory research. However, psychological wellbeing outcomes, such as positivity and fatigue were associated with fat and sugar.

We hypothesised that experiencing stress would associate with increased consumption of fat and sugar, yet neither within-person nor between-person stress or anxiety predicted consumption of fat, saturated fat, or sugar. Previous laboratory studies have shown stress to influence eating behaviour, such as increased consumption of high-fat and high-sugar foods (Zellner et al., 2006). However, artificial environments with provision of specific foods likely moderated dietary choices (Hill et al., 2021). Notably, a similar laboratory study did not present this effect, and only when participants were separated based on emotional eating (assessed via an eating

behaviour questionnaire), was there a stress-induced increase in sweet and fatty foods in the emotional eating group (Oliver et al., 2000). Therefore, individual differences are likely to moderate this relationship, and these have been discussed previously (Hill et al., 2021). Furthermore, the context by which eating occurs can also influence the quantity and type of nutrients consumed (Ruddock et al., 2019). Therefore, future work should investigate contextual factors and how their fluctuation may influence diet choices.

The way in which stress is assessed or intervened may also mediate the relationship between stress and food choices. For example, laboratory-based studies used a stress task intervention, such as mental arithmetic and public speaking, delivering a stress/anxiety-inducing stimulus. However, the present study investigated people in their free-living environment, with no intervention or target of stressful events. It should be acknowledged that this data presented only 7 occurrences of participants reporting to feel ‘extremely’ (5/5) stressed and 5 occurrences of participants reporting to feel ‘extremely’ (5/5) anxious out of 413 completed time points. However, whilst there are few perceptions of extreme stress, the data includes fluctuations in stress perceptions across different days within each participant. Furthermore, this study assessed psychological wellbeing as overall stress/anxiety during the day, which may balance the acute stress perceptions. Previous daily diary studies reported significant associations between stress and eating behaviours yet assessed daily hassles (O'Connor et al., 2008, Zenk et al., 2014, Newman et al., 2006), which again may ascertain a greater and more acute stimulus of stress than experienced in the present study. Furthermore, given that stress associated with flavonoids but not fat and sugar in the present study, perhaps the drive to consume fat and sugar may require higher

levels of stress compared to flavonoids. Therefore, future research should investigate different time points of the day to include a greater variation in stress perceptions, including more extreme perceptions following higher levels of stress.

One daily diary study similarly investigated perceptions of stress, yet assessed cravings, hunger, and goal-congruent eating behaviour and found no associations with stress experienced across a day (Pannicke et al., 2021). Furthermore, previous research has reported stress to be associated with an increase in food consumed as snacks. For example, a 7-day diary study reports that experiencing at least 1 daily hassle associated with consuming more snacks between meals, and more snacks high in fat and sugar (O'Connor et al., 2008). Furthermore, the association between daily hassles and food intake through snacks is reported to be stronger when foods are easily available (Zenk et al., 2014). Therefore, perhaps nutrient intake specifically in snacks has a greater association with stress, and the availability of foods exacerbate the effect of stress on snack consumption. Unfortunately, the design of this study did not allow us to investigate nutrients consumed through snacks compared to main meals or assess the availability of these nutrients. However, future research is warranted to explore the effect of stress on dietary choices, in a free-living environment, and further consider nutrients consumed through snacking.

Fluctuations in positivity associated with saturated fat and sugar intake, whereby feeling less positive associated with increased consumption of saturated fat and sugar. The aversive state reduction hypothesis (Adam and Epel, 2007, Dallman et al., 2005) is a common explanation for stress-induced eating, which may underpin the relationship between positivity and these macronutrients. This hypothesis denotes that

when people are stressed, they consume highly palatable food (i.e., fat and sugar), which triggers a hedonic experience that reverses the aversive, negative feelings associated with stress (Pool et al., 2015). Saturated fat and sugar are highly palatable foods, perhaps consumed to trigger neurophysiological reactions which improve mood, to oppose the perceptions of reduced positivity experienced on an individual level in this study. This pattern of comfort eating in response to reduced psychological wellbeing has been reviewed and poses a serious threat to obesity (Dakanalis et al., 2023). Patterns of emotional eating have mostly been shown in response to negative psychological outcomes (e.g., depression, anxiety, stress) (Dakanalis et al., 2023), and thus, it is noteworthy that we present a significant inverse association with positivity, and not depression, anxiety, or stress. However, in the present study, positivity has a greater variability between days compared to these negative outcomes, perhaps revealing a more nuanced relationship with dietary intake.

The present findings revealed that between-person fatigue positively associated with fat consumption. Previous research has reported associations between fatigue and higher intake of fat in shift workers (Heath et al., 2016). Similarly, laboratory studies which restricted participants' sleep presented increased consumption of fat following sleep restriction (Spaeth et al., 2014, St-Onge et al., 2011). A possible explanation is the impairment in self-control and decision-making following sleep deprivation (Pilcher et al., 2015), which may disrupt dietary choices. Furthermore, lack of sleep and subsequent fatigue can negatively affect cognitive energy (Zohar et al., 2005). Therefore, in response to feeling fatigued, individuals may choose energy-dense foods (i.e., fat) to compensate for the subsequent depletion in cognitive energy. Notably, consumption of high-fat food increased perceptions of fatigue 3 hours post-intake

(Wells et al., 1997). Furthermore, our group have evidenced a greater mood disturbance following a high-fat meal, which was predominantly driven by an increase in fatigue (Baynham et al., 2023a). Therefore, interventions to disrupt this vicious cycle between fatigue and fat consumption should be explored.

We hypothesised that experiencing stress and poorer psychological wellbeing would associate with reduced consumption of fibre (an index of fruit and vegetable intake), given cross-sectional evidence showing higher perceived stress associated with reduced fruit, vegetable, and whole grain food intake (Groesz et al., 2012). Groesz and colleagues suggested this is in part a consequence of the reported increase in consumption of highly palatable food. However, as the present study revealed no association between stress and non-nutritious food (i.e., fat and sugar), perhaps it is unsurprising there was also no association with nutritious food (i.e., fibre). However, the present study did show a positive association between anxiety (between-person) and fibre intake. Given a previously observed inverse relationship between fibre consumption and anxiety outcomes (Aslam et al., 2023), this conflicting association requires further exploration.

Contrary to our hypothesis, the present study revealed within-person perceptions of stress associated with higher flavonoid intake. This contradicts previous cross-sectional research which reported higher consumption of fruit and vegetables (a source of flavonoids) to associate with lower perceived stress (Radavelli-Bagatini et al., 2022), and that green tea intake associates with reduced symptoms of anxiety (Mancini et al., 2017). Further analysis does not reveal which source of flavonoids could be driving this, as fruit and vegetable, tea, nor red wine intake significantly associated with stress.

It is important to acknowledge that flavonoids and red wine were the only dependent variables that exceeded the acceptable skewness range. However, multilevel modelling is more lenient to non-normally distributed data (Maas and Hox, 2004), and the flavonoid skewness in particular was only just outside of range. Given that flavonoids can protect vascular function from the deleterious effects of stress (Baynham et al., 2021), future work should aim to understand the relationship between stress and flavonoid consumption in a real-life setting. Furthermore, perhaps we are underpowered to show which source of flavonoids drives the relationship between stress and flavonoids. Therefore, future work should continue this investigation, and include a greater range of polyphenols in the analyses.

Individual differences have a substantial role in stress perceptions and subsequent eating behaviour (Hill et al., 2021), and thus, inclusion of demographic outcomes and covariates is a strength of this work. These findings presented gender differences, lower levels of daily stress and higher levels of daily energy in females compared to males. Whilst this gender disparity contradicts some research in university students, females utilised more coping mechanisms compared to males (Graves et al., 2021), perhaps reflecting their lower levels of stress in this study. Females also reported to consume more flavonoids and tea compared to males, which may be one of the coping mechanisms adopted during stress. BMI was inversely associated with fibre in all models, in line with previous research suggesting that diets rich in fibre prevent obesity (Waddell and Orfila, 2023). Finally, age was positively associated with fruit and vegetable and tea consumption, which is representative of a UK diet across ages (GOV.UK, 2020). Furthermore, the included sample was representative of the general UK population. For example, the average intake of saturated fat in UK adults (age 19

– 64 years) is 25.1 g (Scientific Advisory Committee on Nutrition, 2019) compared to 30.37 g in the present sample (age 18 – 36 years). Similarly, most UK adults consume an average of 18 – 20 g fibre per day, not dissimilar to the present sample (22.04 g), yet both lower than the recommendation (30 g /day) (National Health Service, 2022). Therefore, these findings have relevance for the general UK population.

7.5.1. Limitations

Whilst inclusion of demographic covariates is a strength, the recruited sample were young and healthy adults which lacks diversity in characteristics such as BMI, age, self-reported health, and perhaps psychological wellbeing. Therefore, future work should investigate the relationship between psychological wellbeing and diet in other populations, such as overweight, older adults, or perhaps patient populations. Furthermore, our sample size was moderate. Yet, the adherence rate was excellent, with 99.8 % of psychological wellbeing assessments and 99.3 % of food diaries completed across the 7-day data collection period, providing a high number of observations. Finally, data collection occurred during the COVID-19 pandemic. Lockdown status was subsequently included as a covariate in our analyses, yet it is important to note that psychological wellbeing and dietary habits were impacted by the pandemic (Smith et al., 2022b, Dicken et al., 2021). For example, whilst dietary intake was not influenced by lockdown status, participants that were in a lockdown during data collection reported to be somewhat more stressed, anxious and depressed, and have less energy compared to participants not experiencing a lockdown. Therefore, future work should continue to consider social isolation in the association between psychological wellbeing and dietary choices.

7.5.2. Future directions

The relationship between stress and nutrient intake in a free-living environment is not clear and hence future exploration of this relationship is warranted. Future research should consider the assessment of moderating variables such as emotional eating, attitudes towards eating, restrained eating, age, weight, and gender (Hill et al., 2021). Furthermore, social norms and the social and environmental context by which stress influences nutrient intake should be investigated (Higgs and Thomas, 2016). Future work should also aim to capture more occurrences of stress exposure across the day and gain a temporal resolution of when stress-induced eating happens. For example, by tracking physiological responses to stress (e.g., HR), as well as stress perceptions and nutrient intake at particular times of the day. As previously mentioned, stress may provoke changes to dietary intake through snacks rather than meals and so future work should assess nutrients consumed specifically from snacks as well as across the whole day. Finally, future work should also include assessments of all polyphenols given the link between flavonoids and stress.

Whilst the relationship between stress and dietary choices is not well understood, it is likely bidirectional. For example, changes in diet can influence mood via effects on the gut microbiome, the brain and inflammatory function, as well as neurotransmitters and neuropeptides (Bremner et al., 2020). More specifically, dietary fat has been implicated in the development of stress-related psychiatric disorders such as depression due to its interference with serotonin synthesis (Markowitz et al., 2008), meanwhile Mediterranean diets have been found to be beneficial on perceived stress and mood (McMillan et al., 2011, Bremner et al., 2020). Therefore, future work should explore

bidirectional associations between stress and dietary intake, to further understanding of this complex relationship.

7.6. Conclusion

The present study revealed that higher perceived stress was not associated with fat and sugar macronutrients. However, feeling less positive and more fatigued associated with consumption of these macronutrients. Stress positively associated with flavonoid consumption, yet the source of flavonoids driving this association is unknown. Both within and between-person associations emerged, with the intake of some nutrients varied with age and gender, presenting a nuanced relationship which requires further investigation. Whilst these findings are observational, they suggest a novel pattern of stress-induced dietary choices compared to previous cross-sectional and laboratory work and, hence, future work should consider the importance of conducting this type of research in free-living environments.

8. The bidirectional relationship between physical activity and stress-related psychological outcomes in healthy, young adults: A daily diary study

8.1. Abstract

Introduction: Physical activity could be an effective strategy to mitigate the negative effects of stress and improve stress-related psychological outcomes, such as our ability to cope with stress. This study investigated the bidirectional associations between stress-related psychological outcomes and physical activity and, explored whether engagement in physical activity was related to the ability to cope with stress.

Methods: Physical activity and stress-related psychological perceptions were collected using a daily diary in 67 young healthy participants over 7 days. Multi-level linear mixed models examined within-and between-person associations between stress-related mood and physical activity.

Results: Fifty-nine participants were included in analyses. Neither stress, anxiety or coping predicted engagement in physical activity. However, within-person daily perceptions of feeling on top of things were associated with higher levels of physical activity. Engagement in physical activity did not predict stress or symptoms of anxiety but, was associated with improved perceptions of coping and feeling on top of things.

Conclusions: Given the bidirectional relationship between physical activity and improved coping with stress and perceptions of feeling on top of things, these findings encourage the use of physical activity as a behavioural intervention to improve our ability to manage stress.

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8.2. Introduction

Stress is extremely prevalent in today's society, with a survey showing 74 % of people have felt so stressed they are unable to cope (Mental Health Foundation, 2018b). Too much stress or an inability to cope with stress can negatively affect our health. Consequences of stress include disruptions to brain function, cognition, the immune system, and cardiovascular health (Yaribeygi et al., 2017). Stress also increases the incidence of depression (Hammen et al., 2009), anxiety-disorders, substance use and suicidal thoughts (Schneiderman et al., 2005). Therefore, it is important to identify modifiable behaviours to mitigate the negative effects of stress on physical and psychological health.

Physical activity (PA) may be an effective strategy to mitigate the negative effects of stress, and improve stress-related outcomes such as anxiety, given its well-established benefits for physical (Anderson and Durstine, 2019) and psychological (Wipfli et al., 2008, Gianfredi et al., 2020) health in healthy and patient populations. Indeed, a systematic review reports improvements in cognitive and mental health in young people following PA interventions (Lubans et al., 2016) and PA is well-established to benefit cardiovascular health (Nystoriak and Bhatnagar, 2018).

PA may also reduce the negative effects of stress and anxiety on health. For example, there is a long-standing cross-stressor adaption hypothesis, that physically active individuals are more resilient, in terms of their physiological reactivity to acute psychological stress (Sothmann et al., 1996). However, when this hypothesis was tested in a real-life setting, it was found that exercise levels did not influence physiological responses to anxiety (Ensari et al., 2020). Several studies have

attempted to understand individuals' psychological state after engaging in PA, with laboratory-based studies showing that walking improves positive affect and promotes calmness and relaxation (Ekkekakis et al., 2000). Yet, the intensity of exercise appears to influence the psychological response, with lower mood during high-intensity compared to low-intensity exercise (Bixby et al., 2001). Similarly, longitudinal studies show that increasing PA on stressful days may buffer the adverse effects of stress on both positive and negative affect (Flueckiger et al., 2016). Finally, physically active individuals do not seem to experience the same stress-induced increase in negative affect shown in underactive individuals (Puterman et al., 2017).

The relationship between stress and PA is complex and likely bidirectional. There is evidence to suggest that during stressful periods, people are less likely to engage in PA (Teisala et al., 2014, Kouvonen et al., 2013). This is supported by Stults-Kolehmainen and Sinha's (2014) review evidencing stress to prospectively predict lower levels of PA. On the other hand, this review also found 17.2 % of studies to show a positive association between stress and PA, suggesting some individuals utilise PA to cope with stress, and 20.1 % of studies to report no association. Importantly, the studies in this review were predominantly cross-sectional, which overlooks the complex and daily fluctuation of both PA and stress, which may be further investigated with different designs.

The different methodologies used to investigate the bidirectional relationship between PA and stress are complementary in establishing a more comprehensive understanding and validation of results. For example, laboratory studies can elucidate the potential mechanisms linking PA and stress, yet are unlikely to be representative

of the PA behaviour performed in daily life, and such artificial settings may impact the psychological responses and even induce stress and anxiety (McAuley et al., 1996). Similarly, longitudinal and cross-sectional studies may be more representative of everyday life, with longitudinal methods having the advantage of tracking changes over long periods of time (Caruana et al., 2015) but both may overlook the daily fluctuations which are typical of PA and stress. Therefore, there is a need to understand individuals' psychological responses to PA, in their free-living environment and, notably, investigate whether there is a post-PA improvement in how individuals can deal with stress.

Daily diaries are a methodology that could be used to examine the bidirectional relationship between PA and stress. Daily diaries use repeated sampling of participant behaviour, feelings, and experiences in real-time, in participants' free-living environment (Shiffman et al., 2008). Capturing data in this way enables the consideration of both between-people (deviations from average group levels across all days) and within-people (deviation from day to day within individuals) associations across the same period (Dunton, 2017). Studies investigating the relationship between PA and stress using such designs have been reviewed (Wright et al., 2023), yet many of these studies do not analyse both between-person differences and within-person fluctuations. Furthermore, Wright et al.'s (2023) review highlights the inconclusiveness of research investigating the relationship between stress and PA, and the possible role of personal and contextual factors that may impact these associations. For example, gender is a factor previously shown to influence the relationship between stress and PA (Stults-Kolehmainen and Sinha, 2014). Similarly, habitual engagement in PA and chronic stress may also play a role in this relationship yet, to our knowledge, no daily

diary studies have examined interaction effects of habitual activity and stress on these associations. Therefore, the present study aims to elucidate these within-person and between-person associations, whilst considering the influence of personal factors (such as gender) and typical behaviour (such as PA and chronic stress). Finally, the majority of studies include stress as a single outcome (Wright et al., 2023). However, it is unlikely that engaging in PA will remove daily stressors, but rather may improve our ability to cope with stress (Childs and de Wit, 2014). For example, a qualitative study has shown that individuals use PA as a coping mechanism to deal with daily stress, as it promotes a sense of accomplishment, enhances confidence and stimulates thinking (White et al., 2023). Consequently, the current study investigated broader psychological outcomes such as anxiety and coping, to further investigate how PA is used to cope with stress in a quantitative and structured way, to better understand complexities within this relationship.

The present study aimed to investigate the association between stress/anxiety and PA and, determine whether PA engagement was related to the ability to cope with stress. It was hypothesised that while stress may be related to reduced PA behaviour, engagement in PA would be associated with reduced feelings of stress as well as greater ability to cope with stress. It was also hypothesised that typically active participants would have a stronger positive association between stress and PA engagement, and PA engagement and coping, compared to less active participants. Similarly, it was hypothesised that stress would be inversely related to PA in participants with higher habitual stress.

8.3. Methods and materials

8.3.1. Participants

Sixty-seven young, healthy adults were recruited via email advertisements. Inclusion criteria were i) aged 18 – 45 years old, ii) able to read English, and iii) no medically diagnosed mental health condition at the time of study participation. Ethical approval was obtained by a University Ethics Committee (ERN17_1755C), and informed consent was obtained online from all participants before enrolment in the study. Participants were awarded course credit marks where applicable.

8.3.2. Procedures

Data collection occurred between February 2021 – August 2021 using an online questionnaire platform (SmartSurvey). If eligible and consenting, participants completed an online questionnaire pack to report on demographic information and typical PA, psychological stress, dietary intake, and other psychological assessments (resilience scale, self-esteem scale, positive and negative affect scale; data not reported here). Following completion of these questionnaires, data collection commenced for 7 days, including 5 weekdays and 2 weekend days (Figure 8.1), starting on a Monday where possible. Each evening, participants received a link to an online questionnaire, which included stress-related mood assessments and recording of PA undertaken during the day.

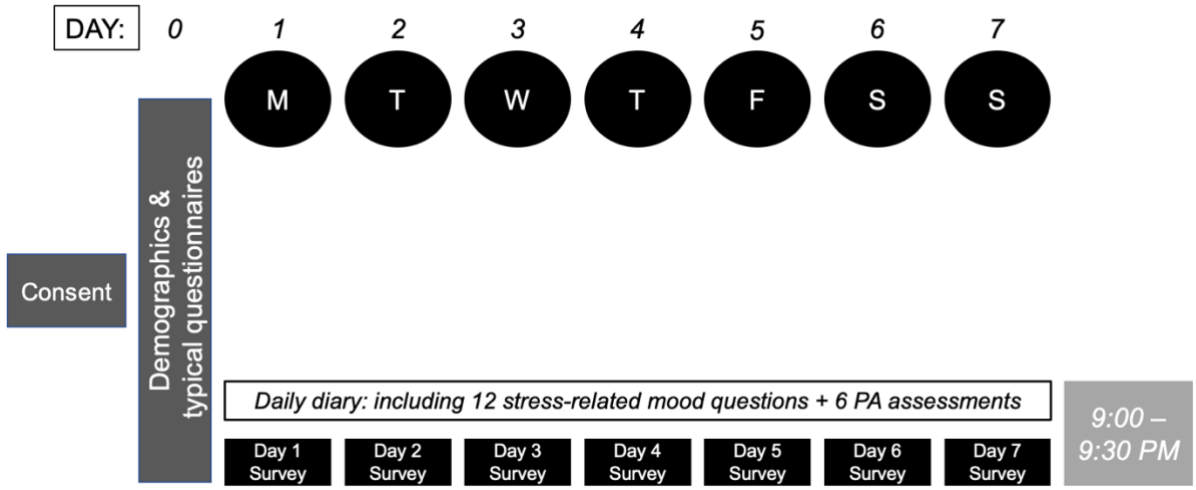

Figure 8.1 Study protocol

Day 0: Online questionnaire pack to obtain demographic and typical information.
Day 1 – 7: Daily diaries for stress-related mood assessments and PA engagement.

8.3.3. Outcome measures

8.3.3.1. Demographic information

Demographic information included questions on age, gender, ethnicity, nationality, height, weight, and general health (long-term conditions/diseases, alcohol consumption and smoking status data were not reported here). Terms ‘male’ and ‘female’ used in this paper refer to self-identified gender; 100 % of participants identified as male or female. Given the time of data collection, COVID-19 lockdown status was also reported by the participants and further checked retrospectively using the UK Government’s official lockdown dates and timestamps, and represented as ‘in a lockdown’ or ‘not in a lockdown’.

8.3.3.2. Typical stress

The 10-item Perceived Stress Scale (PSS) (Cohen et al., 1983) assessed how stressed participants felt over the last month. Each item (e.g., “How often have you felt nervous and stressed?”) was rated on a 5-point Likert scale from 0 (*never*) to 4 (*very often*). Positive items were reverse-scored, and a total score was calculated whereby higher scores indicate higher perceived stress. The scale demonstrated good internal reliability (α = 0.89).

8.3.3.3. Typical physical activity

The international physical activity questionnaire (IPAQ) (Craig et al., 2003) assessed typical PA. Participants were asked how much time in the last week they spent sitting, walking, and engaging in vigorous, moderate, and light PA. Answers were given in days, and more specifically hours and minutes. Scores were converted into a continuous score of total MET (metabolic equivalent) per week as per scoring guidelines (Forde, 2018), providing vigorous, moderate, walking and total MET scores. These total MET scores were divided by 7 to provide a mean PA (METs) per day. The IPAQ has previously shown good levels of validity (Craig et al., 2003).

8.3.3.4. Daily stress-related outcomes

One survey was sent daily (via text message or email, depending on participant preference) at approximately 9.00 – 9.30pm, which took less than 2 minutes to complete. The survey consisted of 12 individual items examining how stressed, positive, cheerful, angry, happy, anxious, calm, depressed, fatigued, and energetic the participant felt, and how well they felt they coped with stress, and how ‘on top of things’

they felt across the day. This was scored on a 5-point scale from 1 (*not at all*) to 5 (*extremely*). There was a (*prefer not to answer*) option. These items were developed by the research group, with the scale and some items based off the PSS. Four items were included in the analysis: (a) stress and (b) anxiety to investigate the association between stress/stress-related anxiety and PA (aim 1), and (c) coping and (d) on top of things, to determine whether PA relates to the ability to cope with stress (aim 2).

8.3.3.5. Daily physical activity

The evening survey also examined PA with 6 items assessing time spent in vigorous, moderate, and light PA, and time spent walking and sitting across the day. In line with the IPAQ, a brief description of activities reflective of these intensities was provided. Participants who had a fitness tracker also entered the number of steps taken that day (71 % participants; data not included). Scores were converted into a continuous score of total METs per day as per IPAQ scoring guidelines (Forde, 2018), providing a vigorous, moderate, walking and total MET score for each day.

8.3.4. Data analysis

After removal of 8 participants (n = 2 > 50 years, n = 1 current mental health condition, n = 1 not completed data collection, n = 4 not started data collection), 59 participants (30 female) were included in the analyses. As all other participants were between the ages of 18 – 36 years, 2 participants > 50 years were excluded so the sample represented young, healthy adults. All analyses were conducted using IBM SPSS Statistics for Windows (version 29.0). Intra-class coefficients (ICC) are reported for daily dependent variables, to show the variance in constructs within participants. One way ANOVAs assessed gender differences across all study variables.

Multi-level linear mixed models examined within-and between-person associations between daily mood and PA. In the first set of models, daily stress (1a), anxiety (1b), coping (1c), and on top of things (1d), and typical stress (PSS, all models) were added as separate predictors with total daily PA as outcomes. In the second set of models, total daily PA and typical PA (IPAQ) were added as predictors with perceptions of stress (2a), anxiety (2b), coping (2c), and on top of things (2d) as separate outcomes. Within-person predictors were person-centred, reflecting variations of a 'daily' assessment point relative to the person's mean value (level 1). At level 2, the 7-day average daily assessments were entered as between-person (grand sample centred), providing 'typical' (or average across the data collection period) levels. Typical PA (IPAQ) and typical stress (PSS) were also entered at level 2 and were grand sample centred. Age, gender, BMI, and COVID-19 status (in lockdown / not in lockdown) were also entered at level 2 as covariates in all models. Two interactions were included in each set of models, investigating the interaction between each predictor and 1) typical stress (PSS) and 2) typical PA (IPAQ). For all models the intercepts were random, and the slopes were fixed. Multilevel models' $\beta \pm SE$ are reported in the text. For all analyses, significance was set at $p < .05$.

To calculate the amount of variance in each dependent variable explained by within- and between-person predictors, a series of models were analysed. The pseudo-R squared models investigated the amount of variance in the dependent variable explained by within-person predictors (R_1^2) and between-person predictors (R_2^2), relative to the empty model (model with covariates only) (Hox et al., 2018).

8.4. Results

8.4.1. Overview of results

Participants completed 412 (99.8 %) of 413 questions assessing mood over the 7-days, 100 % days of PA with 411 (99.5 %) of 413 light PA recordings and 100 % of vigorous, moderate, and walking recordings. The skewness scores for the dependent variables of total PA (1.52) and mood (between –0.68 and 1.77) were within an acceptable range (skewness ± 2) (Gravetter and Wallnau, 2016).

Participant characteristics, daily and typical PA and mood, and PA in minutes and METs are displayed in Table 8.1. Participants were mostly British (81.3 %) and of white ethnicity (88.1 %). Walking made up the largest proportion of total daily PA/METs (53.1 %), followed by vigorous (30.5 %) and then moderate (16.4 %) PA. For subsequent analyses, total PA in METs is used to provide a single outcome of PA.

Table 8.1 Descriptive Statistics and Intraclass Correlation Coefficients for Study Variables

Demographics	M ± SD	ICC	Minimum	Maximum
Age (years)	24.24 ± 3.57	-	18.00	36.00
BMI (kg/m^2)	23.35 ± 2.62	-	19.86	32.02
Health rating /5	3.39 ± 1.03	-	1	5
Gender (n (%))	Female: 30 (51%)			
	Male: 29 (49%)			
Daily PA				
Vigorous MIN/day	20.06 ± 30.12	.193	0	180.00
Moderate MIN/day	21.62 ± 36.73	.189	0	240.00
Light MIN/day	29.72 ± 47.37	.443	0	360.00

Walking MIN/day	84.90 ± 78.86	.513	0	600.00
Vigorous MET/day	160.46 ± 240.94	.193	0	1440.00
Moderate MET/day	86.48 ± 147.04	.189	0	960.00
Walking MET/day	280.16 ± 260.22	.513	0	1980.00
Total MET/day	527.15 ± 388.45	.396	0	2388.00
Daily Mood				
Stress /5	2.08 ± 1.04	.278	1	5
Anxiety /5	2.07 ± 0.98	.361	1	5
Coping /5	3.91 ± 0.97	.406	1	5
On top of things /5	3.56 ± 1.01	.367	1	5
Typical PA (IPAQ)				
Vigorous MET/day	188.28 ± 162.31	-	0	577.14
Moderate MET/day	54.33 ± 77.83	-	0	411.43
Walking MET/day	1145.39 ± 695.52	-	132.00	2772.00
Total MET/day	375.73 ± 248.34	-	34.29	1081.71
Typical stress (PSS)				
PSS /40	17.61 ± 6.33	-	4.00	32.00

n = 59. Note: PA: Physical activity, BMI: Body mass index, MIN: Minutes, MET: Metabolic equivalent, IPAQ: International physical activity questionnaire, PSS: Perceived stress scale, M: Mean, SD: Standard deviation, ICC: Intraclass correlation coefficient.

8.4.2. Gender differences

Gender differences are reported in Table 8.2. Females were younger, had a lower BMI and reported a higher health rating, compared to males. Females reported higher levels of daily moderate and light PA compared to males, and less stress, higher levels of coping and feeling more on top of things compared to males. Females reported higher levels of typical vigorous, moderate, walking, and total PA, and typical stress (PSS), compared to males.

Table 8.2 Gender Differences in Study Variables

Demographics	Males (M ± SD)	Females (M ± SD)	Difference*
Age (years)	24.76 ± 3.65	23.73 ± 3.43	.003
BMI (kg/m^2)	24.07 ± 2.76	22.65 ± 2.28	<.001
Health rating /5	3.28 ± 095	3.50 ± 1.09	.026
Daily PA			
Vigorous MIN/day	19.76 ± 32.43	20.34 ± 27.78	.845
Moderate MIN/day	17.73 ± 32.80	25.38 ± 39.94	.035
Light MIN/day	19.68 ± 37.44	39.62 ± 53.63	<.001
Walking MIN/day	81.83 ± 84.81	87.86 ± 72.72	.438
Total MET/day	499.13 ± 396.03	554.24 ± 379.96	.150
Daily Mood			
Stress /5	2.27 ± 1.06	1.90 ± 0.98	<.001
Anxiety /5	2.00 ± 0.94	2.13 ± 1.02	.153
Coping /5	3.77 ± 1.00	4.05 ± 0.92	.004
On top of things /5	3.41 ± 1.06	3.70 ± 0.94	.003
Typical PA (IPAQ)			
Vigorous MET/day	150.15 ± 156.58	225.14 ± 159.56	<.001
Moderate MET/day	35.47 ± 59.97	72.57 ± 88.23	<.001
Walk MET/day	1039.5 ± 631.46	1227.74 ± 732.63	.014
Total MET/day	293.15 ± 224.97	455.56 ± 244.21	<.001
Typical Stress (PSS)			
PSS /40	15.90 ± 6.02	19.27 ± 6.20	<.001

Note: PA: Physical activity, BMI: Body mass index, MIN: Minutes, MET: Metabolic equivalent, IPAQ: International physical activity questionnaire, PSS: Perceived stress scale, M: Mean, SD: Standard deviation. * P value from ANOVAs.

8.4.3. Stress-related psychological outcomes predicting physical activity

Table 8.3 depicts multilevel models with perceptions of stress, anxiety, coping, and feeling on top of things predicting total daily PA (METs). Total PA in METs is used as a single outcome of PA to reduce the number of analyses. The analyses using individual PA intensities were similar.

In the stress model, within-person (level 1) and between-person (level 2) daily stress did not significantly predict total PA. However, typical stress (PSS, level 2) negatively predicted total PA (–179.48 ± 64.37), whereby a 10-point lower typical stress score (for example moving from high to moderate perceived stress) predicts 179 METs more daily PA (p =.007), which is approx. 22 minutes of vigorous PA or 54 minutes of walking. In the on top of things model, within-person (level 1) daily perceptions of feeling on top of things positively predicted total PA (44.25 ± 20.55), whereby feeling more on top of things compared to your own average predicted 44 METs more daily PA (p =.032). Between-person (level 2) perceptions of feeling on top of things, nor typical stress, did not significantly predict total daily PA. In the coping and anxiety model, within-person and between-person perceptions of coping[1] and anxiety, and typical stress, did not significantly predict total PA.

Despite significant associations of both between-person (level 2) variables (typical stress, PSS) and within-person (level 1) variables (daily on top of things), the amount of variance explained by within-person fluctuations (i.e., R_1^2) was substantially higher

[1] When the coping model is run without PSS as a level 2 predictor, between-person coping significantly predicts total PA (102.21 ± 50.19, p =.034).

than the amount of variance explained by between-person differences (i.e., R_2^2) in each of the outcome measures. For example, within-person fluctuations in feeling on top of things accounted for 18.53 % of the variation in PA, whereas between-person differences in mood indices only accounted for 1.39 % of the variation in PA.

Consistently in each model, BMI positively predicted total daily PA, whereby 1 kg/m^2 increase in BMI predicts 38.57 $\pm$ 13.75 METs more PA in the stress model (p =.007), 39.03 $\pm$ 13.89 METs more PA in the anxiety model (p =.007), 37.10 $\pm$ 13.88 METs more PA in the coping model (p =.010), and 34.56 $\pm$ 13.83 METs more PA in the on top of things model (p =.015). No other covariates significantly predicted total daily PA in any of the models. A 30 MET increase in PA is the equivalent of approx. a 9-minute walk, 8 minutes of moderate PA and 4 minutes of vigorous PA.

Table 8.3 Multilevel Modelling Coefficients of Stress-related Psychological Outcomes Predicting Total Daily Physical Activity

	Total PA β (SE)			
Fixed effects	(1a) Stress	(1b) Anxiety	(1c) Coping	(1d) On top of things
Intercept	**506.55 (64.66)*** **	**519.50 (64.01)*** **	**535.45 (65.33)*** **	**548.01 (64.96)*** **
Covariates				
Age	–12.13 (11.37)	–10.04 (11.31)	–8.13 (11.36)	–6.21 (11.29)
Gender	153.04 (79.63)	125.03 (75.57)	98.42 (79.32)	80.50 (78.76)
BMI	**38.57 (13.75)** **	**39.03 (13.89)** **	**37.10 (13.88)* **	**34.56 (13.83)* **
COVID-19 status	–116.19 (70.27)	–106.45 (70.59)	–107.47 (69.69)	–108.27 (68.72)
Level 1 predictors				
Within-person daily mood	–6.73 (18.97)	–3.91 (21.28)	14.59 (22.83)	**44.25 (20.55)* **
Level 2 predictors				
Between-person daily mood	62.06 (62.22)	–11.28 (63.39)	58.24 (56.43)	93.49 (57.29)
PSS	**–179.48 (64.37)** **	–141.37 (70.86)	–115.06 (65.33)	–92.32 (65.63)
Pseudo R^2				
R_1^2 (%)	15.71	13.80	15.82	18.53
R_2^2 (%)	0.04	0.01	0.12	1.39

Model fit				
AIC	5599.63	5600.68	5599.28	5593.53
-2 LL	5579.63	5580.68	5579.28	5573.53

n = 59. Note. PA: Physical activity, SE: Standard error, BMI: Body mass index, PSS: Perceived stress scale, AIC: Akaike's information criterion, LL: Log likelihood. R_1^2 – variance explained at level 1 (within-person) relative to the variance explained in the covariates only model, R_2^2 – variance explained at level 2 (between-person) relative to the variance explained in the covariates only model. PSS estimates multiplied by 10 in line with total PSS score (/40). * p <.05, ** p <.01, *** p <.001.

8.4.4. Physical activity predicting stress-related psychological outcomes

Table 8.4 depicts multilevel models with total PA predicting perceptions of stress, anxiety, coping, and feeling on top of things. For this model, within-person and between-person daily PA, as well as typical PA (IPAQ), scores have been divided by 100. This makes the data more meaningful, as a 1 MET increase in PA equates to only 18 seconds of walking, 15 seconds of moderate and 8 seconds of vigorous PA. To put this into context, a 100 MET increase in daily PA, could be characterised by 30 minutes of walking, 25 minutes of moderate PA, and 13 minutes of vigorous PA per day. All mood outcomes are scored on a scale of 1 to 5, and so a 1 unit increase in coping for example shifts coping from '*not at all*' to '*a little*' or from '*quite a lot*' to '*extremely*'.

Within-person (level 1) and between-person (level 2) total PA and total typical PA (IPAQ, level 2) did not predict daily symptoms of stress or anxiety. Between-person (level 2) total PA significantly predicted perceptions of coping (0.07 $\pm$ 0.03, p =.036). This means 100 METs more daily PA compared to the group average predicts a 0.07 unit increase in perceptions of coping with stress. Both within-person (0.03 $\pm$ 0.02, p =.032) and between-person (0.09 $\pm$ 0.03, p= .008) total PA significantly predicted feeling on top of things. In other words, doing 100 MET more daily PA compared to your own average predicts a 0.03 unit increase in feeling on top of things. Similarly, doing 100 MET more daily PA compared to the group average predicts a 0.09 unit increase in feeling on top of things.

As in our previous model, the amount of variance explained by within-person (level 1) fluctuations (R_1^2) was substantially higher than the amount of variance explained by between-person (level 2) differences (R_2^2) in each of the outcome measures. For

example, between-person differences in PA indices accounted for 0 % of the variance in stress and anxiety, yet within-person fluctuations in PA accounted for 6.15 % – 18.13 % of the variance in mood indices.

None of the covariates (age, gender, BMI, and COVID-19 status) significantly predicted the mood perceptions in any model.

Table 8.4 Multilevel Modelling Coefficients of Physical Activity Predicting Daily Stress-related Psychological Outcomes

	(2a)	*(2b)*	*(2c)*	*(2d)*
Fixed effects	Stress β (SE)	Anxiety β (SE)	Coping β (SE)	On top of things β (SE)
Intercept	**2.13 (0.15)*** **	**1.92 (0.16)*** **	**3.79 (0.16)*** **	**3.41 (0.16)*** **
Covariates				
Age	0.02 (0.03)	–0.01 (0.03)	–0.02 (0.03)	–0.02 (0.03)
Gender	–0.31 (0.18)	0.17 (0.19)	0.25 (0.20)	0.25 (0.19)
BMI	0.05 (0.03)	0.05 (0.04)	–0.03 (0.04)	–0.02 (0.03)
COVID-19 status	0.30 (0.18)	0.21 (0.19)	–0.07 (0.20)	–0.05 (0.19)
Level 1 predictors				
Within-person daily total PA (METs)	–0.01 (0.02)	–0.00 (0.01)	0.01 (0.01)	**0.03 (0.02)***
Level 2 predictors				
Between-person daily total PA (METs)	–0.01 (0.03)	–0.05 (0.03)	**0.07 (0.03)***	**0.09 (0.03)** **
Total PA/day (IPAQ)	0.06 (0.04)	0.05 (0.04)	–0.04 (0.04)	–0.05 (0.04)
Pseudo R^2				
R_1^2 (%)	6.15	8.90	11.41	18.13
R_2^2 (%)	0	0	0.18	1.51

Model fit				
AIC	1080.51	1011.79	967.19	1023.99
-2 LL	1060.51	991.79	947.19	1003.99

n = 59. Note: PA: Physical activity, SE: Standard error, BMI: Body mass index, MET: Metabolic equivalent, IPAQ: International physical activity questionnaire, AIC: Akaike's information criterion, LL: Log likelihood. R_1^2 – variance explained at level 1 (within-person) relative to the variance explained in the covariates only model, R_2^2 – variance explained at level 2 (between-person) relative to the variance explained in the covariates only model. * p <.05, ** p <.01, *** p <.001.

8.4.5. Interactions

8.4.5.1. Model 1: stress-related psychological outcomes predicting physical activity

Interactions were added to multilevel models of perceptions of stress, anxiety, coping, and feeling on top of things predicting total daily PA. Two interactions were included in each model: 1) an interaction between the within-person daily Mood Symptom × Typical Stress (PSS), and 2) an interaction between the within-person daily Mood Symptom × Typical PA (IPAQ), both predicting total daily PA. There was a significant interaction between within-person Daily Anxiety × Typical Stress (PSS) predicting total daily PA (–7.36 ± 3.47, p =.034), whereby individuals with higher typical stress, had a negative relationship between daily anxiety and total daily PA. Therefore, if participants with higher typical stress have more daily anxiety, they will do less daily PA compared to individuals with lower typical stress. No other interactions predicted total daily PA in any model.

8.4.5.2. Model 2: physical activity predicting stress-related psychological outcomes

Interactions were added to multilevel models of physical activity predicting stress, anxiety, coping, and feeling on top of things. Two interactions were included in each model: 1) an interaction between within-person daily Total PA × Typical PA (IPAQ), and 2) within-person daily Total PA × Typical Stress (PSS). There was a significant interaction between within-person daily Total PA × Typical Stress predicting daily anxiety (–0.05 ± 0.02, p =.027), whereby individuals with a higher typical stress, have a negative relationship between daily PA and anxiety. Therefore, if participants with higher typical stress do more daily PA, they will have a lower anxiety compared to

those who have a lower typical stress. No other interactions were significant in any model.

8.5. Discussion

The present study examined the bidirectional relationship between stress and PA in a young healthy population. Contrary to our hypothesis, neither stress, anxiety, nor coping predicted engagement in PA. However, within-person daily perceptions of feeling on top of things was associated with higher levels of total PA. We further hypothesised that engagement in PA would associate with an improved ability to cope with stress. Our findings reveal that whilst engaging in PA did not predict stress, or symptoms of anxiety, as hypothesised, PA was associated with improved perceptions of coping and feeling on top of things.

8.5.1. Model 1: The associations between stress-related psychological outcomes and PA

We hypothesised that experiencing stress would associate with a reduction in PA, yet neither within-person nor between-person stress predicted daily PA in this study. However, a review of previous daily diary studies investigating this relationship presented mixed findings (Wright et al., 2023). One study depicted that higher stress associated with reduced PA in the subsequent 2.5 hours (Schultchen et al., 2019). However, Schultchen et al. (2019) assessed lagged, within-person associations, whereas the current study investigated concurrent associations, within and between-person, which may explain the discrepancy in findings. Furthermore, the type and intensity of PA (Jones et al., 2017), assessment of stress (Almeida et al., 2020) and individual characteristics, such as coping and exercise behaviour, exercise beliefs and

affect (Stults-Kolehmainen and Sinha, 2014, Wright et al., 2023), are likely to influence this relationship, and future work should assess these moderators. In the present study, typical stress significantly predicted PA, whereby reporting higher perceived stress scores compared to the rest of the sample was associated with lower levels of PA. Cross-sectional research has similarly evidenced a negative association between typical stress and PA (Yoon et al., 2023). Excessive fatigue, emotion-focused coping, and declines in psychological and physical function have been suggested as potential mechanisms (Stults-Kolehmainen and Sinha, 2014). In summary, future exploration of the role of typical stress, as well as daily fluctuations, in stress-induced PA behaviour is warranted.

The novelty of this study is the inclusion of other psychological outcomes which relate to stress. As with stress, daily coping, and anxiety (within and between-person) did not predict PA. An inverse and bidirectional association between PA and anxiety has been corroborated previously (Azevedo Da Silva et al., 2012). However, most evidence used cross-sectional surveys without considering daily fluctuations (Ströhle, 2009) and included samples with mental health conditions (Saxena et al., 2005). Furthermore, Ströhle's (2009) review predominantly illustrates the association between general PA engagement and the development or prevention of mental health disorders (Ströhle, 2009). As the current study excluded mental health conditions, and investigated daily fluctuations in symptoms of anxiety, the relationship between PA and anxiety is likely to be different.

On the other hand, perceptions of feeling on top of things did positively predict total PA, whereby feeling more on top of things compared to your own average associated

with more daily PA. To our knowledge, only one previous study has assessed feeling on top of things, using the first two items of the PSS as daily diary assessments (Schultchen et al., 2019). However, we are the first study to include and report perceptions of feeling on top of things as a separate outcome in the results. A review depicts the disparity in associations between stress and PA, with most studies reporting a negative association, yet some showing no association or even a positive association between stress and PA (Stults-Kolehmainen and Sinha, 2014). Perhaps the terms used to assess stress, and how people cope with stress, may explain the discrepancy in these findings. For example, previous daily diary studies have included daily hassles, social stressors, emotional tension, job demands, and perceptions of stress (Wright et al., 2023). In the present study, perceptions of stress negatively associated with feeling on top of things ($p < .001$), which illustrates the relationship between these variables, and may mediate the relationship between stress and PA. A lack of time and fatigue are reported as the leading two barriers to PA (Koh et al., 2022). Hence, perhaps feeling more on top of things provides additional time and energy to be physically active. These findings suggest that it is not the amount of stress that relates to PA, but how individuals feel they are able to deal with it that may be important. Therefore, to increase PA engagement, future interventions could target people's ability to cope with stress, rather than attempting to reduce stress itself.

8.5.2. Model 2: The associations between PA and stress-related psychological outcomes

Our second aim was to investigate whether PA related to coping with stress. Findings showed that daily PA did not predict perceptions of stress or anxiety. Previous studies

have investigated associations between PA and stress but had inconsistent findings (Wright et al., 2023). For example, between-person walking was associated with lower stress at bed time (Hallman and Lyskov, 2012), and within-person PA associated with lower subsequent stress up to one day later (Abdel Hadi et al., 2022, daSilva et al., 2021, Schultchen et al., 2019, Park et al., 2022), while other studies found no between-person (daSilva et al., 2021, Jones et al., 2017) or within-person (Lee et al., 2022, Sala et al., 2017, Smith et al., 2021) associations. However, these daily diary studies used prospective analyses. Similar to our findings, previous investigation of concurrent associations between PA and stress showed no within or between-person significances (Anderson and Fowers, 2020, Igic et al., 2013, Dalton, 2022, Li et al., 2020, Strahler et al., 2020, Zawadzki et al., 2015). Given that stress is often characterised as 'daily hassles' or 'stressful events' (Buccheri et al., 2018), perhaps it is unlikely that engaging in PA will alter the occurrence of these hassles or events.

The present study investigated people in their free-living environment, with no intervention or target of stressful events (e.g., exam periods, shift work). Consequently, the study had a marginal spread of stress perceptions, with only 7 occurrences of participants reporting to feel 'extremely' (5/5) stressed out of 413 completed time points. Symptoms of anxiety had a similarly moderate spread, with only 5 occurrences of participants feeling 'extremely' anxious. Therefore, perhaps the present study lacks the fluctuation in stressful events, and perceptions of anxiety, to discover more nuanced aspects of the relationship with PA.

The novelty of the present study is the assessment of other psychological outcomes. Within-person and between-person PA predicted feeling more on top of things such

that higher engagement in PA in comparison to your own average, and to the study sample mean, was associated with feeling more on top of things. Between-person PA also positively predicted perceptions of coping with stress. Hence, engaging in PA may not reduce stress but rather improve our ability to cope with stress. These positive psychological outcomes (coping and on top of things) have a greater spread of data compared to negative outcomes (stress and anxiety), perhaps explaining their significant relationship with PA. To our knowledge, other studies have not investigated such stress-related outcomes (Wright et al., 2023). Schultchen et al. (2019) included daily measures of coping and feeling on top of things, but these were not discussed in the results. Given there is minimal previous evidence investigating these psychological outcomes, our conclusions are exploratory. However, participants undertaking an 8-week exercise intervention (Zhang et al., 2019) and in the highest quartile for PA (Wu et al., 2022) have better emotion regulation ability compared to no intervention and the low PA group. Therefore, future research should investigate the benefit of PA on stress perceptions and coping ability using real-life designs.

The current study also included interaction analyses to assess the role of typical stress and PA in the bidirectional relationship between PA and stress. In line with our hypothesis, the findings presented a significant interaction between daily anxiety and typical stress predicting PA, whereby in participants with higher typical stress, increased anxiety associated with reduced PA, compared to participants with lower typical stress. This emphasises the role of typical behaviour and individual differences in the PA and stress relationship, which has been discussed previously (Wright et al., 2023). Furthermore, whilst PA alone did not predict anxiety, there was a significant interaction between daily PA and typical stress predicting anxiety. Thus, in participants

with higher typical stress, an increased engagement in PA associated with reduced anxiety, compared to participants with lower typical stress. This perhaps suggests PA may have a greater benefit for people experiencing higher typical stress. Furthermore, given that stress relates to low levels of PA in chronically stressed populations (Stults-Kolehmainen and Sinha, 2014), future research should investigate the role of typical stress in this relationship. Contrary to our hypothesis, typical PA had no effect on these associations. However, given the included sample had a normal BMI and reported to be relatively healthy, perhaps our sample lacked the range in typical PA engagement and, thus, future research should continue to investigate the role of typical PA behaviour.

Initial analyses presented gender differences, whereby females reported greater PA engagement compared to males. This gender disparity is inconsistent (Gomez et al., 2021, Craft et al., 2014) yet, it has been suggested that females are more likely to over-report PA levels (Prince et al., 2008). In the present study females reported higher typical stress, yet lower daily stress and increased perceptions of feeling on top of things and coping, in line with previous research showing female students to report increased stress yet to use coping strategies more often than males (Graves et al., 2021).

8.5.3. Strengths and implications

Whilst previous research has investigated the impact of stress on PA behaviour, we show for the first time in a real-life setting that engagement in PA positively associated with how we cope with stress. Previous stress-related research has commented on the evolutionary purpose of stress and our subsequent homeostatic response (O'Connor

et al., 2021) thus, making it impossible to eradicate. However, using PA to improve our ability to manage stress, could be a significant behavioural intervention. Another strength is the use of daily diaries to explore these relationships in real-life settings. Although we must be cautious given the observational and self-reporting nature of these findings, our study suggests that PA is likely to improve psychological outcomes related to stress, and future research should investigate this by combining both laboratory-based RCTs and daily diary designs.

8.5.4. Limitations

Data collection occurred during the COVID-19 pandemic. Whilst this has been considered, with lockdown status included as a covariate in our analyses, stress perceptions and the opportunity to be physically active were impacted by the pandemic (Puccinelli et al., 2021). For example, participants that were in a lockdown during data collection reported to engage in less vigorous, light, and total PA compared to participants not in a lockdown. Similarly, participants in a lockdown reported to be more stressed and anxious compared to those not experiencing a lockdown. Therefore, future work should investigate the role of social isolation in the relationship between stress and PA. Furthermore, our sample size was moderate. However, the adherence rate was excellent, with 99.8 % completion of daily mood assessments and 100 % completion of daily PA recordings, providing a high number of observations across the 7-day data collection period. Finally, the recruited sample were young and healthy adults which lacks diversity in characteristics such as BMI, age, health, and perhaps psychological perceptions. For example, BMI positively predicted total PA in each model, yet cross-sectional research illustrates an inverted-U relationship between BMI

and physical fitness in students (Qin et al., 2022). As the present sample had a healthy BMI (approx. 23 kg/m^2), they are not in the BMI range where PA engagement is likely lower (Hemmingsson and Ekelund, 2007). Therefore, future work should investigate the relationship between PA and psychological outcomes in a more representative sample, such as those with an overweight/underweight BMI.

8.6. Conclusion

This study showed that higher typical stress associated with lower PA engagement, yet feeling more on top of things predicted higher levels of PA. Furthermore, engaging in PA positively predicted feeling on top of things and perceived coping. Both within and between-person associations emerged, presenting the nuanced and bidirectional relationship between PA and stress-related psychological outcomes. Whilst these findings are observational, they encourage the use of PA as a behavioural intervention to improve our ability to manage stress.

9. General Discussion

9.1. Overview of findings

Stress can worsen health, by inducing transient impairments in endothelial function and influencing behaviours such as dietary choices and engagement in physical activity. The primary aim of this thesis was to assess the impact of health behaviours (namely diet) on vascular function in the context of mental stress. The findings from this thesis suggest that healthy/unhealthy dietary choices during stress can modify the effects of stress on human vascular function. Specifically, it was shown that:

- Saturated fat consumption can impair the recovery of endothelial function following stress and attenuate cerebral oxygenation during stress.

- Consuming flavonoid-rich cocoa with a meal high in saturated fat can counteract the negative impact of fat consumption on the stress-induced decline in endothelial function, but does not influence cerebral oxygenation during stress following fat consumption.

The second aim of this thesis was to explore the relationship between stress and health behaviours (e.g., diet and physical activity) in a free-living environment. Specifically, it was found that:

- Stress positively associates with flavonoid consumption, yet it is unknown what flavonoid source drives this relationship. There were no consistent associations between stress and fat, saturated fat, and sugar consumption, yet lower positivity and higher fatigue were related to increased consumption of these macronutrients.

- There were no associations between stress and engagement in physical activity. However, engagement in physical activity (PA) associated with improved perceptions of coping with stress and feeling more on top of things.

Therefore, these findings suggest that health behaviours adopted during periods of stress may influence the impact of stress on the vasculature and modify how we psychologically cope with stress.

9.2. Implications of consuming fat during periods of mental stress

The separate effects of stress and fat consumption on endothelial function have been documented previously (Ghiadoni et al., 2000, Vogel et al., 1997). Changes in autonomic activity contribute towards NO-mediated vasodilation during stress (Dietz et al., 1994) yet following stress, elevations in cortisol and inflammatory markers reduce NO bioavailability leading to stress-induced endothelial dysfunction (Broadley et al., 2005, Steptoe et al., 2007, Poitras and Pyke, 2013). To our knowledge, this thesis includes the first investigation of the combined effect of fat consumption and stress on the peripheral vasculature. Chapter 4 demonstrates that whilst fat consumption did not exacerbate the effect of mental stress on endothelial function (FMD), the recovery of endothelial function was impaired. The mechanisms by which fat consumption delays the recovery of FMD following stress are not known, but increases in TAG (as reported in Chapter 4) have been shown to increase oxidative stress, stimulate ET-1 and inflammatory markers, and subsequently reduce NO and impair endothelial function (Bae et al., 2001, Tsai et al., 2004, Man et al., 2020). Chapter 5 reports that fat consumption attenuated the increase in cerebral oxygenation during stress. The implications of these findings are discussed below.

FMD has become increasingly popular in health-related research, as it is predictive of future cardiovascular events in healthy individuals and those with established CVD (Green et al., 2011, Inaba et al., 2010). Therefore, the prolonged impairment in brachial FMD induced by consuming two croissants prior to mental stress may be significant to vascular health. Importantly, we reported this prolonged endothelial dysfunction in young, healthy individuals. It has been well-established that baseline FMD is lower in at-risk populations such as those suffering from obesity and metabolic syndrome (Suzuki et al., 2008), Type 1 Diabetes Mellitus (Lockhart et al., 2011), hypertension (Cetin et al., 2020), and CVD (Inaba et al., 2010). There is also evidence that people with lower FMD have poorer vascular responses to mental stress (Sherwood et al., 1999), so the fat-induced delay in endothelial recovery could be even greater in such populations. Therefore, this should be explored as an area for future research. Future research should, however, consider the most appropriate low-fat intervention as a comparison to the saturated fat intervention. For example, these should be calorie-matched, yet in order to achieve this, the carbohydrate content of the low-fat meal is then often higher than the high-fat meal, as shown in Chapter 4. Carbohydrate ingestion has also been reported to impact vascular function, as the acute increase in insulin can activate eNOS and produce NO, as well as stimulate ET-1 from the endothelium (Gheibi et al., 2020). Thus, future work should continue to investigate the mechanistic effects of carbohydrates on endothelial function and consequently use a low-fat control intervention with a nutrient composition closely matched to the high-fat meal.

Less research has been conducted in exploring cerebral blood flow and/or oxygenation during stress. It is plausible that the reductions in cerebral oxygenation may impact

cognitive performance. For example, decreases in executive function have been shown to correlate with reduced cerebral oxygenation during normobaric hypoxia (Williams et al., 2019). As there were no changes in cortisol, adrenaline, or noradrenaline in this study, reductions in peripheral oxygen saturation and cerebral oxygenation are thought to be responsible for the decrease in cognitive performance, rather than changes in activity of the sympathoadrenal system and HPA axis. However, there is evidence to suggest that glucocorticoid hormones released during stress can also impair cognitive performance, with noradrenaline and cortisol having an additive effect on the decrease in central executive function (Elzinga and Roelofs, 2005). Given executive functions are necessary for the memory, thinking, and control of behaviour (Friedman and Robbins, 2022), these findings could have implications on real life stressful situations, whereby individuals rely on executive function to perform (e.g., at an interview, public presentation or examination). Furthermore, the clinical relevance of an attenuated cerebral oxygenation should be investigated in more detail. One study reported lower cerebral tissue oxygenation in heart failure patients at both rest and during an orthostatic challenge (Kharraziha et al., 2021). Similarly, recent evidence has suggested a role for cerebrovascular dysfunction in the pathogenesis of Alzheimer's disease and dementia (Wang et al., 2022). Therefore, future research should utilise other assessments of cerebral blood flow and function such as magnetic resonance imaging (MRI), transcranial doppler, and electroencephalography (EEG), to understand the consequences of an attenuated cerebral oxygenation and/or blood flow during stress, and whether this is more severe for individuals at higher risk/with CVD.

We live in an increasingly stressful society and unfortunately, many of us face more than one acute bout of stress per day. Previous laboratory-based studies have reported a positive association between stress and fat consumption (Zellner et al., 2006). Given the implications that this could have on vascular health, shown by studies reporting fat consumption to worsen vascular responses to mental stress (Scoping Review, Chapter 2), future work should continue to investigate these associations using different methods and designs. Importantly, exposure to many acute bouts of stress, could lead to chronic and elevated consumption of high-fat foods. Therefore, the prolonged combination of stress and poor diet poses a risk to obesity, future CVD, and long-term health. Crucially, people with obesity have been shown to have reduced vasodilatory responses to stress (Hamer et al., 2007), and also present impairments in endothelial function following mental stress (Rocha et al., 2023a). Therefore, it is important to further this work by investigating populations at risk of CVD or populations with obesity. Furthermore, future research should endeavour to find strategies which can protect vascular health from stress, stress-induced fat consumption and the resulting impact on obesity and CVD risk.

9.3. Can flavonoid consumption protect vascular health from stress?

High-flavanol cocoa has been previously reported to attenuate the decline in endothelial function following stress in a fasted state (Baynham et al., 2021), and the Scoping Review (Chapter 2) presents investigations into cocoa flavanols, nitrate and Emblica Officinalis fruit which could protect vascular function during stress. Chapter 6 explores whether consumption of a high-flavanol cocoa drink, in combination with a meal high in saturated fat (same fat intervention as Chapter 4 and 5), can counteract

the stress-induced decline in endothelial function following fat consumption. It was found that flavonoids attenuate the stress-induced decline in FMD, and the fat-induced delay in the recovery of FMD. Therefore, these findings could have important implications for future use of flavonoid-rich dietary strategies, in both a fasted and postprandial state, to protect the vasculature during periods of stress and from stress-induced consumption of saturated fat. Flavonoids, and specifically cocoa flavanols, have received increasing attention as their effects on endothelial function are well-established in the literature. Whilst cocoa flavanols are a controlled way of delivering flavonoids, the availability of this intervention to the general public should also be considered. The quantity of flavonoids delivered could be achieved through consumption of 5.5 tbsp of unprocessed cocoa or 300 g of berries (Bhagwat et al., 2013). Future work should investigate the impact of flavonoid-rich foods that are more accessible to the public and establish what is the lowest efficacious dose that can be protective in the context of mental stress.

Chapter 6 shows that while flavonoid intake had a beneficial effect on peripheral vascular function, there was no effect of high-flavanol cocoa on cerebral oxygenation or CCA blood flow. Possible reasons for this have been discussed such as timing of flavonoid absorption (delayed absorption due to concomitant intake of fat) and insufficient power (n = 40 required according to retrospective power calculations). Importantly, we suggest that the effect of flavanol consumption on the brain during stress may be smaller than in the periphery. It is possible that the brain vasculature requires a greater stimulus and hence, improvements to cerebral oxygenation could be achieved with a higher dose of flavanols.

Interestingly, previous work has similarly shown that peripheral endothelial function may not be directly related to responses in the cerebrovasculature (Carr et al., 2020). For example, a recent study reported acute high-intensity interval exercise to induce increases in resting peripheral vascular function (brachial FMD), but to have no effect on cerebrovascular reactivity to hypercapnia and hypocapnia (transcranial doppler of middle cerebral artery) (Weston et al., 2022). The peripheral and cerebral vascular systems may be influenced by different mechanisms, which means that improvements in the periphery might not be extrapolated to the brain. Again, the consequence of this in relation to the triggering of stroke vs MI and the development of cerebrovascular vs coronary/peripheral artery disease should be an area for future work.

9.4. Delivering flavonoids in a free-living environment

Chapter 7 includes a daily diary study investigating the relationship between stress and diet. It was shown that stress was positively associated with flavonoid consumption, and further analyses did not reveal what flavonoid source (fruit and vegetables, tea, or red wine) could be driving this. The association between stress and unhealthy nutrients (i.e., fat and sugar) was not clear in a free-living environment. However, previous research reporting a positive association between stress and fat consumption in a free-living environment often assessed fat consumption through snacks rather than main meals (O'Connor et al., 2008, Zenk et al., 2014), which could be an interesting approach for future research. It is interesting that stress associated with flavonoid consumption but not fat and sugar intake. We previously commented on the moderate variability in stress perceptions reported by our sample (only 7 occurrences of participants reporting to feel extremely stressed). However, there were

substantial fluctuations in stress perceptions within each participant. Therefore, it is possible that the drive to consume fat and sugar may require higher levels of stress (i.e., a large stimulus, higher stress threshold), whilst intake of flavonoids may be consumed in response to lower thresholds of stress (i.e., a lower stimulus, lower stress threshold). This is in line with laboratory studies reporting increased fat consumption following acute laboratory induced stress, which tends to be a larger stimulus (Zellner et al., 2006). To our knowledge, there are no studies investigating laboratory stress and subsequent flavonoid consumption. Therefore, investigating whether there is a relationship between levels of stress and food choices may be an interesting research avenue to pursue.

The findings from Chapter 7 suggest that reporting to feel more stressed associated with increased flavonoid consumption. For example, a 5 unit increase in stress (going from not stressed (1/5) to extremely stressed (5/5)), predicted intake of 76 mg flavonoids. We may have been underpowered to reveal which source of flavonoids drives this relationship (i.e., tea, fruit, vegetables). However, the dose of flavonoids delivered in our laboratory study (Chapter 6) is the equivalent of 2 cups of green tea or 300 g berries (Bhagwat et al., 2013). This quantity of tea in particular might be achievable in everyday life and could be an effective way to deliver flavonoids to protect endothelial function from mental stress. Future studies should assess whether lower levels of flavonoids, potentially delivered through tea, could still be protective against mental stress. Future research should also consider other components in tea, such as caffeine and sugar, which may have a negative influence on vascular function when consumed in large quantities. Our Scoping Review (Chapter 2) found no studies which have investigated the impact of tea on vascular function in the context of mental

stress. Tea flavonoids have been reported to improve FMD in healthy participants and CAD patients, following acute and chronic consumption (Duffy et al., 2001, Alexopoulos et al., 2008, Kim et al., 2006, Nagaya et al., 2004), by augmenting NO and reducing ET-1 (Loke et al., 2008) which can contribute to a reduced risk of CVD (Hodgson and Croft, 2010). It is important to acknowledge the diversity in flavonoid composition delivered with tea (green tea: quercetin, (+)-catechin, (-)-epicatechin, epigallocatechin-gallate, black tea: quercetin, theaflavins, proanthocyandins, thearubigens), compared to cocoa ((-)-epicatechin, (+)-catechin), which may have differential effects on vascular function. Studies investigating the effect of tea on the cerebrovasculature are limited, yet one reported a reduction in CBF with caffeine but not tea flavonoids, and no effect on cerebrovascular reactivity (Vidyasagar et al., 2013). Therefore, future work should compare caffeinated and decaffeinated tea, with an identical flavonoid quantity, to understand the separate and combined effects of caffeine and flavonoids on peripheral and cerebral vascular function in the context of mental stress.

9.5. Physical activity as a stimulus to protect vascular function during stress

Chapter 8 used a daily diary design to investigate the relationship between stress and PA. We report that PA associated with improved perceptions of coping with stress. Exercise/PA has been shown to improve vascular health (Green et al., 2004) and mental health (Lubans et al., 2016). Similarly, a 12-week exercise intervention (3 hours/week) and stress management (1 hour/day) practice were reported to improve brachial FMD and decrease inflammatory markers (Dod et al., 2010). The effect of PA on vascular responses to mental stress is less understood. However, aerobically

trained individuals exhibit lower sympathetic nervous system reactivity (e.g., HR) and enhanced cardiovascular efficiency (e.g., decreased recovery time) to psychological stress (Huang et al., 2013), and overweight individuals have a blunted vasodilatory response to mental stress (Hamer et al., 2007). Furthermore, the Scoping Review (Chapter 2) presented two studies which reported chronic exercise training and hypocaloric diet to improve vascular responses to mental stress in obese populations (Ribeiro et al., 2005a, Tonacio et al., 2006a). Therefore, future work should continue this investigation, and specifically examine whether PA interventions can improve vascular function and psychological health during periods of stress, and stress-induced changes in eating behaviour.

Our daily diary study (Chapter 8) did not report significant associations between stress and PA engagement. Interestingly, research reports that some individuals increase their PA engagement during periods of mental stress, whilst others become less active (Wright et al., 2023). Therefore, it is important to understand the commonalities in people who engage in PA during stress such as traits, coping mechanisms, and attitudes to exercise, in order to encourage others to adopt similar PA habits during stressful periods. Using movement analysis (e.g., actigraphy) and tracking psychological perceptions to stress as well as incentives, attitudes, and traits, for example through an app could shed light on these associations. An incentive-based app has been previously shown to induce significant improvements to PA behaviour change (Elliott et al., 2019). Therefore, if a similar app could be utilised during periods of stress to improve coping and perhaps protect vascular function, there may be some benefit to overall physical and psychological health.

9.6. Real world practicalities

In this thesis, dietary intervention studies were designed so that the nutrient peaked in circulation during acute mental stress and over the time point at which stress induces the greatest impairment in endothelial function. PA interventions should be set-up in a similar way, so the benefit of exercise is most protective during the acute stress exposure.

However, perhaps it is unrealistic to assume that people can predict when stress will occur and change their behaviour accordingly (through flavonoid interventions and PA engagement). Whilst there will be some episodes of stress that can be pre-empted such as exams, presentations/seminars, and important meetings, other stressful episodes are unpredictable. Future research should quantify how prevalent this pre-empting of stress is in everyday life compared to unpredictable stress. Furthermore, future work should focus on whether the knowledge about the immediate benefits and/or negative impact of certain food choices during stress, can lead to different changes in behaviour in a controlled laboratory setting as well as in a more ecologically valid free-living environment.

Alternatively, it would be interesting to investigate whether people who have flavonoid-rich diets or are more physically active, can build vascular resilience against stress. Therefore, when stress cannot be anticipated, the positive health behaviours adopted (i.e., flavonoid-rich diet and physically active) may mitigate the negative impact of stress on vascular function. For example, some research has suggested a cross-stressor hypothesis whereby physically active individuals have a better physiological response to mental stress compared to physically inactive individuals (Sothmann et

al., 1996), although the evidence supporting this is mixed. A suggested mechanism is that regular exercise induces adaptations in the cardiovascular system which helps physically fit individuals buffer the adverse response to stress exposure (Sothmann et al., 1996). Similarly, diets rich in flavonoids could induce a similar cardiovascular resilience to stress, given the well-documented improvement in endothelial function following a Mediterranean diet (Rallidis et al., 2009), Vitamin supplementation (Jayedi et al., 2019), and flavonoid consumption (Rees et al., 2018). For example, one study reports that chronic consumption of a Mediterranean diet exhibits enhanced stress resilience in animal models, indicated by lower sympathetic activity, lower cortisol responses, and a more rapid physiological recovery (Shively et al., 2020). However, the translation of this to humans is unknown. Furthermore, a recent study reported that a 4-week psychobiotic diet (high in prebiotic and fermented foods) reduced perceptions of stress (Berding et al., 2023). Therefore, using microbiota-targeted diets to positively modulate gut-brain communication, may build psychological resilience to mental stress. In conclusion, future studies should investigate whether chronic exercise and dietary interventions can improve vascular responses to acute mental stress, as well as psychological perceptions to stress.

9.7. Future directions

Following the findings from this thesis, several relevant research questions and gaps in the literature have been identified, which should be considered as logical steps for future research. The following future directions are suggested below:

1. A young, healthy sample was included in all studies of this thesis. Given the decline in vascular function with age and numerous diseases, as well as a reduced endothelial function in overweight/obese populations and individuals from certain ethnicities (South Asian and Black ethnicities), inclusion of these populations in future research is warranted.

2. This thesis presented saturated fat to affect cerebral and peripheral vasculature, yet flavonoids to only influence the periphery. Future research should investigate this disconnect between brain and peripheral arteries and, specifically the impact of stress and nutrients on the mechanisms which could drive changes in vascular function.

3. This thesis investigated the effect of a controlled cocoa flavanol intervention (delivering 150 mg (-)-epicatechin). Future work should explore a more accessible flavonoid intervention and establish the minimum protective dose of flavonoids in the context of stress and, understand if this dose is different for the brain and periphery.

4. A consideration from these findings is whether people can pre-empt acute stress exposure and change their behaviour accordingly. Future research should try to understand how to drive behaviour change to gain the most vascular protection during stress. For example, the use of mobile apps could be explored for ‘just in time’ behavioural interventions when a person is experiencing stress.

5. Future work should investigate the potential of chronic dietary and exercise interventions to build vascular resilience to acute mental stress. In other words,

can chronic consumption of flavonoids/engagement in physical activity lead to improved vasodilatory responses to acute stress and an attenuated decline in endothelial function following acute stress?

6. The association between stress and nutrient intake in a free-living environment remains inconclusive. Future work should continue this investigation by assessing the bidirectional relationship between stress and nutrient intake, as well as investigate nutrients consumed from snacks, the frequency and stimulus of stress exposures across the day, and gain a better temporal resolution of the relationship between stress and dietary choices (e.g., through an app where both stress and dietary intake could be logged in real time).

9.8. Summary

In conclusion, mental stress and diet provoke acute changes to vascular function. This thesis suggests that the food choices made during periods of stress can worsen or mitigate the effect of mental stress on vascular health. However, changes in peripheral vasculature should not necessarily be extrapolated to cerebral vasculature. The effect of stress on dietary choices in a free-living environment remains largely unknown. However, the positive association between stress and flavonoids should be explored, as the source of flavonoids driving this relationship lends itself as a valuable method to protect vascular function during stress. Finally, physical activity associated with improved perceptions of coping during stress, yet whether physical activity can also improve vascular responses to mental stress is yet to be determined.

List of References

ABDEL HADI, S., MOJZISCH, A., KRUMM, S. & HÄUSSER, J. A. 2022. Day-level relationships between work, physical activity, and well-being: Testing the physical activity-mediated demand-control (pamDC) model. *Work & Stress,* 36**,** 355-376.

ADAM, T. C. & EPEL, E. S. 2007. Stress, eating and the reward system. *Physiology & Behavior,* 91**,** 449-458.

AKINS, J. D., CURTIS, B. M., PATIK, J. C., OLVERA, G., NASIRIAN, A., CAMPBELL, J. C., SHIVA, S. & BROTHERS, R. M. 2021. Blunted hyperemic response to mental stress in young, non-Hispanic black men is not impacted by acute dietary nitrate supplementation. *Journal of applied physiology (Bethesda, Md,* : 1985). 130**,** 1510-1521.

AKOH, C. C. & MIN, D. B. 2008. *Food Lipids: Chemistry, Nutrition, and Biotechnology,* Boca Raton, CRC Press.

ALBERT, M. A., DURAZO, E. M., SLOPEN, N., ZASLAVSKY, A. M., BURING, J. E., SILVA, T., CHASMAN, D. & WILLIAMS, D. R. 2017. Cumulative psychological stress and cardiovascular disease risk in middle aged and older women: Rationale, design, and baseline characteristics. *Am Heart J,* 192**,** 1-12.

ALEXOPOULOS, N., VLACHOPOULOS, C., AZNAOURIDIS, K., BAOU, K., VASILIADOU, C., PIETRI, P., XAPLANTERIS, P., STEFANADI, E. & STEFANADIS, C. 2008. The acute effect of green tea consumption on endothelial function in healthy individuals. *European Journal of Preventive Cardiology,* 15**,** 300-305.

ALMEIDA, D. M., MARCUSSON-CLAVERTZ, D., CONROY, D. E., KIM, J., ZAWADZKI, M. J., SLIWINSKI, M. J. & SMYTH, J. M. 2020. Everyday stress components and physical activity: examining reactivity, recovery and pileup. *J Behav Med,* 43**,** 108-120.

ALONSO, D. & RADOMSKI, M. W. 2003. The nitric oxide-endothelin-1 connection. *Heart Fail Rev,* 8**,** 107-15.

ALSOLMEI, F. A., LI, H., PEREIRA, S. L., KRISHNAN, P., JOHNS, P. W. & SIDDIQUI, R. A. 2019. Polyphenol-Enriched Plum Extract Enhances Myotubule Formation and Anabolism while Attenuating Colon Cancer-induced Cellular Damage in C2C12 Cells. *Nutrients,* 11.

AMERIAN PSYCHOLOGICAL ASSOCIATION. 2013. *Stress and eating* [Online]. American psychological Association. [Accessed 22 February 2024].

ANDERSON, A. R. & FOWERS, B. J. 2020. Lifestyle behaviors, psychological distress, and well-being: A daily diary study. *Social Science & Medicine,* 263**,** 113263.

ANDERSON, E. & DURSTINE, J. L. 2019. Physical activity, exercise, and chronic diseases: A brief review. *Sports Med Health Sci,* 1**,** 3-10.

ANDERSON, T. J., UEHATA, A., GERHARD, M. D., MEREDITH, I. T., KNAB, S., DELAGRANGE, D., LIEBERMAN, E. H., GANZ, P., CREAGER, M. A., YEUNG, A. C. & ET AL. 1995. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol,* 26**,** 1235-41.

ARAIZA, A. M. & LOBEL, M. 2018. Stress and eating: Definitions, findings, explanations, and implications. *Social and Personality Psychology Compass,* 12**,** 1-13.

ASAMANE, E. A., GREIG, C. A. & THOMPSON, J. L. 2020. The association between nutrient intake, nutritional status and physical function of community-dwelling ethnically diverse older adults. *BMC Nutrition,* 6**,** 36.

ASLAM, H., LOTFALIANY, M., SO, D., BERDING, K., BERK, M., ROCKS, T., HOCKEY, M., JACKA, F. N., MARX, W., CRYAN, J. F. & STAUDACHER, H. M. 2023. Fiber intake and fiber intervention in depression and anxiety: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Nutrition Reviews***,** nuad143.

ATKINSON, G. & BATTERHAM, A. M. 2013. The percentage flow-mediated dilation index: A large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vascular Medicine,* 18**,** 354-365.

AYATA, C., SHIN, H. K., DILEKOZ, E., ATOCHIN, D. N., KASHIWAGI, S., EIKERMANN-HAERTER, K. & HUANG, P. L. 2013. Hyperlipidemia disrupts cerebrovascular reflexes and worsens ischemic perfusion defect. *J Cereb Blood Flow Metab,* 33**,** 954-62.

AZEVEDO DA SILVA, M., SINGH-MANOUX, A., BRUNNER, E. J., KAFFASHIAN, S., SHIPLEY, M. J., KIVIMÄKI, M. & NABI, H. 2012. Bidirectional association between physical activity and symptoms of anxiety and depression: the Whitehall II study. *Eur J Epidemiol,* 27**,** 537-46.

BABYAK, M. A., BLUMENTHAL, J. A., HINDERLITER, A., HOFFMAN, B., WAUGH, R. A., COLEMAN, R. E. & SHERWOOD, A. 2010. Prognosis After Change in Left Ventricular Ejection Fraction During Mental Stress Testing in Patients With Stable Coronary Artery Disease. *American Journal of Cardiology,* 105**,** 25-28.

BAE, J.-H., BASSENGE, E., KIM, K.-B., KIM, Y.-N., KIM, K.-S., LEE, H.-J., MOON, K.-C., LEE, M.-S., PARK, K.-Y. & SCHWEMMER, M. 2001. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis,* 155**,** 517-523.

BAIREY MERZ, C. N., DWYER, J., NORDSTROM, C. K., WALTON, K. G., SALERNO, J. W. & SCHNEIDER, R. H. 2002. Psychosocial stress and cardiovascular disease: pathophysiological links. *Behav Med,* 27**,** 141-7.

BALZER, J., RASSAF, T., HEISS, C., KLEINBONGARD, P., LAUER, T., MERX, M., HEUSSEN, N., GROSS, H. B., KEEN, C. L., SCHROETER, H. & KELM, M. 2008. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients - A double-masked, randomized, controlled trial. *Journal of the American College of Cardiology,* 51**,** 2141-2149.

BATISTA, G. M. S., ROCHA, H. N. M., STORCH, A. S., GARCIA, V. P., TEIXEIRA, G. F., MENTZINGER, J., GOMES, E. A. C., VELASCO, L. L., NOBREGA, A. C. L. & ROCHA, N. G. 2020. Ascorbic acid inhibits vascular remodeling induced by mental stress in overweight/obese men. *Life Sci,* 250**,** 117554.

BAYNHAM, R., LUCAS, S. J. E., WEAVER, S. R. C., VELDHUIJZEN VAN ZANTEN, J. J. C. S. & RENDEIRO, C. 2023a. Fat Consumption Attenuates Cortical Oxygenation during Mental Stress in Young Healthy Adults. *Nutrients,* 15**,** 3969.

BAYNHAM, R., VELDHUIJZEN VAN ZANTEN, J. J. C. S., JOHNS, P. W., PHAM, Q. S. & RENDEIRO, C. 2021. Cocoa flavanols improve vascular responses to acute mental stress in young healthy adults. *Nutrients,* 13 (4) (no pagination).

BAYNHAM, R., WEAVER, S. R. C., RENDEIRO, C. & VELDHUIJZEN VAN ZANTEN, J. J. C. S. 2023b. Fat intake impairs the recovery of endothelial function following mental stress in young healthy adults. *Frontiers in Nutrition,* 10.

BERDING, K., BASTIAANSSEN, T. F. S., MOLONEY, G. M., BOSCAINI, S., STRAIN, C. R., ANESI, A., LONG-SMITH, C., MATTIVI, F., STANTON, C., CLARKE, G., DINAN, T. G. & CRYAN, J. F. 2023. Feed your microbes to deal with stress: a

psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population. *Molecular Psychiatry,* 28**,** 601-610.
BERGOVEC, M., MIHATOV, S., PRPIC, H., ROGAN, S., BATARELO, V. & SJEROBABSKI, V. 1992. Acute Myocardial-Infarction among civilians in Zagreb City Area. *Lancet,* 339**,** 303-303.
BERTHIER, F. & BOULAY, F. 2003. Lower myocardial infarction mortality in French men the day France won the 1998 World Cup of football. *Heart,* 89**,** 555-6.
BHAGWAT, S., HAYTOWITZ, D. B., WASSWA-KINTU, S. I. & HOLDEN, J. M. 2013. USDA develops a database for flavonoids to assess dietary intakes. *In:* STUMBO, P. & MCNUTT, S. (eds.) *36th National Nutrient Databank Conference.* Amsterdam: Elsevier Science Bv.
BINGHAM, S. A., WELCH, A. A., MCTAGGART, A., MULLIGAN, A. A., RUNSWICK, S. A., LUBEN, R., OAKES, S., KHAW, K. T., WAREHAM, N. & DAY, N. E. 2001. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr,* 4**,** 847-858.
BIXBY, W. R., SPALDING, T. W. & HATFIELD, B. D. 2001. Temporal Dynamics and Dimensional Specificity of the Affective Response to Exercise of Varying Intensity: Differing Pathways to a Common Outcome. *Journal of Sport and Exercise Psychology,* 23**,** 171-190.
BLACK, P. H. & GARBUTT, L. D. 2002. Stress, inflammation and cardiovascular disease. *Journal of Psychosomatic Research,* 52**,** 1-23.
BLAIR, G. W. S. 1959. An Equation for the Flow of Blood, Plasma and Serum through Glass Capillaries. *Nature,* 183**,** 613-614.
BLOOMFIELD, P. M., FISHER, J. P., SHAW, D. M. & GANT, N. 2023. Cocoa flavanols protect cognitive function, cerebral oxygenation, and mental fatigue during severe hypoxia. *J Appl Physiol (1985),* 135**,** 475-484.
BLUMENTHAL, J. A., JIANG, W., WAUGH, R. A., FRID, D. J., MORRIS, J. J., COLEMAN, R. E., HANSON, M., BABYAK, M., THYRUM, E. T., KRANTZ, D. S. & ET AL. 1995. Mental stress-induced ischemia in the laboratory and ambulatory ischemia during daily life. Association and hemodynamic features. *Circulation,* 92**,** 2102-8.
BONDONNO, N. P., DALGAARD, F., KYRO, C., MURRAY, K., BONDONNO, C. P., LEWIS, J. R., CROFT, K. D., GISLASON, G., SCALBERT, A., CASSIDY, A., TJONNELAND, A., OVERVATH, K. & HODGSON, J. M. 2019. Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort. *Nature Communications,* 10.
BONDONNO, N. P., DALGAARD, F., MURRAY, K., DAVEY, R. J., BONDONNO, C. P., CASSIDY, A., LEWIS, J. R., KYRØ, C., GISLASON, G., SCALBERT, A., TJØNNELAND, A. & HODGSON, J. M. 2021. Higher Habitual Flavonoid Intakes Are Associated with a Lower Incidence of Diabetes. *J Nutr,* 151**,** 3533-3542.
BONVENTO, G., SEYLAZ, J. & LACOMBE, P. 1994. Widespread attenuation of the cerebrovascular reactivity to hypercapnia following inhibition of nitric oxide synthase in the conscious rat. *J Cereb Blood Flow Metab,* 14**,** 699-703.
BOO, Y. C., SORESCU, G., BOYD, N., SHIOJIMA, I., WALSH, K., DU, J. & JO, H. 2002. Shear stress stimulates phosphorylation of endothelial nitric-oxide synthase at Ser1179 by Akt-independent mechanisms: role of protein kinase A. *J Biol Chem,* 277**,** 3388-96.

BORGES, G., OTTAVIANI, J. I., VAN DER HOOFT, J. J. J., SCHROETER, H. & CROZIER, A. 2018. Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings. *Molecular Aspects of Medicine,* 61**,** 18-30.

BOSCH, J. A., DE GEUS, E. J., CARROLL, D., GOEDHART, A. D., ANANE, L. A., VAN ZANTEN, J. J., HELMERHORST, E. J. & EDWARDS, K. M. 2009. A general enhancement of autonomic and cortisol responses during social evaluative threat. *Psychosom Med,* 71**,** 877-85.

BRASSARD, P., LABRECQUE, L., SMIRL, J. D., TYMKO, M. M., CALDWELL, H. G., HOILAND, R. L., LUCAS, S. J. E., DENAULT, A. Y., COUTURE, E. J. & AINSLIE, P. N. 2021. Losing the dogmatic view of cerebral autoregulation. *Physiol Rep,* 9**,** e14982.

BREMNER, J. D., MOAZZAMI, K., WITTBRODT, M. T., NYE, J. A., LIMA, B. B., GILLESPIE, C. F., RAPAPORT, M. H., PEARCE, B. D., SHAH, A. J. & VACCARINO, V. 2020. Diet, Stress and Mental Health. *Nutrients,* 12.

BRICKMAN, A. M., YEUNG, L. K., ALSCHULER, D. M., OTTAVIANI, J. I., KUHNLE, G. G. C., SLOAN, R. P., LUTTMANN-GIBSON, H., COPELAND, T., SCHROETER, H., SESSO, H. D., MANSON, J. E., WALL, M. & SMALL, S. A. 2023. Dietary flavanols restore hippocampal-dependent memory in older adults with lower diet quality and lower habitual flavanol consumption. *Proc Natl Acad Sci U S A,* 120**,** e2216932120.

BRINDLE, R. C., GINTY, A. T., PHILLIPS, A. C. & CARROLL, D. 2014. A tale of two mechanisms: A meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. *Psychophysiology,* 51**,** 964-976.

BRINDLE, R. C., GINTY, A. T., WHITTAKER, A. C., CARROLL, D. & LUCAS, S. J. E. 2018. Assessment of the cerebral pressure-flow relationship using psychological stress to manipulate blood pressure. *Psychophysiology,* 55**,** e13265.

BRITISH HEART FOUNDATION. 2024. *Global Heart and Circulatory Diseases Factsheet* [Online]. Available: https://www.bhf.org.uk/-/media/files/for-professionals/research/heart-statistics/bhf-cvd-statistics-global-factsheet.pdf?rev=f323972183254ca0a1043683a9707a01&hash=5AA21565EEE5D85691D37157B31E4AAA#:~:text=Globally%20it's%20estimated%20that%201,a%20heart%20or%20circulatory%20disease.&text=and%20260%20million%20men. [Accessed 23 April 2024].

BROADLEY, A. J. M., KORSZUN, A., ABDELAAL, E., MOSKVINA, V., JONES, C. J. H., NASH, G. B., RAY, C., DEANFIELD, J. & FRENNEAUX, M. P. 2005. Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. *Journal of the American College of Cardiology,* 46**,** 344-350.

BROTHERS, R. M., FADEL, P. J. & KELLER, D. M. 2019. Racial disparities in cardiovascular disease risk: mechanisms of vascular dysfunction. *Am J Physiol Heart Circ Physiol,* 317**,** H777-h789.

BUCCHERI, T., MUSAAD, S., BOST, K. K. & FIESE, B. H. 2018. Development and assessment of stressful life events subscales – A preliminary analysis. *Journal of Affective Disorders,* 226**,** 178-187.

BUCCI, M., GRATTON, J. P., RUDIC, R. D., ACEVEDO, L., ROVIEZZO, F., CIRINO, G. & SESSA, W. C. 2000. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nat Med,* 6**,** 1362-7.

BURG, M. M., GRAEBER, B., VASHIST, A., COLLINS, D., EARLEY, C., LIU, J., LAMPERT, R. & SOUFER, R. 2009. Noninvasive Detection of Risk for Emotion Provoked Myocardial Ischemia. *Psychosomatic Medicine,* 71**,** 14-20.

BURLEY, C. V., MULLINGER, K. J., THOMAS, K. N., RENDEIRO, C., DEHGHANI, H. & LUCAS, S. J. E. 2022. Imaging Cerebral Blood Flow for Brain Health Measurement. *In:* DELLA SALA, S. (ed.) *Encyclopedia of Behavioral Neuroscience, 2nd edition (Second Edition).* Oxford: Elsevier.

BUTT, E., BERNHARDT, M., SMOLENSKI, A., KOTSONIS, P., FRÖHLICH, L. G., SICKMANN, A., MEYER, H. E., LOHMANN, S. M. & SCHMIDT, H. H. 2000. Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases. *J Biol Chem,* 275**,** 5179-87.

CALDER, P. C. 2015. Functional Roles of Fatty Acids and Their Effects on Human Health. *Journal of Parenteral and Enteral Nutrition,* 39**,** 18S-32S.

CARDILLO, C., KILCOYNE, C. M., CANNON, R. O., 3RD & PANZA, J. A. 1998. Racial differences in nitric oxide-mediated vasodilator response to mental stress in the forearm circulation. *Hypertension (Dallas, Tex. : 1979),* 31**,** 1235-9.

CARDILLO, C., KILCOYNE, C. M., CANNON, R. O., 3RD & PANZA, J. A. 2000. Interactions between nitric oxide and endothelin in the regulation of vascular tone of human resistance vessels in vivo. *Hypertension,* 35**,** 1237-41.

CARDILLO, C., KILCOYNE, C. M., QUYYUMI, A. A., CANNON, R. O., 3RD & PANZA, J. A. 1997. Role of nitric oxide in the vasodilator response to mental stress in normal subjects. *The American journal of cardiology,* 80**,** 1070-4.

CARDILLO, C., KILCOYNE, C.M., CANNON, R.O. & PANZA, J.A. 1998. Racial Differences in Nitric Oxide-Mediated Vasodilator Response to Mental Stress in the Forearm Circulation. *Hypertension,* 31**,** 1235-1239.

CARR, J., HOILAND, R. L., CALDWELL, H. G., COOMBS, G. B., HOWE, C. A., TREMBLAY, J. C., GREEN, D. J. & AINSLIE, P. N. 2020. Internal carotid and brachial artery shear-dependent vasodilator function in young healthy humans. *J Physiol,* 598**,** 5333-5350.

CARROLL, D., EBRAHIM, S., TILLING, K., MACLEOD, J. & SMITH, G. D. 2002. Admissions for myocardial infarction and World Cup football: database survey. *British Medical Journal,* 325**,** 1439-1442.

CARROLL, D., PHILLIPS, A. C. & BALANOS, G. M. 2009. Metabolically exaggerated cardiac reactions to acute psychological stress revisited. *Psychophysiology,* 46**,** 270-275.

CARUANA, E. J., ROMAN, M., HERNÁNDEZ-SÁNCHEZ, J. & SOLLI, P. 2015. Longitudinal studies. *J Thorac Dis,* 7**,** E537-40.

CASSIDY, A., BERTOIA, M., CHIUVE, S., FLINT, A., FORMAN, J. & RIMM, E. B. 2016. Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. *American Journal of Clinical Nutrition,* 104**,** 587-594.

CASSIDY, A., ROGERS, G., PETERSON, J. J., DWYER, J. T., LIN, H. & JACQUES, P. F. 2015. Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults. *Am J Clin Nutr,* 102**,** 172-81.

CETIN, M., ERDOĞAN, T., KIRIŞ, T., ÖZYILDIZ, A. G., ERGÜL, E., DURAKOĞLUGIL, E., DURAK, H., KALAYCIOĞLU, E. & ÇIÇEK, Y. 2020. Endothelial dysfunction, subclinical atherosclerosis and LDL cholesterol are the independent predictors of left atrial functions in hypertension. *The international journal of cardiovascular imaging,* 36**,** 69-77.

CHARAKIDA, M., DE GROOT, E., LOUKOGEORGAKIS, S. P., KHAN, T., LUSCHER, T., KASTELEIN, J. J., GASSER, T. & DEANFIELD, J. E. 2013. Variability and reproducibility of flow-mediated dilatation in a multicentre clinical trial. *Eur Heart J,* 34**,** 3501-7.

CHILDS, E. & DE WIT, H. 2014. Regular exercise is associated with emotional resilience to acute stress in healthy adults. *Front Physiol,* 5**,** 161.

CHU, B., MARWAHA, K., SANVICTORES, T., AWOSIKA, A. O. & AYERS, D. 2022. Physiology, Stress Reaction. *StatPearls.* StatPearls Publishing Copyright © 2024, StatPearls Publishing LLC.

CINES, D. B., POLLAK, E. S., BUCK, C. A., LOSCALZO, J., ZIMMERMAN, G. A., MCEVER, R. P., POBER, J. S., WICK, T. M., KONKLE, B. A., SCHWARTZ, B. S., BARNATHAN, E. S., MCCRAE, K. R., HUG, B. A., SCHMIDT, A. M. & STERN, D. M. 1998. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood,* 91**,** 3527-61.

CLAPP, B. R., HINGORANI, A. D., KHARBANDA, R. K., MOHAMED-ALI, V., STEPHENS, J. W., VALLANCE, P. & MACALLISTER, R. J. 2004. Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc Res,* 64**,** 172-8.

CLERK, L. H., RATTIGAN, S. & CLARK, M. G. 2002. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes,* 51**,** 1138-45.

COHEN, S., KAMARCK, T. & MERMELSTEIN, R. 1983. A global measure of perceived stress. *J Health Soc Behav,* 24**,** 385-96.

CORRETTI, M. C., ANDERSON, T. J., BENJAMIN, E. J., CELERMAJER, D., CHARBONNEAU, F., CREAGER, M. A., DEANFIELD, J., DREXLER, H., GERHARD-HERMAN, M., HERRINGTON, D., VALLANCE, P., VITA, J. & VOGEL, R. 2002. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol,* 39**,** 257-65.

CORTI, R., FLAMMER, A. J., HOLLENBERG, N. K. & LUSCHER, T. F. 2009. Cocoa and cardiovascular health. *Circulation,* 119**,** 1433-41.

COTTER, E. W. & KELLY, N. R. 2018. Stress-related eating, mindfulness, and obesity. *Health Psychol,* 37**,** 516-525.

COTTONE, P., SABINO, V., ROBERTO, M., BAJO, M., POCKROS, L., FRIHAUF, J. B., FEKETE, E. M., STEARDO, L., RICE, K. C., GRIGORIADIS, D. E., CONTI, B., KOOB, G. F. & ZORRILLA, E. P. 2009. CRF system recruitment mediates dark side of compulsive eating. *Proceedings of the National Academy of Sciences,* 106**,** 20016-20020.

CRAFT, B. B., CARROLL, H. A. & LUSTYK, M. K. 2014. Gender Differences in Exercise Habits and Quality of Life Reports: Assessing the Moderating Effects of Reasons for Exercise. *Int J Lib Arts Soc Sci,* 2**,** 65-76.

CRAIG, C. L., MARSHALL, A. L., SJÖSTRÖM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U. L. F., YNGVE, A., SALLIS, J. F. & OJA, P. 2003. International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Medicine & Science in Sports & Exercise,* 35.

D'URZO, K. A., LA ROCQUE, C. L., WILLIAMS, J. S., STUCKLESS, T. J. R., KING, T. J., PLOTNICK, M. D., GURD, B. J., HARKNESS, K. L. & PYKE, K. E. 2019. The impact of acute mental stress on brachial artery flow-mediated dilation in women diagnosed with depression. *Int J Psychophysiol,* 135**,** 113-120.

DAKANALIS, A., MENTZELOU, M., PAPADOPOULOU, S. K., PAPANDREOU, D., SPANOUDAKI, M., VASIOS, G. K., PAVLIDOU, E., MANTZOROU, M. & GIAGINIS, C. 2023. The Association of Emotional Eating with Overweight/Obesity, Depression, Anxiety/Stress, and Dietary Patterns: A Review of the Current Clinical Evidence. *Nutrients,* 15.

DALLMAN, M. F., PECORARO, N. C. & LA FLEUR, S. E. 2005. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain, Behavior, and Immunity,* 19**,** 275-280.

DALTON, E. D. 2022. Exercise-related coping beliefs predict physical activity levels in response to naturally occurring stress: A daily diary study of college students. *J Am Coll Health,* 70**,** 411-419.

DANTAS, P. R. O. F., LIRA, F. A. S., BORBA, V. V. L., COSTA, M. J. C., TROMBETTA, I. C., SANTOS, M. S. B. & SANTOS, A. C. 2011. Vitamin C restores blood pressure and vasodilator response during mental stress in obese children. *Arquivos Brasileiros de Cardiologia,* 96**,** 490-497.

DASILVA, A. W., HUCKINS, J. F., WANG, W., WANG, R., CAMPBELL, A. T. & MEYER, M. L. 2021. Daily perceived stress predicts less next day social interaction: Evidence from a naturalistic mobile sensing study. *Emotion,* 21**,** 1760-1770.

DAVIES, D. J., SU, Z., CLANCY, M. T., LUCAS, S. J., DEHGHANI, H., LOGAN, A. & BELLI, A. 2015. Near-Infrared Spectroscopy in the Monitoring of Adult Traumatic Brain Injury: A Review. *J Neurotrauma,* 32**,** 933-41.

DAVIG, J. P., LARKIN, K. T. & GOODIE, J. L. 2000. Does cardiovascular reactivity to stress measured in the laboratory generalize to thesis and dissertation meetings among doctoral students? *International Journal of Behavioral Medicine,* 7**,** 216-235.

DAYRIT, F. M. 2023. Editorial: Saturated fat: metabolism, nutrition, and health impact. *Front Nutr,* 10**,** 1208047.

DE BOER, D., RING, C., CURLETT, A. C., RIDLEY, M. & CARROLL, D. 2007. Mental stress-induced hemoconcentration and its recovery: a controlled study of time course and mechanisms. *Psychophysiology,* 44**,** 161-9.

DE GEUS, E. J. C., WILLEMSEN, G. H. M., KLAVER, C. & VANDOORNEN, L. J. P. 1995. Ambulatory Measurement of Respiratory Sinus Arrythmia and Respiration Rate. *Biological Psychology,* 41**,** 205-227.

DEL ARCO, A. & MORA, F. 2009. Neurotransmitters and prefrontal cortex-limbic system interactions: implications for plasticity and psychiatric disorders. *J Neural Transm (Vienna),* 116**,** 941-52.

DICKEN, S. J., MITCHELL, J. J., NEWBERRY LE VAY, J., BEARD, E., KALE, D., HERBEC, A. & SHAHAB, L. 2021. Impact of the COVID-19 Pandemic on Diet

Behaviour Among UK Adults: A Longitudinal Analysis of the HEBECO Study. *Front Nutr,* 8**,** 788043.

DICKERSON, S. S. & KEMENY, M. E. 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull,* 130**,** 355-91.

DIETZ, N. M., RIVERA, J. M., EGGENER, S. E., FIX, R. T., WARNER, D. O. & JOYNER, M. J. 1994. Nitric-Oxide Contributes to the Rise in Forearm Blood-Flow During Mental Stress in Humans. *Journal of Physiology-London,* 480**,** 361-368.

DISHY, V., SOFOWORA, G. G., IMAMURA, H., NISHIMI, Y., XIE, H.-G., WOOD, A. J. & STEIN, C. M. 2003. Nitric oxide production decreases after salt loading but is not related to blood pressure changes or nitric oxide-mediated vascular responses. *Journal of hypertension,* 21**,** 153-7.

DOD, H. S., BHARDWAJ, R., SAJJA, V., WEIDNER, G., HOBBS, G. R., KONAT, G. W., MANIVANNAN, S., GHARIB, W., WARDEN, B. E., NANDA, N. C., BETO, R. J., ORNISH, D. & JAIN, A. C. 2010. Effect of intensive lifestyle changes on endothelial function and on inflammatory markers of atherosclerosis. *The American journal of cardiology,* 105**,** 362-7.

DUFFY, S. J., KEANEY JR, J. F., HOLBROOK, M., GOKCE, N., SWERDLOFF, P. L., FREI, B. & VITA, J. A. 2001. Short-and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation,* 104**,** 151-156.

DUNTON, G. F. 2017. Ecological Momentary Assessment in Physical Activity Research. *Exerc Sport Sci Rev,* 45**,** 48-54.

DYSON, K. S., SHOEMAKER, J. K. & HUGHSON, R. L. 2006. Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. *American Journal of Physiology-Heart and Circulatory Physiology,* 290**,** H1446-H1453.

EKKEKAKIS, P., HALL, E. E., VANLANDUYT, L. M. & PETRUZZELLO, S. J. 2000. Walking in (affective) circles: can short walks enhance affect? *J Behav Med,* 23**,** 245-75.

ELLIOTT, M., ECK, F., KHMELEV, E., DERLYATKA, A. & FOMENKO, O. 2019. Physical Activity Behavior Change Driven by Engagement With an Incentive-Based App: Evaluating the Impact of Sweatcoin. *JMIR Mhealth Uhealth,* 7**,** e12445.

ELZINGA, B. M. & ROELOFS, K. 2005. Cortisol-induced impairments of working memory require acute sympathetic activation. *Behav Neurosci,* 119**,** 98-103.

ENSARI, I., SCHWARTZ, J. E., EDMONDSON, D., DURAN, A. T., SHIMBO, D. & DIAZ, K. M. 2020. Testing the cross-stressor hypothesis under real-world conditions: exercise as a moderator of the association between momentary anxiety and cardiovascular responses. *J Behav Med,* 43**,** 989-1001.

EPEL, E., LAPIDUS, R., MCEWEN, B. & BROWNELL, K. 2001. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology,* 26**,** 37-49.

FARD, A., TUCK, C. H., DONIS, J. A., SCIACCA, R., DI TULLIO, M. R., WU, H. D., BRYANT, T. A., CHEN, N.-T., TORRES-TAMAYO, M., RAMASAMY, R., BERGLUND, L., GINSBERG, H. N., HOMMA, S. & CANNON, P. J. 2000. Acute Elevations of Plasma Asymmetric Dimethylarginine and Impaired Endothelial

Function in Response to a High-Fat Meal in Patients With Type 2 Diabetes. *Arterioscler Thromb Vasc Biol,* 20**,** 2039-2044.
FARIDI, Z., NJIKE, V. Y., DUTTA, S., ALI, A. & KATZ, D. L. 2008. Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial. *American Journal of Clinical Nutrition,* 88**,** 58-63.
FARLEY, A., MCLAFFERTY, E. & HENDRY, C. 2012. The cardiovascular system. *Nurs Stand,* 27**,** 35-9.
FAROUQUE, H. M., LEUNG, M., HOPE, S. A., BALDI, M., SCHECHTER, C., CAMERON, J. D. & MEREDITH, I. T. 2006. Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomized double-blind placebo-controlled study. *Clin Sci (Lond),* 111**,** 71-80.
FAUL, F., ERDFELDER, E., LANG, A.-G., & BUCHNER, A. 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods,* 39**,** 175-191.
FINK, G. 2016. Stress, Definitions, Mechanisms, and Effects Outlined: Lessons from Anxiety. *In:* FINK, G. (ed.) *Stress: Concepts, Cognition, Emotion, and Behavior.* San Diego: Academic Press.
FLUECKIGER, L., LIEB, R., MEYER, A. H., WITTHAUER, C. & MATA, J. 2016. The importance of physical activity and sleep for affect on stressful days: Two intensive longitudinal studies. *Emotion,* 16**,** 488-97.
FORDE, C. 2018. Scoring the international physical activity questionnaire (IPAQ). *Dublin: University of Dublin*.
FRANK, S., LINDER, K., KULLMANN, S., HENI, M., KETTERER, C., ÇAVUŞOĞLU, M., KRZEMINSKI, A., FRITSCHE, A., HÄRING, H.-U., PREISSL, H., HINRICHS, J. & VEIT, R. 2012. Fat intake modulates cerebral blood flow in homeostatic and gustatory brain areas in humans. *The American Journal of Clinical Nutrition,* 95**,** 1342-1349.
FREEMAN, L. R., HALEY-ZITLIN, V., ROSENBERGER, D. S. & GRANHOLM, A. C. 2014. Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. *Nutr Neurosci,* 17**,** 241-51.
FRIEDMAN, N. P. & ROBBINS, T. W. 2022. The role of prefrontal cortex in cognitive control and executive function. *Neuropsychopharmacology,* 47**,** 72-89.
GAO, M., JEBB, S. A., AVEYARD, P., AMBROSINI, G. L., PEREZ-CORNAGO, A., CARTER, J., SUN, X. & PIERNAS, C. 2021. Associations between dietary patterns and the incidence of total and fatal cardiovascular disease and all-cause mortality in 116,806 individuals from the UK Biobank: a prospective cohort study. *BMC Med,* 19**,** 83.
GARDENER, H., WRIGHT, C. B., DONG, C., CHEUNG, K., DEROSA, J., NANNERY, M., STERN, Y., ELKIND, M. S. V. & SACCO, R. L. 2016. Ideal Cardiovascular Health and Cognitive Aging in the Northern Manhattan Study. *J Am Heart Assoc,* 5**,** e002731-n/a.
GARDINER, C. K., HAGERTY, S. L. & BRYAN, A. D. 2021. Stress and number of servings of fruit and vegetables consumed: Buffering effects of monetary incentives. *Journal of Health Psychology,* 26**,** 1757-1763.
GELEIJNSE, J. M., LAUNER, L. J., VAN DER KUIP, D. A. M., HOFMAN, A. & WITTEMAN, J. C. M. 2002. Inverse association of tea and flavonoid intakes with

incident myocardial infarction: the Rotterdam Study. *American Journal of Clinical Nutrition,* 75**,** 880-886.

GHEIBI, S., SAMSONOV, A. P., GHEIBI, S., VAZQUEZ, A. B. & KASHFI, K. 2020. Regulation of carbohydrate metabolism by nitric oxide and hydrogen sulfide: Implications in diabetes. *Biochem Pharmacol,* 176**,** 113819.

GHIADONI, L., DONALD, A. E., CROPLEY, M., MILLEN, M. J., OAKLEY, G., TAYLOR, M., O'CONNOR, G., BETTERIDGE, J., KLEIN, N., STEPTOE, A. & DEANFIELD, J. E. 2000. Mental Stress induces Transient endothelial Dysfunction in humans. *Circulation*.

GHIADONI, L., FAITA, F., SALVETTI, M., CORDIANO, C., BIGGI, A., PUATO, M., DI MONACO, A., DE SIATI, L., VOLPE, M., AMBROSIO, G., GEMIGNANI, V., MUIESAN, M. L., TADDEI, S., LANZA, G. A. & COSENTINO, F. 2012. Assessment of flow-mediated dilation reproducibility: a nationwide multicenter study. *J Hypertens,* 30**,** 1399-1405.

GHITESCU, L. & ROBERT, M. 2002. Diversity in unity: the biochemical composition of the endothelial cell surface varies between the vascular beds. *Microsc Res Tech,* 57**,** 381-9.

GIANFREDI, V., BLANDI, L., CACITTI, S., MINELLI, M., SIGNORELLI, C., AMERIO, A. & ODONE, A. 2020. Depression and Objectively Measured Physical Activity: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health,* 17.

GIBBONS, C., FINLAYSON, G., CAUDWELL, P., WEBB, D. L., HELLSTRÖM, P. M., NÄSLUND, E. & BLUNDELL, J. E. 2016. Postprandial profiles of CCK after high fat and high carbohydrate meals and the relationship to satiety in humans. *Peptides,* 77**,** 3-8.

GINTY, A. T., GIANAROS, P. J., DERBYSHIRE, S. W., PHILLIPS, A. C. & CARROLL, D. 2013. Blunted cardiac stress reactivity relates to neural hypoactivation. *Psychophysiology,* 50**,** 219-29.

GOETZ, M. E., JUDD, S. E., SAFFORD, M. M., HARTMAN, T. J., MCCLELLAN, W. M. & VACCARINO, V. 2016. Dietary flavonoid intake and incident coronary heart disease: the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. *American Journal of Clinical Nutrition,* 104**,** 1236-1244.

GOKCE, N. 1998. Impaired flow-mediated dilation following a high fat meal is attributable to a change in basal tone. *Circulation : journal of the American Heart Association. ,* 98**,** 242.

GOKCE, N., KEANEY, J. F., JR., HUNTER, L. M., WATKINS, M. T., NEDELJKOVIC, Z. S., MENZOIAN, J. O. & VITA, J. A. 2003. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol,* 41**,** 1769-75.

GOLDBERG, A. D., BECKER, L. C., BONSALL, R., COHEN, J. D., KETTERER, M. W., KAUFMAN, P. G., KRANTZ, D. S., LIGHT, K. C., MCMAHON, R. P., NOREUIL, T., PEPINE, C. J., RACZYNSKI, J., STONE, P. H., STROTHER, D., TAYLOR, H. & SHEPS, D. S. 1996. Ischemic, hemodynamic, and neurohormonal responses to mental and exercise stress. Experience from the Psychophysiological Investigations of Myocardial Ischemia Study (PIMI). *Circulation,* 94**,** 2402-9.

GOMEZ, G. J., BURR, E. K., DIBELLO, A. M. & FARRIS, S. G. 2021. Understanding sex differences in physical activity behavior: The role of anxiety sensitivity. *Mental Health and Physical Activity,* 20**,** 100392.

GOMEZ-GUZMAN, M., JIMENEZ, R., SANCHEZ, M., ZARZUELO, M. J., GALINDO, P., QUINTELA, A. M., LOPEZ-SEPULVEDA, R., ROMERO, M., TAMARGO, J., VARGAS, F., PEREZ-VIZCAINO, F. & DUARTE, J. 2012. Epicatechin lowers blood pressure, restores endothelial function, and decreases oxidative stress and endothelin-1 and NADPH oxidase activity in DOCA-salt hypertension. *Free Radical Biology and Medicine,* 52**,** 70-79.

GOODWIN, R. D., WEINBERGER, A. H., KIM, J. H., WU, M. & GALEA, S. 2020. Trends in anxiety among adults in the United States, 2008-2018: Rapid increases among young adults. *J Psychiatr Res,* 130**,** 441-446.

GORDON, G. R. J., CHOI, H. B., RUNGTA, R. L., ELLIS-DAVIES, G. C. R. & MACVICAR, B. A. 2008. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature,* 456**,** 745-749.

GOTTDIENER, J. S., KOP, W. J., HAUSNER, E., MCCENEY, M. K., HERRINGTON, D. & KRANTZ, D. S. 2003. Effects of mental stress on flow-mediated brachial arterial dilation and influence of behavioral factors and hypercholesterolemia in subjects without cardiovascular disease. *American Journal of Cardiology,* 92**,** 687-691.

GOUWS, C. A., MCKUNE, A., TEE, N., SOMERSET, S. & MORTAZAVI, R. 2022. Prickly pear juice consumption after fat intake affects postprandial heart rate variability but not traditional risk factors of cardiovascular disease in healthy men. *Nutrition,* 96**,** 111555.

GOV.UK. 2019. *Health matters: health and work* [Online]. Available: https://www.gov.uk/government/publications/health-matters-health-and-work/health-matters-health-and-work#resources [Accessed 27 March 2024].

GOV.UK. 2020. *NDNS: results from years 9 to 11 (combined) – statistical summary* [Online]. Available: https://www.gov.uk/government/statistics/ndns-results-from-years-9-to-11-2016-to-2017-and-2018-to-2019/ndns-results-from-years-9-to-11-combined-statistical-summary#food-consumption [Accessed 2nd April 2024].

GOWDAK, M. M. G., LATERZA, M. C., RONDON, M. U. P. B., TROMBETTA, I. C., PEREIRA, A. C., KRIEGER, J. E. & NEGRAO, C. E. 2010. A high-fat meal impairs muscle vasodilatation response to mental stress in humans with Glu27 beta<inf>2</inf>-adrenoceptor polymorphism. *Lipids in Health and Disease,* 9 (no pagination).

GRASSI, D., NECOZIONE, S., LIPPI, C., CROCE, G., VALERI, L., PASQUALETTI, P., DESIDERI, G., BLUMBERG, J. B. & FERRI, C. 2005. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension,* 46**,** 398-405.

GRATTON, G., WEAVER, S. R., BURLEY, C. V., LOW, K. A., MACLIN, E. L., JOHNS, P. W., PHAM, Q. S., LUCAS, S. J. E., FABIANI, M. & RENDEIRO, C. 2020. Dietary flavanols improve cerebral cortical oxygenation and cognition in healthy adults. *Scientific Reports,* 10**,** 19409.

GRAVES, B. S., HALL, M. E., DIAS-KARCH, C., HAISCHER, M. H. & APTER, C. 2021. Gender differences in perceived stress and coping among college students. *PLoS One,* 16**,** e0255634.

GRAVETTER, F. & WALLNAU, L. B. 2016. Statistics for the Behavioral Sciences. *Cengage Learning*.

GREEN, D. J., JONES, H., THIJSSEN, D., CABLE, N. T. & ATKINSON, G. 2011. Flow-Mediated Dilation and Cardiovascular Event Prediction. *Hypertension,* 57**,** 363-369.

GREEN, D. J., MAIORANA, A., O'DRISCOLL, G. & TAYLOR, R. 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol,* 561**,** 1-25.

GREYLING, A., VAN MIL, A. C. C. M., ZOCK, P. L., GREEN, D. J., GHIADONI, L. & THIJSSEN, D. H. 2016. Adherence to guidelines strongly improves reproducibility of brachial artery flow-mediated dilation. *Atherosclerosis,* 248**,** 196-202.

GROESZ, L. M., MCCOY, S., CARL, J., SASLOW, L., STEWART, J., ADLER, N., LARAIA, B. & EPEL, E. 2012. What is eating you? Stress and the drive to eat. *Appetite,* 58**,** 717-21.

GROVE, R. & PRAPAVESSIS, H. 1992. Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *International Journal of Sport Psychology,* 23**,** 93-109.

GUZIK, T. J., KORBUT, R. & ADAMEK-GUZIK, T. 2003. Nitric oxide and superoxide in inflammation and immune regulation. *J Physiol Pharmacol,* 54**,** 469-87.

HADI, H. A., CARR, C. S. & AL SUWAIDI, J. 2005. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Risk Manag,* 1**,** 183-98.

HALLIWILL, J. R., LAWLER, L. A., EICKHOFF, T. J., DIETZ, N. M., NAUSS, L. A. & JOYNER, M. J. 1997. Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol,* 504 (Pt 1)**,** 211-20.

HALLMAN, D. M. & LYSKOV, E. 2012. Autonomic regulation, physical activity and perceived stress in subjects with musculoskeletal pain: 24-hour ambulatory monitoring. *International Journal of Psychophysiology,* 86**,** 276-282.

HAMER, M., BOUTCHER, Y. N. & BOUTCHER, S. H. 2007. Fatness is related to blunted vascular stress responsivity, independent of cardiorespiratory fitness in normal and overweight men. *International Journal of Psychophysiology,* 63**,** 251-257.

HAMMEN, C., KIM, E. Y., EBERHART, N. K. & BRENNAN, P. A. 2009. Chronic and acute stress and the prediction of major depression in women. *Depress Anxiety,* 26**,** 718-23.

HAMMER, A., KOPPENSTEINER, R., STEINER, S., NIESSNER, A., GOLIASCH, G., GSCHWANDTNER, M. & HOKE, M. 2015. Dark chocolate and vascular function in patients with peripheral artery disease: a randomized, controlled cross-over trial. *Clin Hemorheol Microcirc,* 59**,** 145-53.

HARRIS, C. W., EDWARDS, J. L., BARUCH, A., RILEY, W. A., PUSSER, B. E., REJESKI, W. J. & HERRINGTON, D. M. 2000. Effects of mental stress on brachial artery flow-mediated vasodilation in healthy normal individuals. *American Heart Journal,* 139**,** 405-411.

HEALTH AND SAFETY EXECUTIVE. 2023. *Work-related stress, depression or anxiety statistics in Great Britain* [Online]. Available: https://www.hse.gov.uk/stress/standards/downloads.htm [Accessed 25 March 2024].

HEATH, G., COATES, A., SARGENT, C. & DORRIAN, J. 2016. Sleep Duration and Chronic Fatigue Are Differently Associated with the Dietary Profile of Shift Workers. *Nutrients,* 8**,** 771.

HEISS, C., DEJAM, A., KLEINBONGARD, P., SCHEWE, T., SIES, H. & KELM, M. 2003. Vascular effects of cocoa rich in flavan-3-ols. *Jama-Journal of the American Medical Association,* 290**,** 1030-1031.

HEISS, C., JAHN, S., TAYLOR, M., REAL, W. M., ANGELI, F. S., WONG, M. L., AMABILE, N., PRASAD, M., RASSAF, T., OTTAVIANI, J. I., MIHARDJA, S., KEEN, C. L., SPRINGER, M. L., BOYLE, A., GROSSMAN, W., GLANTZ, S. A., SCHROETER, H. & YEGHIAZARIANS, Y. 2010. Improvement of Endothelial Function With Dietary Flavanols Is Associated With Mobilization of Circulating Angiogenic Cells in Patients With Coronary Artery Disease. *Journal of the American College of Cardiology,* 56**,** 218-224.

HEISS, C., SANSONE, R., KARIMI, H., KRABBE, M., SCHULER, D., RODRIGUEZ-MATEOS, A., KRAEMER, T., CORTESE-KROTT, M. M., KUHNLE, G. G., SPENCER, J. P., SCHROETER, H., MERX, M. W., KELM, M. & FLAVIOLA CONSORTIUM, E. U. T. F. P. 2015. Impact of cocoa flavanol intake on age-dependent vascular stiffness in healthy men: a randomized, controlled, double-masked trial. *Age (Dordr),* 37**,** 9794.

HEMMINGSSON, E. & EKELUND, U. 2007. Is the association between physical activity and body mass index obesity dependent? *Int J Obes (Lond),* 31**,** 663-8.

HIGGINS, J. & GREEN, S. 2011. *Cochrane Handjournal for Systematic Reviews of Interventions,* London: Cochrane Collab.

HIGGS, S. & THOMAS, J. 2016. Social influences on eating. *Current Opinion in Behavioral Sciences,* 9**,** 1-6.

HILL, D., CONNER, M., CLANCY, F., MOSS, R., WILDING, S., BRISTOW, M. & O'CONNOR, D. B. 2021. Stress and eating behaviours in healthy adults: a systematic review and meta-analysis. *Health Psychol Rev***,** 1-25.

HODGSON, J. M. & CROFT, K. D. 2010. Tea flavonoids and cardiovascular health. *Molecular Aspects of Medicine,* 31**,** 495-502.

HOLLENBERG, N. K., FISHER, N. D. & MCCULLOUGH, M. L. 2009. Flavanols, the Kuna, cocoa consumption, and nitric oxide. *J Am Soc Hypertens,* 3**,** 105-12.

HOX, J. J., MOERBEEK, M. & VAN DE SCHOOT, R. 2018. *Multilevel analysis - Techniques and applications* Taylor & Francis.

HUANG, C.-J., WEBB, H. E., ZOURDOS, M. C. & ACEVEDO, E. O. 2013. Cardiovascular reactivity, stress, and physical activity. *Frontiers in Physiology,* 4.

IADECOLA, C. & ZHANG, F. 1994. Nitric oxide-dependent and -independent components of cerebrovasodilation elicited by hypercapnia. *Am J Physiol,* 266**,** R546-52.

IGIC, I., RYSER, S. & ELFERING, A. 2013. Does work stress make you shorter? An ambulatory field study of daily work stressors, job control, and spinal shrinkage. *Journal of Occupational Health Psychology,* 18**,** 469-480.

INABA, Y., CHEN, J. A. & BERGMANN, S. R. 2010. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *International Journal of Cardiovascular Imaging,* 26**,** 631-640.

IUCHI, T., AKAIKE, M., MITSUI, T., OHSHIMA, Y., SHINTANI, Y., AZUMA, H. & MATSUMOTO, T. 2003. Glucocorticoid excess induces superoxide production

in vascular endothelial cells and elicits vascular endothelial dysfunction. *Circ Res,* 92**,** 81-7.

JACKSON, K. G., ARMAH, C. K. & MINIHANE, A. M. 2007. Meal fatty acids and postprandial vascular reactivity. *Biochemical Society Transactions,* 35**,** 451-453.

JAIN, D., SHAKER, S. M., BURG, M., WACKERS, F. J. T., SOUFER, R. & ZARET, B. L. 1998. Effects of Mental Stress on Left Ventricular and Peripheral Vascular Performance in Patients With Coronary Artery Disease. *Journal of the American College of Cardiology,* 31**,** 1314-1322.

JAKULJ, F., ZERNICKE, K., BACON, S. L., VAN WIELINGEN, L. E., KEY, B. L., WEST, S. G. & CAMPBELL, T. S. 2007. A high-fat meal increases cardiovascular reactivity to psychological stress in healthy young adults. *Journal of Nutrition,* 137**,** 935-939.

JAMBRIK, Z., SEBASTIANI, L., PICANO, E., GHELARDUCCI, B. & SANTARCANGELO, E. L. 2005. Hypnotic modulation of flow-mediated endothelial response to mental stress. *International Journal of Psychophysiology,* 55**,** 221-227.

JAYEDI, A., RASHIDY-POUR, A., PAROHAN, M., ZARGAR, M. S. & SHAB-BIDAR, S. 2019. Dietary and circulating vitamin C, vitamin E, β-carotene and risk of total cardiovascular mortality: a systematic review and dose–response meta-analysis of prospective observational studies. *Public health nutrition,* 22**,** 1872-1887.

JOHNSTON, D. W., TUOMISTO, M. T. & PATCHING, G. R. 2008. The Relationship Between Cardiac Reactivity in the Laboratory and in Real Life. *Health Psychol,* 27**,** 34-42.

JONES, M., TAYLOR, A., LIAO, Y., INTILLE, S. S. & DUNTON, G. F. 2017. REAL-TIME SUBJECTIVE ASSESSMENT OF PSYCHOLOGICAL STRESS: ASSOCIATIONS WITH OBJECTIVELY-MEASURED PHYSICAL ACTIVITY LEVELS. *Psychol Sport Exerc,* 31**,** 79-87.

JOYNER, M. J., DIETZ, N.M. & SHEPHERD, J.T. 2001. From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol,* 91**,** 2431-2441.

JOYNER, M. J. & HALLIWILL, J. R. 2000. Sympathetic vasodilatation in human limbs. *The Journal of physiology,* 526 Pt 3**,** 471-80.

KAJIKAWA, M. & HIGASHI, Y. 2022. Obesity and Endothelial Function. *Biomedicines,* 10.

KALMIJN, S. 2000. Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. *J Nutr Health Aging,* 4**,** 202-7.

KAUFMAN, C. L., KAISER, D. R., STEINBERGER, J., KELLY, A. S. & DENGEL, D. R. 2007. Relationships of cardiac autonomic function with metabolic abnormalities in childhood obesity. *Obesity (Silver Spring),* 15**,** 1164-71.

KHARRAZIHA, I., HOLM, H., MAGNUSSON, M., WOLLMER, P., MOLVIN, J., JUJIC, A., FEDOROWSKI, A., BACHUS, E. & HAMREFORS, V. 2021. Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing. *ESC Heart Fail,* 8**,** 586-594.

KIECOLT-GLASER, J. K., FAGUNDES, C. P., ANDRIDGE, R., PENG, J., MALARKEY, W. B., HABASH, D. & BELURY, M. A. 2017. Depression, daily

stressors and inflammatory responses to high-fat meals: when stress overrides healthier food choices. *Mol Psychiatry,* 22**,** 476-482.
KIECOLT-GLASER, J. K., HABASH, D. L., FAGUNDES, C. P., ANDRIDGE, R., PENG, J., MALARKEY, W. B. & BELURY, M. A. 2015. Daily Stressors, Past Depression, and Metabolic Responses to High-Fat Meals: A Novel Path to Obesity. *Biological Psychiatry,* 77**,** 653-660.
KIM, W., JEONG, M. H., CHO, S. H., YUN, J. H., CHAE, H. J., AHN, Y. K., LEE, M. C., CHENG, X., KONDO, T. & MUROHARA, T. 2006. Effect of green tea consumption on endothelial function and circulating endothelial progenitor cells in chronic smokers. *Circulation Journal,* 70**,** 1052-1057.
KIRKUP, W. & MERRICK, D. W. 2003. A matter of life and death: population mortality and football results. *J Epidemiol Community Health,* 57**,** 429-32.
KIRSCHBAUM, C. & HELLHAMMER, D. H. 1994. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology,* 19**,** 313-333.
KOEP, J. L., TAYLOR, C. E., COOMBES, J. S., BOND, B., AINSLIE, P. N. & BAILEY, T. G. 2022. Autonomic control of cerebral blood flow: fundamental comparisons between peripheral and cerebrovascular circulations in humans. *The Journal of Physiology,* 600**,** 15-39.
KOH, Y. S., ASHARANI, P. V., DEVI, F., ROYSTONN, K., WANG, P., VAINGANKAR, J. A., ABDIN, E., SUM, C. F., LEE, E. S., MÜLLER-RIEMENSCHNEIDER, F., CHONG, S. A. & SUBRAMANIAM, M. 2022. A cross-sectional study on the perceived barriers to physical activity and their associations with domain-specific physical activity and sedentary behaviour. *BMC Public Health,* 22**,** 1051.
KOO, T. K. & LI, M. Y. 2016. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med,* 15**,** 155-63.
KOUVONEN, A., VAHTERA, J., OKSANEN, T., PENTTI, J., VÄÄNÄNEN, A. K., HEPONIEMI, T., SALO, P., VIRTANEN, M. & KIVIMÄKI, M. 2013. Chronic workplace stress and insufficient physical activity: a cohort study. *Occup Environ Med,* 70**,** 3-8.
KRANTZ, D. S., QUIGLEY, J. F. & O'CALLAHAN, M. 2001. Mental stress as a trigger of acute cardiac events: the role of laboratory studies. *Ital Heart J,* 2**,** 895-9.
KUCHARCZYK, M., KUREK, A., DETKA, J., SLUSARCZYK, J., PAPP, M., TOTA, K., BASTA-KAIM, A., KUBERA, M., LASON, W. & BUDZISZEWSKA, B. 2016. Chronic mild stress influences nerve growth factor through a matrix metalloproteinase-dependent mechanism. *Psychoneuroendocrinology,* 66**,** 11-21.
LAMPORT, D. J. & WILLIAMS, C. M. 2021. Polyphenols and Cognition In Humans: An Overview of Current Evidence from Recent Systematic Reviews and Meta-Analyses. *Brain Plast,* 6**,** 139-153.
LANE, J. D., GREENSTADT, L., SHAPIRO, D. & RUBINSTEIN, E. 1983. Pulse transit time and blood pressure: an intensive analysis. *Psychophysiology,* 20**,** 45-9.
LEE, S. S., YU, K., CHOI, E. & CHOI, I. 2022. To drink, or to exercise: That is (not) the question! Daily effects of alcohol consumption and exercise on well-being. *Appl Psychol Health Well Being,* 14**,** 555-571.
LEOR, J. & KLONER, R. A. 1996. The Northridge earthquake as a trigger for acute myocardial infarction. *American Journal of Cardiology,* 77**,** 1230-1232.

LEVICK, J. R. 2003. *An introduction to cardiovascular physiology,* London, New York, Arnold ; Distributed in the United States of America by Oxford University Press.
LI, H., SHENG, Z., KHAN, S., ZHANG, R., LIU, Y., ZHANG, Y., YONG, V. W. & XUE, M. 2022. Matrix Metalloproteinase-9 as an Important Contributor to the Pathophysiology of Depression. *Front Neurol,* 13**,** 861843.
LI, S.-H., TIAN, H.-B., ZHAO, H.-J., CHEN, L.-H. & CUI, L.-Q. 2013. The Acute Effects of Grape Polyphenols Supplementation on Endothelial Function in Adults: Meta-Analyses of Controlled Trials. *PLOS ONE,* 8**,** e69818.
LI, Y., DENG, J., LOU, X., WANG, H. & WANG, Y. 2020. A daily diary study of the relationships among daily self-compassion, perceived stress and health-promoting behaviours. *Int J Psychol,* 55**,** 364-372.
LIAO, Y., CHOU, C.-P., HUH, J., LEVENTHAL, A. & DUNTON, G. 2017. Examining acute bi-directional relationships between affect, physical feeling states, and physical activity in free-living situations using electronic ecological momentary assessment. *Journal of Behavioral Medicine,* 40**,** 445-457.
LIND, L., JOHANSSON, K. & HALL, J. 2002. The effects of mental stress and the cold pressure test on flow-mediated vasodilation. *Blood Pressure,* 11**,** 22-27.
LISTON, C., MCEWEN, B. S. & CASEY, B. J. 2009. Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proc Natl Acad Sci U S A,* 106**,** 912-7.
LIU, Z., LIU, J., JAHN, L. A., FOWLER, D. E. & BARRETT, E. J. 2009. Infusing lipid raises plasma free fatty acids and induces insulin resistance in muscle microvasculature. *J Clin Endocrinol Metab,* 94**,** 3543-9.
LOCKHART, C. J., AGNEW, C. E., MCCANN, A., HAMILTON, P. K., QUINN, C. E., MCCALL, D. O., PLUMB, R. D., MCCLENAGHAN, V. C., MCGIVERN, R. C. & HARBINSON, M. T. 2011. Impaired flow-mediated dilatation response in uncomplicated Type 1 diabetes mellitus: influence of shear stress and microvascular reactivity. *Clinical Science,* 121**,** 129-139.
LOKE, W. M., HODGSON, J. M., PROUDFOOT, J. M., MCKINLEY, A. J., PUDDEY, I. B. & CROFT, K. D. 2008. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *American Journal of Clinical Nutrition,* 88**,** 1018-1025.
LOVICK, T. A. 2009. CCK as a modulator of cardiovascular function. *Journal of Chemical Neuroanatomy,* 38**,** 176-184.
LUBANS, D., RICHARDS, J., HILLMAN, C., FAULKNER, G., BEAUCHAMP, M., NILSSON, M., KELLY, P., SMITH, J., RAINE, L. & BIDDLE, S. 2016. Physical Activity for Cognitive and Mental Health in Youth: A Systematic Review of Mechanisms. *PEDIATRICS,* 138.
MAAS, C. & HOX, J. 2004. Robustness Issues in Multilevel Regression Analysis. *Statistica Neerlandica,* 58**,** 127-137.
MAN, A. W. C., LI, H. & XIA, N. 2020. Impact of Lifestyles (Diet and Exercise) on Vascular Health: Oxidative Stress and Endothelial Function. *Oxid Med Cell Longev,* 2020**,** 1496462.
MANACH, C., SCALBERT, A., MORAND, C., RÉMÉSY, C. & JIMÉNEZ, L. 2004. Polyphenols: food sources and bioavailability. *Am J Clin Nutr,* 79**,** 727-47.
MANCINI, E., BEGLINGER, C., DREWE, J., ZANCHI, D., LANG, U. E. & BORGWARDT, S. 2017. Green tea effects on cognition, mood and human brain function: A systematic review. *Phytomedicine,* 34**,** 26-37.

MARKOWITZ, S., FRIEDMAN, M. A. & ARENT, S. M. 2008. Understanding the relation between obesity and depression: causal mechanisms and implications for treatment. *Clinical Psychology: Science and Practice,* 15**,** 1.

MARSLAND, A. L., WALSH, C., LOCKWOOD, K. & JOHN-HENDERSON, N. A. 2017. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity,* 64**,** 208-219.

MASON, T. B., O'CONNOR, S. G., SCHEMBRE, S. M., HUH, J., CHU, D. & DUNTON, G. F. 2019. Momentary affect, stress coping, and food intake in mother-child dyads. *Health Psychol,* 38**,** 238-247.

MATSUMURA, K., YAMAKOSHI, T., NOGUCHI, H., ROLFE, P. & MATSUOKA, Y. 2012. Fish consumption and cardiovascular response during mental stress. *BMC research notes,* 5**,** 288.

MATTHEWS, K. A., MANUCK, S. B. & SAAB, P. G. 1986. Cardiovascular responses of adolescents during a naturally occurring stressor and their behavioral and psychophysiological predictors. *Psychophysiology,* 23**,** 198-209.

MAY, J. M. & HARRISON, F. E. 2013. Role of vitamin C in the function of the vascular endothelium. *Antioxid Redox Signal,* 19**,** 2068-83.

MCAULEY, E., MIHALKO, S. L. & BANE, S. M. 1996. Acute Exercise and Anxiety Reduction: Does the Environment Matter? *Journal of Sport and Exercise Psychology,* 18**,** 408-419.

MCEWEN, B. S. & GIANAROS, P. J. 2010. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci,* 1186**,** 190-222.

MCMILLAN, L., OWEN, L., KRAS, M. & SCHOLEY, A. 2011. Behavioural effects of a 10-day Mediterranean diet. Results from a pilot study evaluating mood and cognitive performance. *Appetite,* 56**,** 143-147.

MCNAIR, D., LORR, M. & DROPPLEMAN, L. 1981. Profile of Mood States Manual, 37–85. *San Diego, California, Educational and Industrial Testing Service*.

MENTAL HEALTH FOUNDATION. 2018a. *Stress: Are we coping?* [Online]. London: Mental Health Foundation. [Accessed].

MENTAL HEALTH FOUNDATION. 2018b. *Stress: statistics. [online]* [Online]. Mental Health Foundation. Available: https://www.mentalhealth.org.uk/explore-mental-health/statistics/stress-statistics [Accessed 27th July 2022].

MIDDLEKAUFF, H. R., NGUYEN, A. H., NEGRAO, C. E., NITZSCHE, E. U., HOH, C. K., NATTERSON, B. A., HAMILTON, M. A., FONAROW, G. C., HAGE, A. & MORIGUCHI, J. D. 1997. Impact of acute mental stress on sympathetic nerve activity and regional blood flow in advanced heart failure: implications for 'triggering' adverse cardiac events. *Circulation,* 96**,** 1835-42.

MILLER, A. A. & SPENCER, S. J. 2014. Obesity and neuroinflammation: A pathway to cognitive impairment. *Brain, Behavior, and Immunity,* 42**,** 10-21.

MILLER, K. B., HURST, W. J., PAYNE, M. J., STUART, D. A., APGAR, J., SWEIGART, D. S. & OU, B. 2008. Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *J Agric Food Chem,* 56**,** 8527-33.

MILLIS, R. M., AUSTIN, R. E., BOND, V., FARUQUE, M., GORING, K. L., HICKEY, B. M., BLAKELY, R. & DEMEERSMAN, R. E. 2009. Effects of high-carbohydrate and high-fat dietary treatments on measures of heart rate variability and sympathovagal balance. *Life Sciences,* 85**,** 141-145.

MONAHAN, K. D., FEEHAN, R. P., KUNSELMAN, A. R., PRESTON, A. G., MILLER, D. L. & LOTT, M. E. J. 2011. Dose-dependent increases in flow-mediated dilation following acute cocoa ingestion in healthy older adults. *Journal of Applied Physiology,* 111**,** 1568-1574.

MOORE, C. J. & CUNNINGHAM, S. A. 2012. Social Position, Psychological Stress, and Obesity: A Systematic Review. *Journal of the Academy of Nutrition and Dietetics,* 112**,** 518-526.

MORENO-ULLOA, A., ROMERO-PEREZ, D., VILLARREAL, F., CEBALLOS, G. & RAMIREZ-SANCHEZ, I. 2014. Cell membrane mediated (-)-epicatechin effects on upstream endothelial cell signaling: evidence for a surface receptor. *Bioorg Med Chem Lett,* 24**,** 2749-52.

MULLIGAN, A. A., LUBEN, R. N., BHANIANI, A., PARRY-SMITH, D. J., O'CONNOR, L., KHAWAJA, A. P., FOROUHI, N. G. & KHAW, K. T. 2014. A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open,* 4**,** e004503.

MUNIYAPPA, R., HALL, G., KOLODZIEJ, T. L., KARNE, R. J., CRANDON, S. K. & QUON, M. J. 2008. Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension2. *The American Journal of Clinical Nutrition,* 88**,** 1685-1696.

NAGASAWA, Y., ISHIDA, M., KOMURO, Y., USHIODA, S., HU, L. & SAKATANI, K. 2020. Relationship Between Cerebral Blood Oxygenation and Electrical Activity During Mental Stress Tasks: Simultaneous Measurements of NIRS and EEG. *In:* RYU, P.-D., LAMANNA, J. C., HARRISON, D. K. & LEE, S.-S. (eds.) *Oxygen Transport to Tissue XLI.* Cham: Springer International Publishing.

NAGAYA, N., YAMAMOTO, H., UEMATSU, M., ITOH, T., NAKAGAWA, K., MIYAZAWA, T., KANGAWA, K. & MIYATAKE, K. 2004. Green tea reverses endothelial dysfunction in healthy smokers. *Heart,* 90**,** 1485-1486.

NAPPO, F., ESPOSITO, K., CIOFFI, M., GIUGLIANO, G., MOLINARI, A. M., PAOLISSO, G., MARFELLA, R. & GIUGLIANO, D. 2002. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: Role of fat and carbohydrate meals. *J Am Coll Cardiol,* 39**,** 1145-1150.

NAQVI, T. Z. & HYUHN, H. K. 2009. Cerebrovascular mental stress reactivity is impaired in hypertension. *Cardiovasc Ultrasound,* 7**,** 32.

NATIONAL HEALTH SERVICE. 2020. *Statistics on Obesity, Physical Activity and Diet, England* [Online]. Available: https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-obesity-physical-activity-and diet/england-2020/part-6-diet-copy [Accessed 01 August 2023].

NATIONAL HEALTH SERVICE. 2022. *How to get more fibre into your diet* [Online]. [Accessed 2nd April 2024].

NEWENS, K. J. 2011. DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. *American Journal of Clinical Nutrition,* 94**,** 742-749.

NEWLIN, D. B. 1981. Relationships of pulse transmission times to pre-ejection period and blood pressure. *Psychophysiology,* 18**,** 316-21.

NEWMAN, E., O'CONNOR, D. B. & CONNER, M. 2006. Daily hassles and eating behaviour: The role of cortisol reactivity status. *Psychoneuroendocrinology,* 32**,** 125-132.

NYSTORIAK, M. A. & BHATNAGAR, A. 2018. Cardiovascular Effects and Benefits of Exercise. *Front Cardiovasc Med,* 5**,** 135.
O'CONNOR, D. B., JONES, F., CONNER, M., MCMILLAN, B. & FERGUSON, E. 2008. Effects of daily hassles and eating style on eating behavior. *Health Psychol,* 27**,** S20-31.
O'CONNOR, D. B., THAYER, J. F. & VEDHARA, K. 2021. Stress and Health: A Review of Psychobiological Processes. *Annu Rev Psychol,* 72**,** 663-688.
OGOH, S., BROTHERS, M., BARNES, Q., EUBANK, W. L., HAWKINS, M. N., PURKAYASTHA, S., O-YURVATI, A. & RAVEN, P. B. 2005. The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol,* 569**,** 697-704.
OLIVER, G. & WARDLE, J. 1999. Perceived Effects of Stress on Food Choice. *Physiology & Behavior,* Vol. 66**,** pp. 511–515.
OLIVER, G., WARDLE, J. & GIBSON, E. 2000. Stress and Food Choice: A Laboratory Study. *Psychosomatic medicine,* 62**,** 853-65.
ORMSHAW, N. G., JUNEJO, R. T. & MARSHALL, J. M. 2018. Forearm vasodilator responses to environmental stress and reactive hyperaemia are impaired in young South Asian men. *Eur J Appl Physiol,* 118**,** 979-988.
PAINE, N. J., BOSCH, J. A. & VAN ZANTEN, J. J. 2012. Inflammation and vascular responses to acute mental stress: implications for the triggering of myocardial infarction. *Curr Pharm Des,* 18**,** 1494-501.
PAINE, N. J., RING, C., ALDRED, S., BOSCH, J. A., WADLEY, A. J. & VELDHUIJZEN VAN ZANTEN, J. J. 2013a. Eccentric-exercise induced inflammation attenuates the vascular responses to mental stress. *Brain Behav Immun,* 30**,** 133-42.
PAINE, N. J., RING, C., BOSCH, J. A., DRAYSON, M. T., ALDRED, S. & VELDHUIJZEN VAN ZANTEN, J. J. 2014. Vaccine-induced inflammation attenuates the vascular responses to mental stress. *Int J Psychophysiol,* 93**,** 340-8.
PAINE, N. J., RING, C., BOSCH, J. A., MCINTYRE, D. & VELDHUIJZEN VAN ZANTEN, J. J. C. S. 2013b. The effect of acute mental stress on limb vasodilation is unrelated to total peripheral resistance. *Psychophysiology,* 50**,** 680-690.
PALMER, R. M., ASHTON, D. S. & MONCADA, S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature,* 333**,** 664-6.
PANNICKE, B., KAISER, T., REICHENBERGER, J. & BLECHERT, J. 2021. Networks of stress, affect and eating behaviour: Anticipated stress coping predicts goal-congruent eating in young adults. *The International Journal of Behavioral Nutrition and Physical Activity,* 18.
PAPIER, K., AHMED, F., LEE, P. & WISEMAN, J. 2015. Stress and dietary behaviour among first-year university students in Australia: Sex differences. *Nutrition,* 31**,** 324-330.
PARK, S. H., PETRUNOFF, N. A., WANG, N. X., VAN DAM, R. M., SIA, A., TAN, C. S. & MÜLLER-RIEMENSCHNEIDER, F. 2022. Daily park use, physical activity, and psychological stress: A study using smartphone-based ecological momentary assessment amongst a multi-ethnic Asian cohort. *Mental Health and Physical Activity,* 22**,** 100440.

PATIK, J. C., LENNON, S. L., FARQUHAR, W. B. & EDWARDS, D. G. 2021. Mechanisms of Dietary Sodium-Induced Impairments in Endothelial Function and Potential Countermeasures. *Nutrients,* 13.

PATIK, J. C., TUCKER, W. J., CURTIS, B. M., NELSON, M. D., NASIRIAN, A., PARK, S. & BROTHERS, R. M. 2018. Fast-food meal reduces peripheral artery endothelial function but not cerebral vascular hypercapnic reactivity in healthy young men. *Physiol Rep,* 6**,** e13867.

PEAIRS, A. D., RANKIN, J. W. & LEE, Y. W. 2011. Effects of acute ingestion of different fats on oxidative stress and inflammation in overweight and obese adults. *Nutrition Journal,* 10**,** 122.

PERDOMO, S. J., WARD, J., LIU, Y., VIDONI, E. D., SISANTE, J. F., KIRKENDOLL, K., BURNS, J. M. & BILLINGER, S. A. 2020. Cardiovascular disease risk is associated with middle cerebral artery blood flow velocity in older adults. *Cardiopulm Phys Ther J,* 31**,** 38-46.

PILCHER, J. J., MORRIS, D. M., DONNELLY, J. & FEIGL, H. B. 2015. Interactions between sleep habits and self-control. *Front Hum Neurosci,* 9**,** 284.

PLOTNICK, G. D., CORRETTI, M. C. & VOGEL, R. A. 1997. Effect of Antioxidant Vitamins on the Transient Impairment of Endothelium—Dependent Brachial Artery Vasoactivity Following a Single High-Fat Meal. *JAMA,* 278**,** 1682-1686.

PLOTNICK, M. D., D'URZO, K. A., GURD, B. J. & PYKE, K. E. 2017a. The influence of vitamin C on the interaction between acute mental stress and endothelial function. *European journal of applied physiology,* 117**,** 1657-1668.

PLOTNICK, M. D., D'URZO, K. A., GURD, B. J. & PYKE, K. E. 2017b. The influence of vitamin C on the interaction between acute mental stress and endothelial function. *Eur J Appl Physiol,* 117**,** 1657-1668.

POITRAS, V. J. & PYKE, K. E. 2013. The impact of acute mental stress on vascular endothelial function: evidence, mechanisms and importance. *Int J Psychophysiol,* 88**,** 124-35.

POITRAS, V. J., SLATTERY, D. J., LEVAC, B. M., FERGUS, S., GURD, B. J. & PYKE, K. E. 2014. The combined influence of fat consumption and repeated mental stress on brachial artery flow-mediated dilatation: A preliminary study. *Experimental Physiology,* 99**,** 715-728.

POOL, E., DELPLANQUE, S., COPPIN, G. & SANDER, D. 2015. Is comfort food really comforting? Mechanisms underlying stress-induced eating. *Food Research International,* 76**,** 207-215.

POWELL-WILEY, T. M., POIRIER, P., BURKE, L. E., DESPRÉS, J.-P., GORDON-LARSEN, P., LAVIE, C. J., LEAR, S. A., NDUMELE, C. E., NEELAND, I. J., SANDERS, P., ST-ONGE, M.-P. & NULL, N. 2021. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation,* 143**,** e984-e1010.

PRASAD, M., KHANNA, P., KATYAL, V. K. & VERMA, R. 2020. Acute Psychological Stress is a Trigger for Stroke: A Case-Crossover Study. *J Stroke Cerebrovasc Dis,* 29**,** 104799.

PRINCE, S. A., ADAMO, K. B., HAMEL, M. E., HARDT, J., GORBER, S. C. & TREMBLAY, M. 2008. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. *International Journal of Behavioral Nutrition and Physical Activity,* 5**,** 56.

PUCCINELLI, P. J., DA COSTA, T. S., SEFFRIN, A., DE LIRA, C. A. B., VANCINI, R. L., NIKOLAIDIS, P. T., KNECHTLE, B., ROSEMANN, T., HILL, L. & ANDRADE, M. S. 2021. Reduced level of physical activity during COVID-19 pandemic is associated with depression and anxiety levels: an internet-based survey. *BMC Public Health,* 21**,** 425.

PUTERMAN, E., WEISS, J., BEAUCHAMP, M. R., MOGLE, J. & ALMEIDA, D. M. 2017. Physical activity and negative affective reactivity in daily life. *Health Psychol,* 36**,** 1186-1194.

PUZSEROVA, A. & BERNATOVA, I. 2016a. Blood Pressure Regulation in Stress: Focus on Nitric Oxide-Dependent Mechanisms. *Physiol. Res,* 65**,** S309-S342.

PUZSEROVA, A. & BERNATOVA, I. 2016b. Blood pressure regulation in stress: focus on nitric oxide-dependent mechanisms. *Physiological research,* 65**,** S309-S342.

QIN, G., QIN, Y. & LIU, B. 2022. Association between BMI and health-related physical fitness: A cross-sectional study in Chinese high school students. *Frontiers in Public Health,* 10.

RADAVELLI-BAGATINI, S., SIM, M., BLEKKENHORST, L. C., BONDONNO, N. P., BONDONNO, C. P., WOODMAN, R., DICKSON, J. M., MAGLIANO, D. J., SHAW, J. E., DALY, R. M., HODGSON, J. M. & LEWIS, J. R. 2022. Associations of specific types of fruit and vegetables with perceived stress in adults: the AusDiab study. *Eur J Nutr,* 61**,** 2929-2938.

RAITAKARI, O. T., LAI, N., GRIFFITHS, K., MCCREDIE, R., SULLIVAN, D. & CELERMAJER, D. S. 2000. Enhanced peripheral vasodilation in humans after a fatty meal. *Journal of the American College of Cardiology,* 36**,** 417-422.

RALLIDIS, L. S., LEKAKIS, J., KOLOMVOTSOU, A., ZAMPELAS, A., VAMVAKOU, G., EFSTATHIOU, S., DIMITRIADIS, G., RAPTIS, S. A. & KREMASTINOS, D. T. 2009. Close adherence to a Mediterranean diet improves endothelial function in subjects with abdominal obesity. *Am J Clin Nutr,* 90**,** 263-8.

RAMIREZ-SANCHEZ, I., MAYA, L., CEBALLOS, G. & VILLARREAL, F. 2010. (-)-Epicatechin Activation of Endothelial Cell Endothelial Nitric Oxide Synthase, Nitric Oxide, and Related Signaling Pathways. *Hypertension,* 55**,** 1398-U198.

RAUBER, F., LOUZADA, M. L. D. C., MARTINEZ STEELE, E., DE REZENDE, L., F. M. , MILLETT, C., MONTEIRO, C., A. & LEVY, R., B. 2019. Ultra-processed foods and excessive free sugar intake in the UK: a nationally representative cross-sectional study. *BMJ Open,* 9**,** e027546.

RAZ, N., RODRIGUE, K. M., KENNEDY, K. M. & ACKER, J. D. 2007. Vascular health and longitudinal changes in brain and cognition in middle-aged and older adults. *Neuropsychology,* 21**,** 149-57.

REES, A., DODD, G. F. & SPENCER, J. P. E. 2018. The Effects of Flavonoids on Cardiovascular Health: A Review of Human Intervention Trials and Implications for Cerebrovascular Function. *Nutrients,* 10.

REICHENBERGER, J., PANNICKE, B., AREND, A.-K., PETROWSKI, K. & BLECHERT, J. 2021. Does stress eat away at you or make you eat? EMA measures of stress predict day to day food craving and perceived food intake as a function of trait stress-eating. *Psychology & Health,* 36**,** 129-147.

RENDEIRO, C., DONG, H., SAUNDERS, C., HARKNESS, L., BLAZE, M., HOU, Y., BELANGER, R. L., ALTIERI, V., NUNEZ, M. A., JACKSON, K. G., CORONA, G., LOVEGROVE, J. A. & SPENCER, J. P. 2016. Flavanone-rich citrus beverages counteract the transient decline in postprandial endothelial function

in humans: a randomised, controlled, double-masked, cross-over intervention study. *Br J Nutr,* 116**,** 1999-2010.

RENDEIRO, C., GUERREIRO, J. D. T., WILLIAMS, C. M. & SPENCER, J. P. E. 2012. Flavonoids as modulators of memory and learning: molecular interactions resulting in behavioural effects. *Proceedings of the Nutrition Society,* 71**,** 246-262.

RIBEIRO, M. M., SILVA, A. G., SANTOS, N. S., GUAZZELLE, I., MATOS, L. N., TROMBETTA, I. C., HALPERN, A., NEGRAO, C. E. & VILLARES, S. M. 2005a. Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children. *Circulation,* 111**,** 1915-23.

RIBEIRO, M. M., SILVA, A. G., SANTOS, N. S., GUAZZELLE, I., MATOS, L. N. J., TROMBETTA, I. C., HALPERN, A., NEGRAO, C. E. & VILLARES, S. M. F. 2005b. Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children. *Circulation,* 111**,** 1915-23.

ROBBINS, R. J., LEONCZAK, J., LI, J., JOHNSON, J. C., COLLINS, T., KWIK-URIBE, C. & SCHMITZ, H. H. 2012. Determination of flavanol and procyanidin (by degree of polymerization 1-10) content of chocolate, cocoa liquors, powder(s), and cocoa flavanol extracts by normal phase high-performance liquid chromatography: collaborative study. *J AOAC Int,* 95**,** 1153-60.

ROBERTS, C. J., CAMPBELL, I. C. & TROOP, N. 2014a. Increases in Weight during Chronic Stress are Partially Associated with a Switch in Food Choice towards Increased Carbohydrate and Saturated Fat Intake. *Eur. Eat. Disorders Rev,* 22**,** 77-82.

ROBERTS, C. J., CAMPBELL, I. C. & TROOP, N. 2014b. Increases in Weight during Chronic Stress are Partially Associated with a Switch in Food Choice towards Increased Carbohydrate and Saturated Fat Intake: Stress Food Choice Weight Increase. *European eating disorders review,* 22**,** 77-82.

ROBINSON, E., FLEMING, A. & HIGGS, S. 2014. Prompting healthier eating: testing the use of health and social norm based messages. *Health Psychology,* 33**,** 1057.

ROCHA, H. N. M., BATISTA, G. M. S., STORCH, A. S., GARCIA, V. P., TEIXEIRA, G. F., MENTZINGER, J., GOMES, E. A. C., CAMPOS, M. O., NOBREGA, A. C. L. & ROCHA, N. G. 2023a. Mental stress induces endothelial dysfunction by AT1R-mediated redox imbalance in overweight/ obese men. *Brazilian Journal of Medical and Biological Research,* 56**,** e12547.

ROCHA, H. N. M., TEIXEIRA, G. F., BATISTA, G. M. S., STORCH, A. S., GARCIA, V. P., MENTZINGER, J., GOMES, E. A. C., CAMPOS, M. O., NÓBREGA, A. C. L. & ROCHA, N. G. 2023b. AT1R blocker prevents mental stress induced retrograde blood flow in overweight/obese men. *Physiol Rep,* 11**,** e15566.

RODRIGUEZ-MATEOS, A., HEZEL, M., AYDIN, H., KELM, M., LUNDBERG, J. O., WEITZBERG, E., SPENCER, J. P. E. & HEISS, C. 2015. Interactions between cocoa flavanols and inorganic nitrate: Additive effects on endothelial function at achievable dietary amounts. *Free Radical Biology and Medicine,* 80**,** 121-128.

RODRIGUEZ-MATEOS, A., VAUZOUR, D., KRUEGER, C. G., SHANMUGANAYAGAM, D., REED, J., CALANI, L., MENA, P., DEL RIO, D. & CROZIER, A. 2014. Bioavailability, bioactivity and impact on health of dietary

flavonoids and related compounds: an update. *Archives of Toxicology,* 88**,** 1803-1853.

ROSSI, R., NUZZO, A., ORIGLIANI, G. & MODENA, M. G. 2008. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol,* 51**,** 997-1002.

RUDDOCK, H. K., BRUNSTROM, J. M., VARTANIAN, L. R. & HIGGS, S. 2019. A systematic review and meta-analysis of the social facilitation of eating. *The American Journal of Clinical Nutrition,* 110**,** 842-861.

RUF, A., NEUBAUER, A. B., KOCH, E. D., EBNER-PRIEMER, U., REIF, A. & MATURA, S. 2023. Individual differences in the dietary response to stress in ecological momentary assessment: Does the individual-difference model need expansion? *Applied Psychology: Health and Well-Being,* 15**,** 629-649.

RUIZ-GAYO, M., GONZÁLEZ, M. C. & FERNÁNDEZ-ALFONSO, S. 2006. Vasodilatory effects of cholecystokinin: New role for an old peptide? *Regulatory Peptides,* 137**,** 179-184.

SALA, M., BROSOF, L. C., ROSENFIELD, D., FERNANDEZ, K. C. & LEVINSON, C. A. 2017. Stress is associated with exercise differently among individuals with higher and lower eating disorder symptoms: An ecological momentary assessment study. *Int J Eat Disord,* 50**,** 1413-1420.

SAMIERI, C., PERIER, M. C., GAYE, B., PROUST-LIMA, C., HELMER, C., DARTIGUES, J. F., BERR, C., TZOURIO, C. & EMPANA, J. P. 2018. Association of Cardiovascular Health Level in Older Age With Cognitive Decline and Incident Dementia. *JAMA,* 320**,** 657-664.

SÁNCHEZ–FERNÁNDEZ, C., GONZÁLEZ, C., MERCER, L. D., BEART, P. M., RUIZ–GAYO, M. & FERNÁNDEZ-ALFONSO, M. S. 2003. Cholecystokinin Induces Cerebral Vasodilatation via Presynaptic CCK2 Receptors: New Implications for the Pathophysiology of Panic. *Journal of Cerebral Blood Flow & Metabolism,* 23**,** 364-370.

SANDOO, A., VELDHUIJZEN VAN ZANTEN, J. J. C. S., METSIOS, G. M., CARROLL, D. & KITAS, G. D. 2010. The Endothelium and Its Role in Regulating Vascular Tone. *The Open Cardiovascular Medicine Journal,* 4**,** 302-312.

SANSONE, R., OTTAVIANI, J. I., RODRIGUEZ-MATEOS, A., HEINEN, Y., NOSKE, D., SPENCER, J. P., CROZIER, A., MERX, M. W., KELM, M., SCHROETER, H. & HEISS, C. 2017. Methylxanthines enhance the effects of cocoa flavanols on cardiovascular function: randomized, double-masked controlled studies. *American Journal of Clinical Nutrition,* 105**,** 352-360.

SANSONE, R., RODRIGUEZ-MATEOS, A., HEUEL, J., FALK, D., SCHULER, D., WAGSTAFF, R., KUHNLE, G. G., SPENCER, J. P., SCHROETER, H., MERX, M. W., KELM, M., HEISS, C. & FLAVIOLA CONSORTIUM, E. U. T. F. P. 2015. Cocoa flavanol intake improves endothelial function and Framingham Risk Score in healthy men and women: a randomised, controlled, double-masked trial: the Flaviola Health Study. *Br J Nutr,* 114**,** 1246-55.

SANTOS, K. S. D., JUNIOR, O., TAVARES, I. R. G., VOLINO-SOUZA, M., OLIVEIRA, G. V. & ALVARES, T. D. S. 2023. A single dose of microencapsulated cocoa supplementation attenuated eccentric exercise-induced endothelial dysfunction. *Int J Food Sci Nutr,* 74**,** 373-381.

SANTOS-GARCÍA, D., BLANCO, M., SERENA, J., RODRÍGUEZ-YÁÑEZ, M., LEIRA, R. & CASTILLO, J. 2011. Impaired brachial flow-mediated dilation is a predictor of a new-onset vascular event after stroke. *Cerebrovasc Dis,* 32**,** 155-62.

SAXENA, S., VAN OMMEREN, M., TANG, K. C. & ARMSTRONG, T. P. 2005. Mental health benefits of physical activity. *Journal of Mental Health,* 14**,** 445-451.

SCARBOROUGH, P., BHATNAGAR, P., WICKRAMASINGHE, K., SMOLINA, K., MITCHELL, C. & RAYNER, M. 2010. *Coronary Heart Disease Statistics 2010* [Online]. London, UK. [Accessed 23 April 2024].

SCHNEIDERMAN, N., IRONSON, G. & SIEGEL, S. D. 2005. Stress and health: psychological, behavioral, and biological determinants. *Annu Rev Clin Psychol,* 1**,** 607-28.

SCHROETER, H., HEISS, C., BALZER, J., KLEINBONGARD, P., KEEN, C. L., HOLLENBERG, N. K., SIES, H., KWIK-URIBE, C., SCHMITZ, H. H. & KELM, M. 2006. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci U S A,* 103**,** 1024-9.

SCHULTCHEN, D., REICHENBERGER, J., MITTL, T., WEH, T. R. M., SMYTH, J. M., BLECHERT, J. & POLLATOS, O. 2019. Bidirectional relationship of stress and affect with physical activity and healthy eating. *British Journal of Health Psychology,* 24**,** 315-333.

SCIENTIFIC ADVISORY COMMITTEE ON NUTRITION. 2019. *Saturated Fats and health - GOV.UK, Scientific Advisory Committee on Nutrition: reports and position statements* [Online]. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/814995/SACN_report_on_saturated_fat_and_health.pdf [Accessed 01 August 2023].

SELYE, H. 1974. *Stress without distress.*, Springer. .

SHARMA, S., HRYHORCZUK, C. & FULTON, S. 2012. Progressive-ratio Responding for Palatable High-fat and High-sugar Food in Mice. *JoVE***,** e3754.

SHERWOOD, A., ALLEN, M. T., FAHRENBERG, J., KELSEY, R. M., LOVALLO, W. R. & VANDOORNEN, L. J. P. 1990. Methodological Guidelines for Impedance Cardiography. *Psychophysiology,* 27**,** 1-23.

SHERWOOD, A., JOHNSON, K., BLUMENTHAL, J. A. & HINDERLITER, A. L. 1999. Endothelial function and hemodynamic responses during mental stress. *Psychosom Med,* 61**,** 365-70.

SHIFFMAN, S., STONE, A. A. & HUFFORD, M. R. 2008. Ecological momentary assessment. *Annu Rev Clin Psychol,* 4**,** 1-32.

SHIMABUKURO, M., CHINEN, I., HIGA, N., TAKASU, N., YAMAKAWA, K. & UEDA, S. 2007. Effects of dietary composition on postprandial endothelial function and adiponectin concentrations in healthy humans: A crossover controlled study. *Am J Clin Nutr,* 86**,** 923-928.

SHIN, Y., PARK, S. & CHOUE, R. 2009. Comparison of time course changes in blood glucose, insulin and lipids between high carbohydrate and high fat meals in healthy young women. *Nutr Res Pract,* 3**,** 128-33.

SHIVELY, C. A., APPT, S. E., CHEN, H., DAY, S. M., FRYE, B. M., SHALTOUT, H. A., SILVERSTEIN-METZLER, M. G., SNYDER-MACKLER, N., UBERSEDER, B., VITOLINS, M. Z. & REGISTER, T. C. 2020. Mediterranean diet, stress resilience, and aging in nonhuman primates. *Neurobiol Stress,* 13**,** 100254.

SHOEMAKER, L. N., WILSON, L. C., LUCAS, S. J. E., MACHADO, L. & COTTER, J. D. 2019. Cerebrovascular regulation is not blunted during mental stress. *Exp Physiol,* 104**,** 1678-1687.

SMITH, K. E., MASON, T. B., WANG, W. L., SCHUMACHER, L. M., PELLEGRINI, C. A., GOLDSCHMIDT, A. B. & UNICK, J. L. 2022a. Dynamic associations between anxiety, stress, physical activity, and eating regulation over the course of a behavioral weight loss intervention. *Appetite,* 168**,** 105706.

SMITH, K. E., O'CONNOR, S. M., MASON, T. B., WANG, S., DZUBUR, E., CROSBY, R. D., WONDERLICH, S. A., SALVY, S. J., FEDA, D. M. & ROEMMICH, J. N. 2021. Associations between objective physical activity and emotional eating among adiposity-discordant siblings using ecological momentary assessment and accelerometers. *Pediatr Obes,* 16**,** e12720.

SMITH, K. J. & AINSLIE, P. N. 2017. Regulation of cerebral blood flow and metabolism during exercise. *Exp Physiol,* 102**,** 1356-1371.

SMITH, L. E., AMLÔT, R., FEAR, N. T., MICHIE, S., RUBIN, G. J. & POTTS, H. W. W. 2022b. Psychological wellbeing in the English population during the COVID-19 pandemic: A series of cross-sectional surveys. *Journal of Psychiatric Research,* 153**,** 254-259.

SOMEYA, N., ENDO, M. Y., FUKUBA, Y., HIROOKA, Y. & HAYASHI, N. 2010. Effects of a mental task on splanchnic blood flow in fasting and postprandial conditions. *European Journal of Applied Physiology,* 108**,** 1107-1113.

SOROND, F. A., LIPSITZ, L. A., HOLLENBERG, N. K. & FISHER, N. D. L. 2008. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatric Disease and Treatment,* 4**,** 433-440.

SOTHMANN, M. S., BUCKWORTH, J., CLAYTOR, R. P., COX, R. H., WHITE-WELKLEY, J. E. & DISHMAN, R. K. 1996. Exercise training and the cross-stressor adaptation hypothesis. *Exercise and Sport Sciences Reviews,* 24**,** 267–287.

SPAETH, A. M., DINGES, D. F. & GOEL, N. 2014. Sex and race differences in caloric intake during sleep restriction in healthy adults1234. *The American Journal of Clinical Nutrition,* 100**,** 559-566.

SPIEKER, L. E., HÜRLIMANN, D., RUSCHITZKA, F., CORTI, R., ENSELEIT, F., SHAW, S., HAYOZ, D., DEANFIELD, J. E., LÜSCHER, T. F. & NOLL, G. 2002. Mental Stress Induces Prolonged Endothelial Dysfunction via Endothelin-A Receptors. *Circulation,* 105**,** 2817-2820.

ST-ONGE, M.-P., ROBERTS, A. L., CHEN, J., KELLEMAN, M., O'KEEFFE, M., ROYCHOUDHURY, A. & JONES, P. J. H. 2011. Short sleep duration increases energy intakes but does not change energy expenditure in normal-weight individuals123. *The American Journal of Clinical Nutrition,* 94**,** 410-416.

STEER, P., SARABI, D. M., KARLSTRÖM, B., BASU, S., BERNE, C., VESSBY, B. & LIND, L. 2003. The effect of a mixed meal on endothelium-dependent vasodilation is dependent on fat content in healthy humans. *Clin Sci (Lond),* 105**,** 81-7.

STEINBERG, H. O., TARSHOBY, M., MONESTEL, R., HOOK, G., CRONIN, J., JOHNSON, A., BAYAZEED, B. & BARON, A. D. 1997. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest,* 100**,** 1230-1239.

STEPTOE, A., HAMER, M. & CHIDA, Y. 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun,* 21**,** 901-12.
STEPTOE, A. & KIVIMÄKI, M. 2012. Stress and cardiovascular disease. *Nat Rev Cardiol,* 9**,** 360-70.
STRAHLER, J., NATER, U. M. & SKOLUDA, N. 2020. Associations between Health Behaviors and Factors on Markers of Healthy Psychological and Physiological Functioning: a Daily Diary Study. *Ann Behav Med,* 54**,** 22-35.
STRIKE, P. C. & STEPTOE, A. 2003. Systematic review of mental stress-induced myocardial ischaemia. *European Heart Journal,* 24**,** 690-703.
STRIKE, P. C. & STEPTOE, A. 2005. Behavioral and emotional triggers of acute coronary syndromes: a systematic review and critique. *Psychosom Med,* 67**,** 179-86.
STRÖHLE, A. 2009. Physical activity, exercise, depression and anxiety disorders. *Journal of Neural Transmission,* 116**,** 777-784.
STULTS-KOLEHMAINEN, M. A. & SINHA, R. 2014. The effects of stress on physical activity and exercise. *Sports Med,* 44**,** 81-121.
STUPIN, A., DRENJANCEVIC, I., SUSNJARA, P., DEBELJAK, Z., KOLOBARIC, N., JUKIC, I., MIHALJEVIC, Z., MARTINOVIC, G. & SELTHOFER-RELATIC, K. 2021. Is there association between altered adrenergic system activity and microvascular endothelial dysfunction induced by a 7-day high salt intake in young healthy individuals. *Nutrients,* 13 (5) (no pagination).
SUNDSTRÖM, J. & VASAN, R. S. 2006. Circulating biomarkers of extracellular matrix remodeling and risk of atherosclerotic events. *Current Opinion in Lipidology,* 17.
SUPPAN, E., PICHLER, G., BINDER-HESCHL, C., SCHWABERGER, B. & URLESBERGER, B. 2022. Three Physiological Components That Influence Regional Cerebral Tissue Oxygen Saturation. *Front Pediatr,* 10**,** 913223.
SUZUKI, T., HIRATA, K., ELKIND, M. S., JIN, Z., RUNDEK, T., MIYAKE, Y., BODEN-ALBALA, B., DI TULLIO, M. R., SACCO, R. & HOMMA, S. 2008. Metabolic syndrome, endothelial dysfunction, and risk of cardiovascular events: the Northern Manhattan Study (NOMAS). *American heart journal,* 156**,** 405-410.
TANIDA, M., KATSUYAMA, M. & SAKATANI, K. 2007. Relation between mental stress-induced prefrontal cortex activity and skin conditions: a near-infrared spectroscopy study. *Brain Res,* 1184**,** 210-6.
TEISALA, T., MUTIKAINEN, S., TOLVANEN, A., ROTTENSTEINER, M., LESKINEN, T., KAPRIO, J., KOLEHMAINEN, M., RUSKO, H. & KUJALA, U. M. 2014. Associations of physical activity, fitness, and body composition with heart rate variability-based indicators of stress and recovery on workdays: a cross-sectional study. *J Occup Med Toxicol,* 9**,** 16.
TENTOLOURIS, N., TSIGOS, C., PEREA, D., KOUKOU, E., KYRIAKI, D., KITSOU, E., DASKAS, S., DAIFOTIS, Z., MAKRILAKIS, K., RAPTIS, S. A. & KATSILAMBROS, N. 2003. Differential effects of high-fat and high-carbohydrate isoenergetic meals on cardiac autonomic nervous system activity in lean and obese women. *Metabolism,* 52**,** 1426-1432.
THIJSSEN, D. H. J., BLACK, M. A., PYKE, K. E., PADILLA, J., ATKINSON, G., HARRIS, R. A., PARKER, B., WIDLANSKY, M. E., TSCHAKOVSKY, M. E. & GREEN, D. J. 2011. Assessment of flow-mediated dilation in humans: a

methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology,* 300**,** H2-H12.

THIJSSEN, D. H. J., BRUNO, R. M., VAN MIL, A., HOLDER, S. M., FAITA, F., GREYLING, A., ZOCK, P. L., TADDEI, S., DEANFIELD, J. E., LUSCHER, T., GREEN, D. J. & GHIADONI, L. 2019. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J,* 40**,** 2534-2547.

THIJSSEN, D. H. J., DAWSON, E. A., TINKEN, T. M., CABLE, N. T. & GREEN, D. J. 2009. Retrograde Flow and Shear Rate Acutely Impair Endothelial Function in Humans. *Hypertension,* 53**,** 986-992.

THIJSSEN, D. H. J., MAIORANA, A. J., O'DRISCOLL, G., CABLE, N. T., HOPMAN, M. T. E. & GREEN, D. J. 2010. Impact of inactivity and exercise on the vasculature in humans. *European Journal of Applied Physiology,* 108**,** 845-875.

THOMAS, K. N., LEWIS, N. C., HILL, B. G. & AINSLIE, P. N. 2015. Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. *Am J Physiol Regul Integr Comp Physiol,* 309**,** R707-20.

TODA, N. & NAKANISHI-TODA, M. 2011a. How mental stress affects endothelial function. *Pflugers Arch,* 462**,** 779-94.

TODA, N. & NAKANISHI-TODA, M. 2011b. How mental stress affects endothelial function. *Pflugers Archiv : European journal of physiology,* 462**,** 779-94.

TONACIO, A. C., TROMBETTA, I. C., RONDON, M. U., BATALHA, L. T., KUNIYOSHI, F. H., LATERZA, M. C., SUZUKI, P. H., GOWDAK, M. M., BARRETTO, A. C., HALPERN, A., VILLARES, S. M. & NEGRAO, C. E. 2006a. Effects of diet and exercise training on neurovascular control during mental stress in obese women. *Brazilian Journal of Medical & Biological Research,* 39**,** 53-62.

TONACIO, A. C., TROMBETTA, I. C., RONDON, M. U. P. B., BATALHA, L. T., KUNIYOSHI, F. H. S., LATERZA, M. C., SUZUKI, P. H., GOWDAK, M. M. G., BARRETTO, A. C. P., HALPERN, A., VILLARES, S. M. F. & NEGRAO, C. E. 2006b. Effects of diet and exercise training on neurovascular control during mental stress in obese women. *Brazilian Journal of Medical and Biological Research,* 39**,** 53-62.

TRICCO, A. C., LILLIE, E., ZARIN, W., O'BRIEN, K. K., COLQUHOUN, H., LEVAC, D., MOHER, D., PETERS, M. D. J., HORSLEY, T., WEEKS, L., HEMPEL, S., AKL, A. E., CHANG, C., MCGOWAN, J., STEWART, L., HARTLING, L., ALDCROFT, A., WILSON, M. G., GARRITTY, C., LEWIN, S., GODFREY, C. M., MACDONALD, M. T., LANGLOIS, E. V., SOARES-WEISER, K., MORIARTY, J., CLIFFORD, T., TUNÇALP, Ö. & STRAUS, S. E. 2018. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Annals of Internal Medicine,* 169**,** 467-473.

TSAI, W. C., LI, Y. H., LIN, C. C., CHAO, T. H. & CHEN, J. H. 2004. Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci (Lond),* 106**,** 315-9.

TURNER, J. R. 1994. *Cardiovascular reactivity and stress: Patterns of physiological response,* New York, NY, US, Plenum Press.

TZENIOS, N., TAZANIOS, M., CHAHINE, M. AND BINTI JAMAL, P 2023. The Relationship between Fat Consumption and Mood Enhancement: A Comprehensive Review. *Special Journal of the Medical Academy and other Life Sciences,* 1.

USHARANI, P., SUDHARANI, E., KIRANKISHORE, K. & RAVEENDRANATH, P. 2017. Evaluation of the effect of a standardized aqueous extract of the fruits of Emblica officinalis on mental stress induced cardiovascular changes in healthy human subjects. *International Journal of Pharmaceutical Sciences and Research,* 8**,** 4138-4146.

VALLANCE, P., COLLIER, J. & MONCADA, S. 1989. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet,* 2**,** 997-1000.

VAN DER KOOIJ, M. A., FANTIN, M., REJMAK, E., GROSSE, J., ZANOLETTI, O., FOURNIER, C., GANGULY, K., KALITA, K., KACZMAREK, L. & SANDI, C. 2014. Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. *Nature Communications,* 5**,** 4995.

VELDHUIJZEN VAN ZANTEN, J. J. C. S., DE BOER, D., HARRISON, L. K., RING, C., CARROLL, D., WILLEMSEN, G. & DE GEUS, E. J. C. 2002. Competitiveness and hemodynamic reactions to competition. *Psychophysiology,* 39**,** 759-766.

VELDHUIJZEN VAN ZANTEN, J. J. C. S., KITAS, G. D., CARROLL, D. & RING, C. 2008. Increase in systemic vascular resistance during acute mental stress in patients with rheumatoid arthritis with high-grade systemic inflammation. *Biological Psychology,* 77**,** 106-110.

VELDHUIJZEN VAN ZANTEN, J. J. C. S., RING, C., BURNS, V. E., EDWARDS, K. M., DRAYSON, M. & CARROLL, D. 2004. Mental stress-induced hemoconcentration: Sex differences and mechanisms. *Psychophysiology,* 41**,** 541-551.

VELDHUIJZEN VAN ZANTEN, J. J. C. S., RING, C., CARROLL, D. & KITAS, G. D. 2005. Increased C reactive protein in response to acute stress in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases,* 64**,** 1299.

VIDYASAGAR, R., GREYLING, A., DRAIJER, R., CORFIELD, D. R. & PARKES, L. M. 2013. The effect of black tea and caffeine on regional cerebral blood flow measured with arterial spin labeling. *J Cereb Blood Flow Metab,* 33**,** 963-8.

VLACHOPOULOS, C., AZNAOURIDIS, K., ALEXOPOULOS, N., ECONOMOU, E., ANDREADOU, I. & STEFANADIS, C. 2005. Effect of dark chocolate on arterial function in healthy individuals. *American Journal of Hypertension,* 18**,** 785-791.

VOGEL, R. A., CORRETTI, M. C. & PLOTNICK, G. D. 1997. Effect of a Single High-Fat Meal on Endothelial Function in Healthy Subjects. *Am J Cardiol,* 79**,** 350-354.

VOGIATZOGLOU, A., MULLIGAN, A. A., BHANIANI, A., LENTJES, M. A. H., MCTAGGART, A., LUBEN, R. N., HEISS, C., KELM, M., MERX, M. W., SPENCER, J. P. E., SCHROETER, H., KHAW, K. T. & KUHNLE, G. G. C. 2015a. Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). *Free Radic Biol Med,* 84**,** 1-10.

VOGIATZOGLOU, A., MULLIGAN, A. A., LENTJES, M. A., LUBEN, R. N., SPENCER, J. P., SCHROETER, H., KHAW, K. T. & KUHNLE, G. G. 2015b. Flavonoid intake in European adults (18 to 64 years). *PLoS One,* 10**,** e0128132.

WADDELL, I. S. & ORFILA, C. 2023. Dietary fiber in the prevention of obesity and obesity-related chronic diseases: From epidemiological evidence to potential molecular mechanisms. *Critical Reviews in Food Science and Nutrition,* 63**,** 8752-8767.

WADLEY, A. J., VELDHUIJZEN VAN ZANTEN, J. J. & ALDRED, S. 2013. The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. *Age (Dordr),* 35**,** 705-18.

WADLEY, A. J., VELDHUIJZEN VAN ZANTEN, J. J. C. S., PAINE, N. J., DRAYSON, M. T. & ALDRED, S. 2014. Underlying inflammation has no impact on the oxidative stress response to acute mental stress. *Brain, Behavior, and Immunity,* 40**,** 182-190.

WAGNER, J. A., TENNEN, H., FINAN, P. H., WHITE, W. B., BURG, M. M. & GHUMAN, N. 2012. Lifetime History of Depression, Type 2 Diabetes, and Endothelial Reactivity to Acute Stress in Postmenopausal Women. *International Journal of Behavioral Medicine,* 19**,** 503-511.

WALLERATH, T., WITTE, K., SCHÄFER, S. C., SCHWARZ, P. M., PRELLWITZ, W., WOHLFART, P., KLEINERT, H., LEHR, H. A., LEMMER, B. & FÖRSTERMANN, U. 1999. Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proc Natl Acad Sci U S A,* 96**,** 13357-62.

WANG, D., RAO, H., WETMORE, G., FURLAN, P., KORCZYKOWSKI, M., DINGES, D. & DETRE, J. 2005. Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 17804-9.

WANG, J., ZHAO, D. Y., TIANO, S., ESTEBAN-FERNANDEZ, A., YUAN, B., SMITH, C., BRATHWAITE, J., JLAYER, Z., WU, Q. L., SIMON, J. E., TRAGESER, K. J. & PASINETTI, G. M. 2020. Prophylactic effect of flavanol rich preparation metabolites in promoting resilience to a mouse model of social stress. *Translational Psychiatry,* 10.

WANG, S., TANG, C., LIU, Y., BORDER, J. J., ROMAN, R. J. & FAN, F. 2022. Impact of impaired cerebral blood flow autoregulation on cognitive impairment. *Front Aging,* 3**,** 1077302.

WANG, X., LIU, B., XIE, L., YU, X., LI, M. & ZHANG, J. 2016. Cerebral and neural regulation of cardiovascular activity during mental stress. *BioMedical Engineering OnLine,* 15**,** 160.

WANG, Z., YANG, T. & FU, H. 2021. Prevalence of diabetes and hypertension and their interaction effects on cardio-cerebrovascular diseases: a cross-sectional study. *BMC Public Health,* 21**,** 1224.

WARDLE, J., CHIDA, Y., GIBSON, E. L., WHITAKER, K. L. & STEPTOE, A. 2011. Stress and Adiposity: A Meta-Analysis of Longitudinal Studies. *Obesity,* 19**,** 771-778.

WELLS, A. S., READ, N. W., UVNAS-MOBERG, K. & ALSTER, P. 1997. Influences of Fat and Carbohydrate on Postprandial Sleepiness, Mood, and Hormones. *Physiology & Behavior,* 61**,** 679-686.

WEST, S. G., KRICK, A. L., KLEIN, L. C., ZHAO, G., WOJTOWICZ, T. F., MCGUINESS, M., BAGSHAW, D. M., WAGNER, P., CEBALLOS, R. M., HOLUB, B. J. & KRIS-ETHERTON, P. M. 2010. Effects of diets high in walnuts and flax oil on hemodynamic responses to stress and vascular endothelial function. *Journal of the American College of Nutrition,* 29**,** 595-603.

WESTON, M. E., KOEP, J. L., LESTER, A. B., BARKER, A. R. & BOND, B. 2022. The acute effect of exercise intensity on peripheral and cerebral vascular function in healthy adults. *J Appl Physiol (1985),* 133**,** 461-470.

WESTPHAL, S. & LULEY, C. 2011. Flavanol-rich cocoa ameliorates lipemia-induced endothelial dysfunction. *Heart and Vessels,* 26**,** 511-515.
WHITE, R. L., RYAN, D., YOUNG, C., ELSTON, R. & ROSSI, T. 2023. How does the context of physical activity influence perceived mood and wellbeing after exercise? *Mental Health and Physical Activity,* 24**,** 100504.
WIDMER, R. J. & LERMAN, A. 2014. Endothelial dysfunction and cardiovascular disease. *Glob Cardiol Sci Pract,* 2014**,** 291-308.
WILBERT-LAMPEN, U., NICKEL, T., LEISTNER, D., GÜTHLIN, D., MATIS, T., VÖLKER, C., SPER, S., KÜCHENHOFF, H., KÄÄB, S. & STEINBECK, G. 2010. Modified Serum Profiles of Inflammatory and Vasoconstrictive Factors in Patients With Emotional Stress-Induced Acute Coronary Syndrome During World Cup Soccer 2006. *Journal of the American College of Cardiology,* 55**,** 637-642.
WILBERT-LAMPEN, U., TRAPP, A., MODRZIK, M., FIEDLER, B., STRAUBE, F. & PLASSE, A. 2006. Effects of corticotropin-releasing hormone (CRH) on endothelin-1 and NO release, mediated by CRH receptor subtype R2: a potential link between stress and endothelial dysfunction? *Journal of psychosomatic research,* 61**,** 453-60.
WILKINSON, I. B., MACCALLUM, H., COCKCROFT, J. R. & WEBB, D. J. 2002. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *Br J Clin Pharmacol,* 53**,** 189-92.
WILLIAMS, T. B., CORBETT, J., MCMORRIS, T., YOUNG, J. S., DICKS, M., ANDO, S., THELWELL, R. C., TIPTON, M. J. & COSTELLO, J. T. 2019. Cognitive performance is associated with cerebral oxygenation and peripheral oxygen saturation, but not plasma catecholamines, during graded normobaric hypoxia. *Exp Physiol,* 104**,** 1384-1397.
WILLIE, C. K., TZENG, Y.-C., FISHER, J. A. & AINSLIE, P. N. 2014a. Integrative regulation of human brain blood flow. *The Journal of Physiology,* 592**,** 841-859.
WILLIE, C. K., TZENG, Y. C., FISHER, J. A. & AINSLIE, P. N. 2014b. Integrative regulation of human brain blood flow. *J Physiol,* 592**,** 841-59.
WINOCUR, G. & GREENWOOD, C. E. 2005. Studies of the effects of high fat diets on cognitive function in a rat model. *Neurobiol Aging,* 26 Suppl 1**,** 46-9.
WIPFLI, B. M., RETHORST, C. D. & LANDERS, D. M. 2008. The anxiolytic effects of exercise: a meta-analysis of randomized trials and dose-response analysis. *J Sport Exerc Psychol,* 30**,** 392-410.
WORLD HEALTH ORGANISATION. 2020. *Global Health Estimates for 2019* [Online]. Available:http://www.who.int/news/item/09-12-2020-who-reveals-leading-causes-of-death-and-disability-worldwide-2000-2019 [Accessed 23 April 2024].
WORLD HEALTH ORGANISATION. 2021. *Cardiovascular diseases (cvds)* [Online]. Available:https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases(cvds)#:~:text=Cardiovascular%20diseases%20(CVDs)%20are%20the,%2D%20and%20middle%2Dincome%20countries. [Accessed 12 May 2023].
WORLD HEALTH ORGANISATION. 2022. *WHO European Regional Obesity Report 2022* [Online]. [Accessed 27 March 2024].
WRIGHT, L. J., WILLIAMS, S. E. & VELDHUIJZEN VAN ZANTEN, J. J. C. S. 2023. Associations between physical activity, sedentary behaviour, and stress using ecological momentary assessment: A scoping review. *Mental Health and Physical Activity,* 24**,** 100518.

WU, J., ZHU, L., DONG, X., SUN, Z., CAI, K., SHI, Y. & CHEN, A. 2022. Relationship between Physical Activity and Emotional Regulation Strategies in Early Adulthood: Mediating Effects of Cortical Thickness. *Brain Sciences,* 12**,** 1210.

YAMAKAWA, K., MATSUNAGA, M., ISOWA, T., KIMURA, K., KASUGAI, K., YONEDA, M., KANEKO, H. & OHIRA, H. 2009. Transient responses of inflammatory cytokines in acute stress. *Biological Psychology,* 82**,** 25-32.

YARIBEYGI, H., PANAHI, Y., SAHRAEI, H., JOHNSTON, T. P. & SAHEBKAR, A. 2017. The impact of stress on body function: A review. *Excli j,* 16**,** 1057-1072.

YOON, E. S., SO, W.-Y. & JANG, S. 2023. Association between Perceived Psychological Stress and Exercise Behaviors: A Cross-Sectional Study Using the Survey of National Physical Fitness. *Life,* 13**,** 2059.

ZANSTRA, Y. J. & JOHNSTON, D. W. 2011. Cardiovascular reactivity in real life settings: measurement, mechanisms and meaning. *Biol Psychol,* 86**,** 98-105.

ZAWADZKI, M. J., SMYTH, J. M. & COSTIGAN, H. J. 2015. Real-Time Associations Between Engaging in Leisure and Daily Health and Well-Being. *Ann Behav Med,* 49**,** 605-15.

ZELLNER, D. A., LOAIZA, S., GONZALEZ, Z., PITA, J., MORALES, J., PECORA, D. & WOLF, A. 2006. Food selection changes under stress. *Physiol Behav,* 87**,** 789-793.

ZENK, S. N., HOROI, I., MCDONALD, A., CORTE, C., RILEY, B. & ODOMS-YOUNG, A. M. 2014. Ecological momentary assessment of environmental and personal factors and snack food intake in African American women. *Appetite,* 83**,** 333-341.

ZHANG, Y., FU, R., SUN, L., GONG, Y. & TANG, D. 2019. How Does Exercise Improve Implicit Emotion Regulation Ability: Preliminary Evidence of Mind-Body Exercise Intervention Combined With Aerobic Jogging and Mindfulness-Based Yoga. *Front Psychol,* 10**,** 1888.

ZIMMERMAN, B., KUNDU, P., ROONEY, W. D. & RABER, J. 2021. The Effect of High Fat Diet on Cerebrovascular Health and Pathology: A Species Comparative Review. *Molecules,* 26.

ZOHAR, D., TZISCHINSKY, O., EPSTEIN, R. & LAVIE, P. 2005. The Effects of Sleep Loss on Medical Residents' Emotional Reactions to Work Events: a Cognitive-Energy Model. *Sleep,* 28**,** 47-54.