CARDIOVASCULAR HEALTH, DISEASE AND FUNCTION: Contributions of stress and diet, and consequences of type 2 diabetes

by

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ABSTRACT

Identifying the contributions to, and consequences of, impaired cardiovascular health and function is critical to inform effective prevention and treatment strategies. Mental stress and a high-fat diet are independent predictors of cardiovascular disease, and impaired vascular endothelial function (assessed via flow-mediated dilation; FMD) may be a common mechanistic link. Exercise can mitigate cardiovascular risk, but cardiovascular dysfunction (i.e. impaired oxygen delivery) can reduce exercise tolerance and decrease adherence. This may be characteristic of persons with Type 2 Diabetes (T2D), but this has never been investigated within the typical constellation of co-morbidities and associated medications in this population.

**Purpose:** 1) To evaluate whether the combined experience of mental stress and fat consumption (versus either stimulus alone) exacerbates: postprandial lipemia, stress responsiveness, and endothelial dysfunction. 2) To determine whether T2D (within the typical cluster of co-morbidities and medications) results in impaired exercising muscle oxygen delivery and associated reduced small muscle mass exercise tolerance.

**Methods:** In healthy persons, FMD was assessed before and hourly for 4-hours post-consumption of a high-fat or low-fat meal, with hourly mental stress (mental arithmetic, speech) or control (counting) tasks. In persons with T2D and matched Controls, forearm critical force (fCF_impulse) was used as an indicator of exercise tolerance; exercising muscle blood flow was measured during fCF_impulse and during the adjustment and steady state of submaximal rest-to-exercise and exercise-to-exercise transitions. Ultrasound was used to measure FMD and exercising muscle blood flow.

**Results:** 1) Repeated mental stress tasks did not impact postprandial lipemia following either meal; 2) Meal fat content did not influence hemodynamic stress responsiveness; 3) Meal fat content did not affect FMD, but endothelial function was modestly greater when the postprandial state was accompanied by mental stress; 4) Relative to matched Controls, representative persons with T2D did not have impaired small muscle mass exercise tolerance (fCF_impulse) or muscle blood flow (rate of adjustment, or amount during submaximal/maximal steady state).

**Conclusions:** These findings challenge the assertions that mental stress and fat consumption are universally detrimental, and that T2D, on top of the typical constellation of co-morbidities and medications, has an impact on exercising muscle blood flow and exercise tolerance.
CO-AUTHORSHIP

This dissertation is the work of Veronica J. Poitras, in collaboration with her supervisors, Dr. Kyra E. Pyke and Dr. Michael E. Tschakovsky.

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The definitive version is available at www.blackwell-synergy.com and www.expophysiol.org. © 2014 The Authors. Experimental Physiology © 2014 The Physiological Society. VJP and KEP contributed to the conception and design of the experiments. VJP led all data collection, with contributions from KEP and DJS. VJP analyzed and interpreted the data, with contributions from KEP. VJP wrote the manuscript, and all authors critically revised it for important intellectual content and approved the final version of the manuscript.
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Lack of independent effect of type 2 diabetes beyond characteristic co-morbidities and medications on small muscle mass exercising muscle blood flow and exercise tolerance

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<td>ACE inhibitor</td>
<td>angiotensin-converting enzyme inhibitor</td>
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<tr>
<td>Ach</td>
<td>acetylcholine</td>
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<td>angiotensin II</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BF</td>
<td>blood flow</td>
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<tr>
<td>BH₄</td>
<td>tetrahydrobiopterin</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<td>BOLD</td>
<td>blood oxygen level dependent</td>
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<td>BV</td>
<td>blood volume</td>
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<td>C</td>
<td>control</td>
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<tr>
<td>CAD</td>
<td>coronary artery disease</td>
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<td>CaO₂</td>
<td>arterial oxygen content</td>
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<td>CBR</td>
<td>carotid baroreflex</td>
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<td>CHO</td>
<td>carbohydrates</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>CME</td>
<td>continuous moderate exercise</td>
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<td>CO</td>
<td>cardiac output</td>
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<td>cold pressor test</td>
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<td>cardiac rehabilitation</td>
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<td>CRH</td>
<td>corticotropin-releasing hormone</td>
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<td>CSAR</td>
<td>cardiac sympathetic afferent reflex</td>
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<td>CV</td>
<td>coefficient of variation</td>
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<td>cardiovascular disease</td>
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<td>DBP</td>
<td>diastolic blood pressure</td>
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<td>DEX</td>
<td>dexamethasone</td>
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<td>DHA</td>
<td>docosahexaenoic acid</td>
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<td>DICOM</td>
<td>digital imaging and communications in medicine</td>
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<td>ecSOD</td>
<td>extracellular superoxide dismutase</td>
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<td>EDL</td>
<td>extensor digitorum longus</td>
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<td>EF</td>
<td>endothelial function</td>
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<td>EFI</td>
<td>endothelial function index</td>
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<td>ELISA</td>
<td>enzyme-linked immunoassay</td>
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<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<td>EPA</td>
<td>eicosapentaenoic acid</td>
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<td>Epi</td>
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<td>equation</td>
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<td>ET-1</td>
<td>endothelin-1</td>
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<td>ETₐ</td>
<td>endothelin A receptor</td>
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<td>EX</td>
<td>exercise</td>
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<td>F</td>
<td>female</td>
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<tr>
<td>FAD, FADH₂</td>
<td>oxidized and reduced flavin adenine dinucleotide</td>
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<tr>
<td>FFBF</td>
<td>forearm blood flow</td>
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<tr>
<td>FC</td>
<td>fat consumption</td>
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<tr>
<td>fCF_{impulse}</td>
<td>forearm critical force</td>
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<td>FFA</td>
<td>free fatty acid</td>
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<tr>
<td>F₁</td>
<td>force impulse</td>
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<td>FMD</td>
<td>flow-mediated dilation</td>
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<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>FO</td>
<td>follicular</td>
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<tr>
<td>FVK</td>
<td>forearm vascular conductance</td>
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NO – nitric oxide
NO_2^- – nitrite
NO_3^- – nitrate
NS – not significant
NT – nitrotyrosine
O_2 – oxygen
O_2^- – superoxide
OGGT – oral glucose tolerance test
ON – on-transient
ONO_2^- – peroxynitrite
oxLDL – oxidized low density lipoprotein
P_2y – endothelial purinergic receptor
PAD – peripheral artery disease
PAR – physical activity recall
PCapO_2 – partial pressure of oxygen in the capillaries
PCr – phosphocreatine
Pi – inorganic phosphate
PIR – peak initial response
PmyoO_2 – muscle oxygenation (partial pressure of oxygen in myocytes)
PUFA – polyunsaturated fatty acids
R_1 – early recovery (2-3 min post-stress)
R_2 – late recovery (7-8 min post-stress)
RBC – red blood cell
RH – reactive hyperemia
RLP-C – remnant-like particle cholesterol
RM – repeated measures
ROS – reactive oxygen species
RPE – rating of perceived exertion
S – stress, stress task, stress condition
SA – stearic acid
SBP – systolic blood pressure
SD – standard deviation
SDT – status during testing
SF – saturated fat
SNA – sympathetic nervous system activity
SNAP - S-nitroso-N-acetyl-D,L-penicillamine
SNO – S-nitrosothiol
SNP – sodium nitroprusside
SNS – sympathetic nervous system
SOD – superoxide dismutase
SS – shear stress, steady state
SV – stroke volume
τ – “tau”; time constant
T – time
T1D – type 1 diabetes
T2D, T2DM – type 2 diabetes (mellitus)
TA – tibialis anterior
TBARS – thiobarbituric reactive substances
TCA – tricarboxylic acid cycle
TD – time delay
TG – triglyceride
TPR – total peripheral resistance
VCAM – vascular cellular adhesion molecule
VLDL – very low density lipoprotein
\dot{VO}_2 – oxygen consumption
vWF – von Willebrand factor
W’ – curvature constant of the force-time relationship
WC – waist circumference
Chapter 1

GENERAL INTRODUCTION
“Cardiovascular disease” (CVD) is an overarching term that refers to all diseases that affect the structure and function of the heart and/or blood vessels (including heart attack, stroke, coronary artery disease, hypertension and atherosclerosis), and it is a major cause of morbidity and mortality worldwide (12). In Canada alone, someone dies of CVD every 7 minutes (accounting for 29% of all deaths) (18, 49), and CVD costs the Canadian economy more than $20.9 billion each year in terms of physician services, hospital costs, lost wages, and reduced productivity (53). CVD has a complex etiology that involves both genetic and “lifestyle” factors such as stress, consumption of a high-fat diet, smoking, and physical inactivity (12, 40, 45). Identifying the physiological mechanisms linking these factors with the development of CVD will advance our understanding of these complex relationships and help to inform public health recommendations aimed at reducing the risk of CVD.

Type 2 diabetes (T2D) is another disease of epidemic proportions; it is projected to affect more than 277 million people worldwide by the year 2030 (57). Although it is by definition a metabolic disorder (23), the primary cause of morbidity and mortality in this population is cardiovascular disease; CVD accounts for up to 80% of deaths in persons with T2D (47). In this regard, while physical inactivity is a known risk factor for CVD (12), physical activity is considered a critical lifestyle factor that can aid in delaying or preventing its development (36, 58), and it is of particular importance in the management of T2D (1). However, evidence is accumulating to suggest that persons with T2D have reduced exercise tolerance (4, 29, 37, 38), which may be due at least in part to impaired cardiovascular support of exercising muscle [i.e. reduced exercising muscle blood flow and compromised oxygen delivery (26, 29), which could contribute to the progression of fatigue (2, 24)]. In other words, physical activity can mitigate cardiovascular risk, but a dysfunctional cardiovascular system can reduce exercise tolerance, and thereby decrease the ability or willingness of persons to engage in physical activity. This could result in a self-perpetuating reduction in functional capacity and cardiovascular health. Elucidating whether and/or how oxygen delivery is impaired during exercise in T2D, and whether
this influences exercise tolerance, could provide a foundation for novel treatment approaches
designed to improve patient adherence to physical activity in the management of the disease.

Therefore, within the broad theme of cardiovascular health, disease and function, this
dissertation has a dual focus: 1) Contributions to CVD: investigation of mechanisms linking two
lifestyle factors (stress and fat intake) with the development of CVD, and 2) Cardiovascular
consequences of T2D: investigation of exercising muscle oxygen delivery and exercise tolerance
in persons with T2D (see Figure 1-1 for an illustration of these themes). A systematic
exploration of the contributions to, and consequences of, impaired cardiovascular health
and function is vital to guide the advancement of effective prevention and treatment
strategies.

1.1 FOCUS 1. Contributions to CVD: Investigation of mechanisms linking stress
and high fat meal consumption with the development of CVD

Mental stress and consumption of a high-fat diet are “lifestyle” factors that have been
independently linked to the development of CVD (40, 52). While the mechanisms are not fully
understood, recent studies have revealed that postprandial blood lipid excursions (5, 35) and the
magnitude (“reactivity”) and duration (“recovery”) of acute stress responses (10) are positively
associated with cardiovascular risk status and incident cardiovascular events, signifying that acute
physiological responses to these stimuli contribute to the chronic relationships between dietary
fat, mental stress and CVD. Temporary impairments in vascular endothelial cell function may be
a common underlying mechanism; when experienced separately, both acute mental stress (7, 17)
and consumption of a high-fat meal (16, 55) have been found to evoke transient (45 min to 4 h)
endothelial dysfunction. This is important because the proper functioning of endothelial cells is
essential for the maintenance of vascular health (11, 15), and a dysfunctional endothelium

1“Mental stress” may be defined as a state of threatened homeostasis, either perceived or real, provoked by
a psychological stressor (e.g. fear, anxiety, social defeat, humiliation, and disappointment) (6, 32).
Figure 1-1. Simplified model of dissertation themes and hypotheses tested.

(A) Contributions to cardiovascular disease: Proposed model of the pathways linking mental stress and fat consumption to the development of cardiovascular disease. (B) Possible cardiovascular consequences of Type 2 Diabetes: Proposed cycle whereby decrements in cardiovascular function (which could be present in persons with T2D) may result in reduced exercising muscle oxygen ($O_2$) delivery and impaired exercise tolerance, with subsequently lower levels of physical activity and exacerbation and/or perpetuation of cardiovascular health and function issues.

$+$ = augments; $?$ = hypothesized link.
contributes to the development of atherosclerosis (8, 13, 25). Thus temporary impairments following repeated exposures to stress or fat intake could accumulate over time to result in a clinically relevant loss of endothelial vasoprotection and contribute to the development of atherosclerotic CVD.

Mechanistically, mental stress-induced impairments in endothelial function derive from the physiological stress response [i.e. the sympathetic nervous system and hypothalamic-pituitary-adrenal axis (7, 19)], while fat-consumption-induced impairments result primarily from elevated blood lipids and an associated increase in oxidative stress (56). Importantly, there is evidence to suggest that the combined experience of mental stress and fat consumption could exacerbate endothelial dysfunction versus either stimulus alone; stress could exaggerate the rise in blood lipid levels following fat consumption (i.e. postprandial lipemia) (3, 28), and consumption of a high-fat meal could amplify stress responsiveness (14, 21). Therefore, experiencing mental stress in conjunction with consumption of high-fat foods may worsen the impairment of endothelial function and augment cardiovascular risk, compared to experiences of either stimulus in isolation.

These potential interactions are important because activation of the physiological stress response and ingestion of fat often occur together in everyday life (31). However, no previous studies have investigated the combined impact of high-fat meal consumption and repeated episodes of mental stress on vascular endothelial function, and no studies have examined the time course of a fat-stress response interaction. This is important because metabolic parameters change for several hours following meal consumption. In addition, the effect of fat consumption on an appropriate index of hemodynamic stress “recovery” has not been investigated; this is important because this aspect of stress responsiveness affords additional predictive value beyond that of the magnitude (“reactivity”) of the response alone in terms of CVD risk (10, 50). Therefore, the objectives and hypotheses of Focus 1 were the following.
1.1.1 Objectives

1. To investigate the combined effects of mental stress and fat consumption on postprandial lipemia and endothelial function, as assessed via brachial artery flow-mediated dilation (FMD).

2. To determine the impact of a high-fat meal on the magnitude and stability of hemodynamic reactivity to, and recovery from, varied mental stressors over a 4 hour period.

1.1.2 Hypotheses

1. High-fat meal consumption followed by repeated episodes of mental stress would result in a greater impairment of endothelial function (FMD) versus either high-fat meal consumption alone or repeated episodes of mental stress following a low-fat meal.

2. Mental stress in combination with high-fat meal consumption would evoke a larger postprandial lipemia versus consumption of a high-fat meal alone.

3. Stress reactivity (magnitude) and recovery (duration) would be augmented following consumption of a high- versus low-fat meal, and these differences would be greatest at 3-4 hours post-meal consumption when postprandial lipemia is expected to peak.

1.2 FOCUS 2. Cardiovascular consequences of T2D: investigation of exercising muscle oxygen delivery and exercise tolerance in persons with T2D

It is commonly stated that persons with T2D have exaggerated intolerance to exercise (39), and that this contributes to poor adherence to physical activity (54), which is an important treatment modality (46, 51). This tenet is based largely on observations of slowed oxygen uptake kinetics and reduced peak oxygen consumption capacity (\(\dot{V}O_2\)peak) relative to (self-reported) activity-matched Controls (4, 29, 37, 38). Importantly however, there is no evidence of reduced
exercise tolerance when matched for \( \dot{V}O_2 \) peak, and since \( \dot{V}O_2 \) kinetics and peak respond normally to exercise training in this population (39) it is unclear how much of the “impairment” in tolerance is due to sedentary behaviour versus endogenous disease-related dysfunction.

If in fact exaggerated exercise intolerance is present in persons with T2D, it has been proposed that reduced exercising muscle blood flow and associated impaired oxygen delivery, factors which are known to increase fatigue progression (2, 24), are at least partly responsible (26, 29). T2D is characterized by vascular pathologies (20, 22, 33, 48) which could impact vascular responsiveness in exercising muscle, blunting oxygen delivery and exercise tolerance. However, key knowledge gaps exist.

First, persons with T2D typically also present with obesity (44), hypertension (43) and dyslipidemia (9), and take associated medications to manage these co-morbidities in addition to T2D-specific medications (27, 47). Yet no previous studies have investigated the whether there is an impact of T2D on exercising muscle oxygen delivery within the constellation of prevalent co-morbidities and medications that accompany this disease. Thus, previous findings lack ecological validity and clinical relevance for this population. Second, if present, the degree to which compromised oxygen supply might explain exercise intolerance in this population is unknown. Third, both the initial dynamic adjustment and the eventual steady state of muscle oxygen delivery can impact myocellular oxygenation, and thus each phase of the response must be considered with regard to a potential muscle oxygen delivery impairment. However, the dynamics of the oxygen delivery response per se have only been examined in one study (30), and this was done in terms of vascular conductance (versus blood flow specifically) and only in the transition from rest to a single exercise intensity. While this is an important initial investigation, our understanding remains limited because: a) impairment may be intensity-dependent in T2D (38), b) propensity for impairment may be different in rest-to-exercise versus exercise-to-exercise transitions (41, 42), and c) much of daily living involves transitioning between different intensities of physical activity, making exercise-to-exercise transitions particularly relevant.
Lastly, impaired oxygen delivery could result from peripheral vascular dysfunction, but it could also be due to vasoconstriction secondary to impaired cardiac function (34, 59), and thus to isolate peripheral vascular mechanisms requires the use of a small muscle mass modality that would be unlikely to be impacted by central hemodynamic responses. If present, reduced muscle blood flow and small muscle mass exercise tolerance would be one factor contributing to whole-body exercise intolerance in this population. With this as a foundation, the specific objectives and hypotheses of the second research focus were the following.

1.2.1 Objectives

1. To assess small muscle mass (forearm) exercising muscle blood flow and exercise tolerance (via a forearm critical force test) in representative persons with T2D versus Control subjects matched for age, body mass index (BMI), aerobic fitness, co-morbidities, and non-T2D medications.

2. To characterize the oxygen delivery response during submaximal single-leg exercise in persons with T2D [in terms of: (a) the dynamics of the rest-to-exercise response, (b) the dynamics of a low-to-moderate intensity exercise transition, and (c) the steady state response at each intensity] and to determine whether and/or how this is impaired relative to age, sex, aerobic fitness, co-morbidity and non-T2D medication matched Controls.

1.2.2 Hypotheses

1. Persons with T2D would have reduced exercising forearm muscle blood flow and associated reduced exercise tolerance (forearm critical force).

2. Persons with T2D would have reduced exercising leg muscle blood flow, in terms of both the rate and amount of adjustment, compared to the Control group.
1.3 References


49. **Statistics Canada.** Mortality, Summary List of Causes. 84F0209X. 2011.

51. **Stewart KJ.** Exercise Training and the Cardiovascular Consequences of Type 2 Diabetes and Hypertension: Plausible Mechanisms for Improving Cardiovascular Health. *JAMA* 288: 1622-1631, 2002.


Chapter 2 – LITERATURE REVIEW PART I:

The impact of acute mental stress on vascular endothelial function:

Evidence, mechanisms and importance

Published As:

2.1 Abstract

Cardiovascular disease is a principle cause of morbidity and mortality worldwide, and it has a complex etiology that involves lifestyle factors such as psychosocial stress. Recent evidence suggests that temporary impairments in vascular endothelial cell function may contribute to the relationship between stress and cardiovascular disease. Indeed, impaired endothelial function has been observed to occur transiently (lasting up to 1.5 h) following mental stress, and such periods of impairment could accumulate to become clinically relevant over the long term. The finding of acute stress induced endothelial dysfunction is not universal however, and both physiological (e.g. sympathetic nervous system and hypothalamic-pituitary-adrenal axis reactivity), and methodological factors contribute to the conflicting results. A clear understanding of the interaction between stress response activation and endothelial function is critical to elucidating the complexities of the relationship between psychosocial stress and cardiovascular disease. Therefore, the purpose of this review is: 1) to briefly describe the importance of vascular endothelial function and how it is assessed, 2) to review the literature investigating the impact of acute mental stress on endothelial function in humans, identifying factors that may explain contradictory results, and 3) to summarize our current understanding of the mechanisms that may mediate an acute mental stress-endothelial function interaction.
2.2 Introduction

The physiological response to mental stress is much the same as that to physical stressors and involves adaptive changes that act to preserve health and life, such as: mobilization of fuels, inhibition of functions that are nonessential to survival (e.g. growth and reproduction), and redistribution of blood flow from the viscera to the skeletal muscles (Widmaier et al. 2006). These responses to stress are mediated primarily by the stress hormone cortisol [a glucocorticoid secreted via the hypothalamic-pituitary-adrenal (HPA) axis; Figure 2-1] and by sympathetic nervous system activity (SNA) (Widmaier et al. 2006). While these responses may be useful in the short-term, chronic activation of stress response pathways may be maladaptive.

Several studies have identified a link between increased psychosocial stress and increased risk of cardiovascular disease [reviewed in (Rozanski et al. 1999; Black and Garbutt 2002; Greenwood et al. 1996; McEwen and Stellar 1993; Pickering 2001)], however the physiological mechanisms that underlie this connection remain somewhat unclear. In the past decade, evidence supporting a deleterious impact of stress on arterial endothelial cell function has begun to accumulate (Broadley et al. 2005; Ghiadoni et al. 2000; Gottdiener et al. 2003; Lind et al. 2002; Sarabi and Lind 2001; Spieker et al. 2002) and this is of critical importance as there is a growing consensus that endothelial dysfunction precedes and plays a role in the development of atherosclerosis (Celermajer et al. 1992; Drexler and Hornig 1999; Kawashima and Yokoyama 2004). Therefore, endothelial dysfunction may provide an important physiological link between psychosocial stress and cardiovascular disease (Rozanski et al. 1999).

The immediate impact of acute stress on endothelial function may be an important component of the chronic interaction between stress and cardiovascular health. In the short term, stress-induced endothelial dysfunction could contribute to an existing potential for myocardial or peripheral ischemia (Krantz et al. 1996; Rozanski et al. 1988; Gottdiener et al. 1994; Krantz et al. 2000; Sheps et al. 2002; Peix et al. 2006). In addition, if brief stressful experiences produce
Figure 2-1. The hypothalamic-pituitary-adrenal (HPA) axis.
The axis is activated by neural inputs including those related to stressful stimuli (e.g. mental stress) and non-stress inputs like circadian rhythms. Cortisol exerts negative feedback control over the system as indicated by dashed arrows. ACTH = adrenocorticotropic hormone; CRH = corticotropin-releasing hormone. Adapted from Widmaier et al., (2006) (Fig. 11-18, page 366) (Widmaier et al. 2006).
temporary but prolonged periods of endothelial dysfunction (Broadley et al. 2005; Gottdiener et al. 2003; Lind et al. 2002; Sarabi and Lind 2001; Spieker et al. 2002), repeat exposure could cumulatively result in a clinically relevant loss of vasoprotection (Black and Garbutt 2002) (Figure 2-2). Although several studies have identified an impairment in endothelial function during or following acute stressors (Broadley et al. 2005; Gottdiener et al. 2003; Lind et al. 2002; Sarabi and Lind 2001; Spieker et al. 2002), this finding is not universal (Szijgyarto et al. 2012; Dyson et al. 2006; Harris et al. 2000; Jambrik et al. 2005). The purpose of this review is therefore: 1) to provide a brief synopsis of the importance of vascular endothelial function and how it is assessed, 2) to review the literature investigating the impact of acute mental stress on endothelial function in humans, identifying factors that may explain conflicting results and 3) to summarize our current understanding of the mechanisms that may mediate an acute mental stress-endothelial function interaction. Readers are directed to Toda and Nakanishi-Toda (2011) for a review that covers the impact of chronic stress on endothelial function.

### 2.3 Vascular endothelial function: importance and assessment

The endothelium comprises the innermost lining of the blood vessels. Only one cell thick, the endothelium is a dynamic tissue that is responsible for many functions including vasoregulation, influencing vascular architecture, and determining the nature of the interaction between the blood and the vessel wall (Behrendt and Ganz 2002; Lockhart et al. 2006; Luscher and Noll 1995). Under healthy conditions, a properly functioning endothelium promotes vasodilation (Behrendt and Ganz 2002; Luscher and Noll 1995), inhibits vascular smooth muscle cell migration and proliferation (Cornwell et al. 1994; Garg and Hassid 1989; Sarkar et al. 1996), and prevents platelet aggregation and the adhesion of platelets and monocytes to the walls of the blood vessels (Gauthier et al. 1995; Kubes et al. 1991; Radomski et al. 1987a, 1987c).
Figure 2-2. Suggested model of the relationship between acute mental stress, endothelial dysfunction, and cardiovascular disease. Endothelial dysfunction represents a physiological mechanistic link between mental stress and cardiovascular disease. CRH = corticotropin-releasing hormone; ET-1 = Endothelin-1; FFAs = free fatty acids; NO = nitric oxide; ROS = reactive oxygen species; SNA = sympathetic nervous system activity.
These actions are due at least in part to endothelially derived nitric oxide (NO) (Behrendt and Ganz 2002; Cornwell et al. 1994; Garg and Hassid 1989; Gauthier et al. 1995; Joannides et al. 1995; Kubes et al. 1991; Lockhart et al. 2006; Luscher and Noll 1995; Radomski et al. 1987b; Radomski et al. 1987a; Radomski et al. 1987c; Sarkar et al. 1996). In contrast, a dysfunctional endothelium mediates vasoconstriction [mainly via increased production of endothelin-1 (ET-1)] (Yanagisawa et al. 1988), an increase in smooth muscle cell proliferation, and adhesion of platelets and monocytes (Cooke and Dzau 1997; Luscher and Noll 1995). Thus while a functional endothelium exerts vasoprotective effects, a dysfunctional endothelium mediates conditions that are known to contribute to ischemic events and the pathogenesis of atherosclerosis (Lockhart et al. 2006; Luscher and Noll 1995). Indeed, endothelial dysfunction predicts cardiovascular events in both healthy individuals (Shechter et al. 2009; Yeboah et al. 2007; Yeboah et al. 2009; Shimbo et al. 2007; Rossi et al. 2008) and those with established cardiovascular disease (Karatzis et al. 2006; Patti et al. 2005; Meyer et al. 2005; Fischer et al. 2005; Gokce et al. 2003; Brevetti et al. 2003; Modena et al. 2002; Neunteufl et al. 2000; Heitzer et al. 2001; Schachinger et al. 2000; Suwaidi et al. 2000; Perticone et al. 2001).

Evaluation of endothelial function exploits the endothelium’s vasoregulatory ability in response to a standardized stimulus (Lockhart et al. 2006; Drexler and Hornig 1999). Specifically, human endothelial function is usually assessed by either 1) measuring the magnitude of increase in flow (an index of resistance vessel dilation) or conduit artery diameter elicited by stepwise infusions of endothelium-dependent vasodilator substances (such as acetylcholine; ACh), or 2) by measuring the increase in arterial diameter following an increase in blood flow associated shear stress (endothelium dependent flow mediated dilation; FMD). The most common methodology employed to increase shear stress for FMD assessment is a reactive hyperemia protocol, achieved with the release of a temporary limb occlusion (Celermajer et al. 1992; Thijssen et al. 2011). Handgrip exercise and distal skin warming procedures have also
been used to elevate conduit artery shear stress for FMD evaluation (Szigyarto et al. 2012; Pyke et al. 2008; Bellien et al. 2010; Grzelak et al. 2010). In such tests of endothelial function, healthy arteries generally dilate ~5-15%, and blood flow increases several fold (Walther et al. 2004). The brachial artery is the most common site of FMD evaluation and brachial artery FMD has been shown to correlate with that of the more clinically relevant coronary arteries (Takase et al. 1998; Anderson et al. 1995; Takase et al. 2005). Additionally, control tests of vascular smooth muscle responsiveness to exogenous delivery of NO (i.e. endothelium-independent dilation) are often done in conjunction with these assessments (Lockhart et al. 2006).

Several recent reviews detail the methodological considerations associated with FMD assessment (Pyke and Tschakovsky 2005; Thijssen et al. 2011; Harris et al. 2010). Unless otherwise stated, the FMD studies that are reviewed in Section 2.4 used a standard technique with cuff placement distal to the ultrasound probe, and a 3-5 min occlusion without ischemic handgrip exercise (Pyke and Tschakovsky 2005). Several studies measured arterial diameter continuously for 2+ min following cuff release, in line with current recommendations (Thijssen et al. 2011) while others measured diameter at discrete time points, starting at 60 s post-cuff release (Broadley et al. 2005; Ghiadoni et al. 2000; Jambrik et al. 2004b; Jambrik et al. 2005; Jambrik et al. 2004a). While measurement at 60 s may result in missing the true peak FMD response (Black et al. 2008), studies with an analogous design and continuous diameter measurements have found similar results (Spieker et al. 2002). Furthermore, Szigyarto and colleagues (2012) performed continuous diameter analysis and reported no impact of acute stress on the time to peak diameter measurement. Taken together this indicates that in the reviewed FMD studies (Section 2.4), methodological issues associated with the FMD protocol itself are unlikely to be important with respect to study conclusions regarding the impact of mental stress on endothelial function, and therefore will not be discussed further.
Although several vasodilators can be released by the endothelium in response to an increase in shear stress, NO has received the most attention, likely due to its early identification as a key mediator of FMD (Joannides et al. 1995) and its established vasoprotective properties (Behrendt and Ganz 2002; Cornwell et al. 1994; Garg and Hassid 1989; Gauthier et al. 1995; Kubes et al. 1991; Luscher and Noll 1995; Radomski et al. 1987b; Radomski et al. 1987a; Radomski et al. 1987c; Sarkar et al. 1996). The basic pathways of NO release and degradation that interact to determine the net role of this molecule in receptor- and shear-mediated vasodilation are shown in Figure 2-3. Thus in this context, “endothelial dysfunction” may be defined as “a state in which there is a reduction in the bioactivity of NO and other endothelially derived vasodilator molecules and an increase in activity of mediators that favor vasoconstriction” (Lockhart et al. 2006).

2.4 The impact of acute mental stress on vascular endothelial function

The majority of studies have found that an acute experience of mental stress adversely affects endothelial function (Broadley et al. 2005; Ghiadoni et al. 2000; Gottdiener et al. 2003; Lind et al. 2002; Sarabi and Lind 2001; Spieker et al. 2002), although as identified above, this finding is not unanimous (Dyson et al. 2006; Harris et al. 2000; Szijgyarto et al. 2012) (Table 2-1). Importantly, the cardiovascular response to mental stress can include increased limb blood flow during an acute stressor (Dietz et al. 1994). This blood flow response to stress has been shown to be at least partially dependent on NO (Dietz et al. 1994) and is reduced in some populations with existing endothelial dysfunction (Middlekauff et al. 1997; Cardillo et al. 1998). This suggests that the endothelium is stimulated by mental stress and that its response reflects existing levels of endothelial function. Observations of these endothelial dependent hemodynamic responses during mental stress have previously been grouped with findings of stress-induced endothelial dysfunction (i.e. reduced FMD or reduced responses to an infused
Figure 2-3. Basic pathways of endothelial nitric oxide (NO) generation and degradation.

NO is synthesized from its precursor, L-arginine, electrons carried by NADPH and molecular oxygen (O₂), via the enzyme endothelial nitric oxide synthase (eNOS) and other cofactors including tetrahydrobiopterin (BH₄). The bioavailability of NO is determined by the balance between the inflow and outflow of NO to and from the interstitial space. The “inflow” of NO is represented by the endothelial production of NO, while the “outflow” of NO is represented by the rate of NO degradation, which occurs as a function of its half-life and via its reaction with reactive oxygen species (ROS), such that the greater the concentration of ROS, the faster the breakdown of NO. In addition to directly scavenging NO, ROS can oxidize BH₄, which leads to eNOS uncoupling (i.e. production of more ROS instead of NO by eNOS), such that initial increases in ROS potentiate increasing oxidative disruptions through an amplification pathway. Thus a reduced NO bioavailability may be the result of either a reduced NO production, an increased NO degradation, or both (Walther et al. 2004; Green et al. 2004). Ach = acetylcholine; ecSOD = extracellular superoxide dismutase; eNOS = endothelial NO synthase; L-Arg = L-arginine; M = muscarinic receptor; NADPH = nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; ROS = reactive oxygen species. Illustration adapted from Gielen and Hambrecht (2001) and Wallace et al. (2010).
Table 2-1. A summary of studies that have examined the effect of mental stress on endothelial function in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Effect of MS on EF</th>
<th>Key Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broadley et al., (2005)</td>
<td>36; healthy</td>
<td>Brachial artery FMD measured at baseline and ~15 min post-MS following metyrapone (cortisol inhibitor) or placebo. *MS task: speech task</td>
<td>↓</td>
<td>• FMD fell from 4.5 ± 0.7% to 1.4 ± 1.1% with MS in the placebo group *Pretreatment with metyrapone prevented the MS-induced reduction in FMD</td>
</tr>
<tr>
<td>Dyson et al., (2006)</td>
<td>11 M; healthy</td>
<td>Brachial artery FMD measured at baseline and during MS. *MS task: mental arithmetic</td>
<td>↔</td>
<td>• Norepinephrine, epinephrine, and cortisol not elevated by MS task *Protocol elicited the same hyperemic stimulus pre- and post-MS</td>
</tr>
<tr>
<td>Eriksson et al., (2007)</td>
<td>14 M, 17 F; healthy</td>
<td>Endothelium-dependent (MCh) and independent (SNP) vasodilation assessed before and during MS, with or without local β-blockade, α-blockade or neurogenic blockade. *MS task: mental arithmetic</td>
<td>↓</td>
<td>• MCh induced resistance vessel vasodilation reduced during MS, but SNP induced dilation not affected by MS *MS effect was prevented by infusion of a β-blocker or neurogenic blocker, but not an α-blocker</td>
</tr>
<tr>
<td>Ghiadoni et al., (2000)</td>
<td>18 M; 10 healthy, 8 with T2DM</td>
<td>Brachial artery FMD and response to sublingual GTN (endothelium-independent vasodilator) measured before and after (30, 90, and 240 min) MS. *MS task: speech task</td>
<td>↓</td>
<td>• Healthy: FMD fell (5.0 ± 2.1% to 2.8 ± 2.3%) 30-min post-MS, still impaired 90-min post-MS; No effect of MS on GTN response *T2DM: no change in FMD</td>
</tr>
<tr>
<td>Gottdiener et al., (2003)</td>
<td>17 M, 21 F; 20 with hypercholesterolemia</td>
<td>Brachial artery FMD measured before and during MS. *MS task: anger recall speech task or mental arithmetic</td>
<td>↔ FMD ↓ dilation AUC</td>
<td>• FMD was 4.50 ± 2.65% in the control condition and 4.03 ± 2.04% during MS *FMD during MS inversely related to hostile affect, *r = -0.57</td>
</tr>
<tr>
<td>Harris et al., (2000)</td>
<td>10 M, 11 F; healthy</td>
<td>Brachial artery FMD measured before and during MS. *MS task: mental arithmetic</td>
<td>↑</td>
<td>• FMD increased significantly from 5.05 ± 2.20 to 8.68 ± 3.00% with MS *Baseline diameter decreased with MS</td>
</tr>
<tr>
<td>Jambrik et al., (2004b)</td>
<td>20; healthy; 10 LOW, 10 HI HYP</td>
<td>Brachial artery FMD measured before and immediately and 12 min post-MS. *MS task: mental arithmetic</td>
<td>↓ in LOW ↔ in HI</td>
<td>• FMD reduced immediately and 12 min post-MS in LOW, but unaltered by MS in HI</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Protocol</td>
<td>Effect of MS on EF</td>
<td>Key Results</td>
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<tr>
<td>Jambrik et al., (2004a)</td>
<td>6 M, 4 F; healthy; 5 LOW, 5 HI HYP</td>
<td>Brachial artery FMD measured before and immediately after 20 min of real or sham acupuncture, and 8 min after a subsequent MS task. MS task: mental arithmetic</td>
<td>↓ in LOW</td>
<td>• FMD reduced at 8 min post-MS in LOW, but unaltered by MS in HI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↔ in HI</td>
<td>• Acupuncture did not influence the impact of MS in either group</td>
</tr>
<tr>
<td>Jambrik et al., (2005)</td>
<td>10 M, 7 F; healthy; 9 LOW, 8 HI HYP</td>
<td>Brachial artery FMD measured before and immediately post-MS while under hypnosis, and 11 min post-MS following a standard awakening procedure. MS task: mental arithmetic</td>
<td>↓ in LOW</td>
<td>• FMD reduced immediately post-MS while under hypnosis, and 11 min post-MS following awakening procedure in LOW, but unaltered by MS at either time point in HI</td>
</tr>
<tr>
<td>Lind et al., (2002)</td>
<td>10 M, 8 F; healthy</td>
<td>Brachial artery FMD measured before and during MS. MS task: mental arithmetic</td>
<td>↓ (norm to BF)</td>
<td>• FMD did not decrease during MS unless expressed normalized to the blood flow post cuff release</td>
</tr>
<tr>
<td>Sarabi and Lind, (2001)</td>
<td>4 M, 6 F; healthy</td>
<td>Endothelium-dependent (MCh) and independent (SNP) forearm resistance vessel vasodilation assessed at rest, and during MS. MS task: mental arithmetic</td>
<td>↓</td>
<td>• MCh induced resistance vessel vasodilation reduced during MS, but SNP induced dilation not affected by MS</td>
</tr>
<tr>
<td>Spieker et al., (2002)</td>
<td>23; healthy</td>
<td>Radial artery FMD and nitroglycerin-induced vasodilation (endothelium-independent) measured before and after (10, 30 and 45 min) MS during infusion of an endothelin A (ET,A) receptor antagonist (BQ-123) or placebo. MS task: responding to flashing coloured lights</td>
<td>↓</td>
<td>• FMD reduced for ~ 45 min post-MS</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• FMD decreased significantly from 8.0 ± 1.1% to 4.1 ± 1.0% 10 min post-MS</td>
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<td></td>
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<td></td>
<td></td>
<td>• Infusion of BQ-123 abolished FMD impairment with MS (8.6 ± 1.3% vs. 9.4 ± 1.4%)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• No effect of MS on response to nitroglycerin</td>
</tr>
<tr>
<td>Szigyarto et al., (2012)</td>
<td>16 M; healthy</td>
<td>Brachial artery FMD measured pre and post-stress in response to reactive hyperemia (RH) and handgrip exercise (HGEX) induced increases in shear stress. MS task: speech task and mental arithmetic</td>
<td>↓ RH-FMD ↔ HGEX-FMD</td>
<td>• Decrease in RH-FMD from pre- to post-stress (5.2 ± 0.6% pre-stress to 4.1 ± 0.5%. P=0.071) became significant with the removal of one outlier.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Cortisol levels did not increase with the stress task</td>
</tr>
</tbody>
</table>

AUC = area under the curve; BF = blood flow; CPT = cold pressor test; EF = endothelial function; ET,A = endothelin A receptor; F = female; FMD = flow mediated dilation; GTN = glycerol trinitrite; HI = high hypnotic susceptibility; HYP = hypnotizability; LOW = low hypnotic susceptibility; M = male; MCh = methacholine; MS = mental stress; SNP = sodium nitroprusside; T2DM = type 2 diabetes mellitus; ↑ = increase; ↓ = decrease; ↔ = no change.
endothelium dependent dilator during or after stress versus during an unstressed condition) (Toda and Nakanishi-Toda 2011). However, these two sets of observations are distinct in that the former is not informative regarding the ability of acute mental stress to cause altered endothelial function, and therefore will not be covered in this review.

The acute stress response follows a time course such that increased sympathetic activity and associated hemodynamic responses occur shortly following stressor onset, while an elevation in cortisol, the primary effector of the HPA axis, peaks approximately 20-40 min post stressor onset (Kirschbaum et al. 1995; Schommer et al. 2003; Rimmeele et al. 2007). This is often after hemodynamic responses have returned to baseline following a brief stress stimulus (Spieker et al. 2002; Ghiadoni et al. 2000). Therefore, the stress associated physiological signals that interact with endothelial function vary over the course of the stress response (Figure 2-4).

### 2.4.1 Vascular endothelial function following acute mental stress

Endothelial dysfunction following acute mental stress was first shown by Ghiadoni and colleagues (2000). They measured brachial artery FMD and endothelium-independent vasodilation [sublingual glycerol trinitrite (GTN), an exogenous NO donor] in healthy volunteers and participants with Type 2 Diabetes Mellitus (T2DM) both before and after (30, 90, and 240 minutes) a mental stress test (speech task). In healthy participants, FMD was found to fall significantly from a mean of 5.0 ± 2.1% to 2.8 ± 2.3% at 30 minutes post-mental stress, to remain impaired at 90 minutes post-mental stress, and to have returned to baseline after 240 minutes. Interestingly, mental stress reduced FMD in the healthy volunteers to levels comparable to those at baseline in the T2DM group [i.e. a population characterized by chronic endothelial dysfunction (Kingwell et al. 2003)]. This suggests that acute stress causes a meaningful reduction in FMD magnitude. In contrast, mental stress had no effect on endothelium-independent vasodilation. It is noteworthy that stress did not impair FMD in the T2DM group. This is likely because significant baseline levels of endothelial dysfunction prevented the detection of a further
Figure 2.4. The stress response timeline.

Top – schematic approximate timeline of key variables in the physiological stress response. Bottom – studies examining human endothelial function at various time points relative to the physiological response. CO = cardiac output, CRH = corticotropin releasing hormone, ET-1 = endothelin-1, epi = epinephrine, norepi = norepinephrine, HR = heart rate, MAP = mean arterial pressure, SNA = sympathetic nervous activity, TPR = total peripheral resistance, FMD = flow mediated dilation, EF = endothelial function, AUC = area under the curve, Norm FMD = FMD that has been normalized to the magnitude of the blood flow stimulus post occlusion cuff release, HGEX-FMD = FMD stimulated by handgrip exercise induced increases in shear stress. All other FMD stimulated by reactive hyperemia induced increase in shear stress. ↔ indicates no change in FMD from the pre-stress period; an asterisk (↔*) indicates that this response was only observed in subjects characterized as having high hypnotizability. ↓ indicates reduced FMD from the pre-stress period. ↑ indicates increased FMD from the pre-stress period.
impairment following mental stress. However, more recently, Wagner et al. (2012) identified decreased post-stress brachial artery FMD in postmenopausal women with a lifetime history of depression and T2DM.

Similar impairment in conduit artery endothelial function following mental stress has been demonstrated in other studies of healthy subjects (Table 2-1) (Broadley et al. 2005; Spiker et al. 2002), such that impaired FMD has been observed immediately (Jambrik et al. 2004b; Jambrik et al. 2005) and from ~10 minutes (Jambrik et al. 2004b; Jambrik et al. 2005; Jambrik et al. 2004a; Broadley et al. 2005; Ghiadoni et al. 2000; Spiker et al. 2002) to 90 minutes (Ghiadoni et al. 2000) post-stress. These studies have examined both men and women from their late teens to middle age. Importantly, although not reported in all studies (Broadley et al. 2005; Spiker et al. 2002; Jambrik et al. 2004b; Jambrik et al. 2005; Jambrik et al. 2004a) the shear stress stimulus following cuff release pre- vs. post-stress appears to be stable (Ghiadoni et al. 2000; Szijgyarto et al. 2012) and therefore the lower post-stress FMD is likely not explained by stimulus differences. There have been no studies to date examining resistance vessel endothelial function post-mental stress. This is an important gap in the literature because conduit artery and resistance vessel endothelial function are not necessarily in good agreement (Herrington et al. 2001; Green et al. 2006).

The finding of impaired FMD post-stress is not universal. First, in studies reporting a mean decrease in FMD post-stress, all individual subjects do not always exhibit FMD impairment (Ghiadoni et al. 2000; Szijgyarto et al. 2012). Second, Jambrik and colleagues (2004b) demonstrated that susceptibility to impaired FMD with mental stress was contingent on the psychological trait of hypnotizability. In their study, FMD was decreased immediately and 12 minutes after a mental stress task (mental arithmetic) in participants with low hypnotic susceptibility, but was unaltered from baseline in those with high hypnotic susceptibility. This finding has been replicated by this group (Jambrik et al. 2005; Jambrik et al. 2004a). However,
hormonal and hemodynamic variables were not measured and the mechanisms of protection from stress induced FMD impairment are therefore unclear. Finally, a recent study by our group (Szijgyarto et al. 2012) identified that while FMD stimulated by a standard reactive hyperemia stimulus was impaired post-stress, dilation stimulated by handgrip exercise-induced increases in shear stress was not. This suggests that the impact of mental stress varies with the shear stress profile and the transduction pathways that are engaged.

As a whole these studies demonstrate that there is heterogeneity in the propensity for post-stress conduit artery endothelial dysfunction and while mechanisms governing this variability are incompletely understood at present, psychosocial characteristics and the nature of the shear stress stimulus profile appear to play a role. Understanding the factors that influence vulnerability to stress induced endothelial dysfunction may be important in predicting the long term impact of stress on vascular health.

2.4.2 Vascular endothelial function during acute stress

Results from studies investigating endothelial function during mental stress tasks are more variable and interpretation of results may be complicated by the superimposition of endothelial function tests (which have vessel diameter and blood flow as vascular outcome variables) on the hemodynamic/vasoactive effects of the stressor itself. Lind and colleagues (2002) found that a mental arithmetic stress task did not result in an impaired FMD response (control 9.3% vs. during the arithmetic task 10.1%). However, the authors noted that this finding may have been due to the greater hyperemia stimulus observed in the mental stress condition versus the control condition (Lind et al. 2002). Indeed, when normalized to the magnitude of the hyperemic stimulus [FMD/blood flow stimulus (blood flow used as a surrogate for shear stress in this investigation)] the FMD response was attenuated in the stress condition. Conversely, in a protocol that permitted precise matching of the hyperemic stimulus in the control and stress conditions, Dyson and colleagues (2006) found that mental arithmetic had no effect on brachial
artery FMD. In this study however, the increases in systolic and diastolic blood pressure were more modest than those reported by Lind and colleagues (2002), and other key markers of stress (norepinephrine, epinephrine, and cortisol) were not elevated by the mental stress task (not measured by Lind et. al. 2002) (Dyson et al. 2006). Thus it is possible that the mental stress stimulus was not of adequate magnitude to evoke an impairment in endothelial function.

Gottdiener et al. (2003) also investigated brachial artery FMD during acute mental stress. They found that the area under the curve of the diameter post-cuff-release was significantly lower in the stress vs. control condition. This finding is interesting and might indicate that the dynamics of the FMD response (i.e. rate of dilation and rate of reconstriction) are influenced by the stress task. However, these results are difficult to compare to those of other studies because it is typically the peak percent change in diameter that is reported as the FMD response and index of endothelial function. The peak percent FMD reported by Gottdiener and colleagues (2003) was quite similar in the control and stress conditions (4.50% vs. 4.03% respectively). Interestingly, Gottdiener and colleagues found that the magnitude of FMD impairment observed during stress varied positively with hostility, again suggesting variability in the propensity for stress induced impairment of FMD. However, similar to the findings of Jambrik and colleagues regarding hypnotizability (Jambrik et al. 2004b; Jambrik et al. 2005), the mechanisms underlying this relationship are unclear as no physiological correlate of hostility was reported.

In contrast to the findings reviewed thus far, Harris and colleagues (2000) found that FMD increased vs. the control condition during a mental arithmetic stress task (5.05 ± 2.20 to 8.68 ± 3.00%). It is possible that the seemingly beneficial effect of mental stress on FMD would disappear or even reverse in direction if FMD were indexed to the shear stress stimulus, as found by Lind and colleagues (2002), however no measurements of brachial artery blood flow or shear stress were reported to allow this determination. Harris and colleagues (2000) did report that the baseline diameter was significantly smaller in the mental stress condition versus control [not
found by Lind et al. (2002), Dyson et al. (2006) or Grottiener et al. (2003)]. Indeed, this reduction in baseline diameter, suggesting baseline conduit artery vasoconstriction, is largely responsible for the larger calculated %FMD response in the stress condition as peak diameter was similar in the control and stress conditions. The functional significance of the larger calculated %FMD response following mental stress is more difficult to interpret given the differences in baseline diameter. If mental stress causes persistent vasoconstriction this may also be associated with structural or functional consequences independent from endothelial function.

Sarabi and Lind (2001) investigated forearm resistance vessel endothelial function during a mental stress task. They found that the decrease in vascular resistance with infusion of the endothelium dependent vasodilator methacholine (MCh) during mental stress (arithmetic task) was blunted relative to the unstressed control condition while vascular resistance was similar between control and mental stress conditions superimposed on sodium nitroprusside (SNP) infusion (endothelial independent vasodilator; an exogenous NO donor). Similar findings of reduced resistance vessel dilation to endothelial dependent vasodilator infusion during mental stress have been reported by others (Eriksson et al. 2007).

In summary, while the studies examining resistance vessel endothelial function during acute stress have consistently reported impairment (Sarabi and Lind 2001; Eriksson et al. 2007) studies of conduit artery endothelial function during mental stress have yielded conflicting results. As described above when mental stress is superimposed on occlusion cuff release this may result in a distinct reactive hyperemia profile vs. pre-stress. This may explain some of the variability in the impact of mental stress on FMD as not all studies have accounted for changes in the shear stress stimulus magnitude (Gottdiener et al. 2003; Harris et al. 2000). In contrast, the majority of studies demonstrate an adverse effect of mental stress on conduit artery endothelial function when measured shortly following the stress experience (Broadley et al. 2005; Ghiadoni et al. 2000; Spieker et al. 2002). Future studies are required to assess resistance vessel
endothelial function post-stress. Regardless of the time point of measurement (during vs. post-stress), the impact of acute stress on endothelial function may depend on individual characteristics (e.g. hostility or hypnotizability) however the physiological underpinnings of these stress-trait-endothelial function interactions require further study.

One further consideration is that the laboratory mental stress tasks employed in the reviewed studies may be less potent stimulators of the stress response than real life events (Rozanski et al. 1999) and as a result may underestimate the impact of acute stress on endothelial function. The length of the imposed stress tasks may also be important as physiological stress reactivity characteristics depend on stressor duration (Ring et al. 2002). As the stress tasks in the reviewed studies were all of similar short duration [2-8 min, with the exception being a 20 min task with a similar influence on FMD (Szijgyarto et al. 2012)], it is not possible to evaluate the potential impact of task length on endothelial function from the present data. This may be most relevant to evaluation of endothelial function during stress tasks and deserves further study as bouts of acute stress vary in duration in real life. The divergence of responses to mental stress underscores the need to understand the mechanistic basis of stress-endothelial function interactions.

2.5 Putative mechanisms underlying the impact of acute mental stress on vascular endothelial function

As noted, the primary physiological responses to mental stress involve the HPA axis and SNA (Widmaier et al. 2006); thus corresponding endothelial dysfunction resulting from mental stress is likely related to the actions of these physiological responses. Namely, acute mental stress may adversely affect endothelial function via the actions of corticotropin-releasing hormone, cortisol, and enhanced SNA. Pro-inflammatory cytokine levels may also increase acutely post-stress and may also be involved (Huang et al. 2011; Aschbacher et al. 2012; Prather et al. 2009). While considerable evidence indicating involvement of these factors is reviewed
below, it is unlikely that there is a straightforward dose-response relationship between indices of stress reactivity and stress-induced decrements in endothelial function; indeed studies have largely failed to demonstrate this (Ghiadoni et al. 2000; Spieker et al. 2002; Szijgyarto et al. 2012). In addition, the interval between the stress experience and endothelial function evaluation (i.e. during or at a specific time following stress) is likely to influence the primary mechanisms involved (Figure 2-4).

2.5.1 Corticotropin-releasing hormone (CRH)

CRH is the major regulatory hormone of the HPA axis (Figure 2-1) (Wilbert-Lampen et al. 2006b); its release from the hypothalamus occurs within seconds following exposure to stress (Brunson et al. 2001). CRH may contribute to endothelial dysfunction via stimulation of ET-1 vasoconstrictor release from the endothelium (Wilbert-Lampen et al. 2006b), and by increasing cell adhesion (Wilbert-Lampen et al. 2006a).

Wilbert-Lampen and colleagues (2006b) examined the effect of CRH on ET-1 and NO release using an in vitro model with human microvascular endothelial cells and human coronary artery endothelial cells. They demonstrated that CRH stimulated ET-1 release in both types of cultured human endothelial cells in a time- and dose-dependent manner, but that NO release was not affected. The effect of CRH on ET-1 release was completely abolished by a CRH-receptor antagonist, signifying a functional involvement of these receptors (specifically, receptor subtype CRH-R2).

However, while there are documented increases in CRH in the peripheral circulation during physical stressors [e.g. abdominal surgery (Donald et al. 1993), myocardial infarction (Donald et al. 1994), and prolonged exercise (Inder et al. 1998)], and during chronic psychosocial stress during pregnancy (Weinstock 2005), peripheral CRH levels are usually undetectable during mental stress (O'Connor et al. 2000). Since the lack of detectable increase in CRH may be due to rapid uptake, catabolism or binding (O'Connor et al. 2000), further work with careful
measurement of plasma CRH is needed. Therefore it is currently unclear to what extent this mechanism may link acute mental stress and impaired endothelial function.

2.5.2 Cortisol

In humans, plasma cortisol concentrations range from 40 to 80 ng·ml\(^{-1}\) under normal conditions (Rogers et al. 2002). Cortisol levels follow a circadian rhythmicity, characterized by maximal plasma levels in the morning (Linkowski et al. 1993). Aside from this natural variation, plasma cortisol concentrations may increase as much as 10-fold in response to severe stress (Rogers et al. 2002), with peak concentration occurring at approximately 20 (Kirschbaum et al. 1995; Schommer et al. 2003; Rimmele et al. 2007) to 40 minutes post-onset of stress (Ghiadoni et al. 2000; Kirschbaum et al. 1992), and with levels remaining elevated for as long as an hour after only a 10-minute bout of stress (Nicolson 2008). Plasma and salivary cortisol levels are highly related (Dorn et al. 2007), and both measures are frequently used in research studies. Several lines of evidence suggest that this elevation in cortisol may contribute to the reduction in endothelial function observed with acute mental stress.

First, and probably most compelling, pharmacological inhibition of cortisol production has been shown to prevent acute mental stress-induced endothelial dysfunction (Broadley et al. 2005). Broadley and colleagues (2005) measured brachial artery FMD in 36 healthy volunteers at baseline and during mental stress (a speech task) following administration of an oral cortisol inhibitor (metyrapone) or a placebo. They found that in the placebo condition mental stress significantly reduced FMD compared to baseline (from 4.5 ± 0.7 to 1.4 ± 1.1%), but that metyrapone reduced pre-stress plasma cortisol, blunted the cortisol response to mental stress, and completely abolished the reduction in FMD with mental stress. This provides strong evidence for a role of cortisol in the mental stress-induced reduction in endothelial function.

Second, short-term administration of oral cortisol has been shown to reduce resistance vessel endothelial function (Mangos et al. 2000). Mangos and colleagues (2000) assessed
forearm endothelium-dependent and –independent vasodilatory function (via respective intra-arterial infusions of ACh and SNP) before and after 2 or 5 days of oral cortisol or placebo treatment in 14 healthy volunteers. They found that after 2 days of oral cortisol (20 mg hydrocortisone acetate every 6 hours) there was a trend towards a reduction in forearm blood flow responses to ACh, and after 5 days of cortisol administration this reduction became significant. In contrast, cortisol had no effect on endothelium-independent vasodilation, as assessed by SNP. After 5 days of administration cortisol levels increased (387 ± 30 to 849 ± 54 nmol/L) to levels similar to those observed in response to acute psychological stress (Kirschbaum et al. 1993).

In addition, correlative evidence suggests that cortisol contributes to the mental stress-mediated reduction in endothelial function. For instance, FMD exhibits diurnal variation that coincides with that of cortisol, such that FMD tends to be low in the morning when cortisol levels are elevated (Ringqvist et al. 2000). Harris and colleagues (2003) demonstrated that high scores on measures of anxiety/depression, conditions in which basal cortisol is elevated, were associated with impaired brachial artery FMD in healthy postmenopausal women (Harris et al. 2003). Similarly, Akaza and colleagues (2010) observed that patients with Cushing’s syndrome, a condition in which cortisol is chronically elevated, had reduced FMD compared to healthy matched controls. Furthermore, FMD improved in these patients following medical or surgical correction of the hypercortisolism, and the magnitude of improvement correlated inversely with morning cortisol levels ($r = -0.85$), such that lower morning cortisol levels were associated with greater improvements in FMD.

It is of note however that some evidence of impairment has been observed during acute mental stress, and this is a time when cortisol is unlikely to have yet become elevated (Gottdiener et al. 2003; Lind et al. 2002; Sarabi and Lind 2001). In addition Szigyarto et al. (2012) did not observe an increase in salivary cortisol levels, but did find that FMD was modestly impaired 10
min post-stress. Taken together this suggests that although cortisol is likely play a role in mental stress associated endothelial dysfunction it is unlikely to be the only factor involved.

2.5.2.1 Cortisol and NO production

Cortisol may contribute to mental stress-induced endothelial dysfunction via direct effects on NO bioavailability by inhibiting NO synthesis. For example, Wallerath and colleagues (1999) demonstrated that incubation of human umbilical vein endothelial cells with several glucocorticoids for 18-36 hours down-regulated endothelium NO synthase (eNOS), the enzyme responsible for NO synthesis in the endothelium (Figure 2-3), by reducing eNOS mRNA and protein expression to 60-70% of control. This downregulation of eNOS was prevented by co-incubation with the glucocorticoid receptor antagonist mifepristone, signifying a functional involvement of glucocorticoid receptors. Similar findings have been reported by others (Rogers et al. 2002). This finding is not universal however; Nickel et al. (2009) found that a 6-hour incubation of human microvascular endothelial cells with dexamethasone (DEX; a glucocorticoid) did not reduce NO production.

Extending their findings to in vivo rat and mice models, Wallerath and colleagues (1999) provided evidence that oral consumption of a glucocorticoid resulted in a significant reduction in plasma nitrite and nitrate [NO$_2$/$NO_3$; markers of NO bioavailability (Brown et al. 2000)]. Functionally, this translated to significantly reduced vasodilation in the resistance arterioles in response to the endothelium-dependent vasodilator ACh, but not to the endothelium-independent vasodilator S-nitroso-N-acetyl-D,L-penicillamine (SNAP).

Taken together, these data indicate that cortisol may reduce the bioavailability of NO. However, the time course of eNOS expression changes may limit the role of this mechanism in explaining the immediate impact of elevated cortisol on FMD post-stress.
2.5.2.2 Cortisol and reactive-oxygen species

In addition to inhibiting NO synthesis, cortisol may contribute to greater NO degradation via enhancement of reactive oxygen species (ROS) production (Iuchi et al. 2003). As shown in Figure 2-3, ROS reduce NO availability directly [resulting in formation of peroxynitrite, itself a potent oxidant (Harris and Matthews 2004)], and indirectly by oxidizing tetrahydrobiopterin (BH₄), a critical cofactor for eNOS, resulting in eNOS uncoupling [i.e. in the absence of sufficient BH₄ cofactor, oxygen becomes the substrate for eNOS instead of L-arginine, resulting in the production of more ROS in lieu of NO (Forstermann and Munzel 2006; Forstermann 2006)]. This creates an amplification pathway, whereby the initial increase in ROS potentiates increasing oxidative disruptions (secondary to peroxynitrite); the greater the production of ROS, the greater the NO degradation.

Iuchi and colleagues (2003) found that short-term glucocorticoid therapy reduced forearm blood flow responses to reactive hyperemia in comparison to pre-treatment levels. The resistance vessel vasodilatory response to occlusion (the degree of which determines the magnitude of hyperemia) is a complex response that is probably at least partially endothelially mediated (Dakak et al. 1998). Administration of a single oral dose of 2 grams of vitamin C, a ROS scavenger, almost normalized blood flow responses to pre-treatment levels, indicating that the glucocorticoid-induced decrement in blood flow was mediated in part by an increased production of ROS.

Additionally, it was shown that human umbilical vein endothelial cells treated with DEX increased production of hydrogen peroxide (a ROS) by up to 166.5 ± 3.3% of control values, and that intracellular peroxynitrite increased and NO decreased with a similar time course (Iuchi et al. 2003). Furthermore, administration of a glucocorticoid receptor antagonist (RU486) concomitantly with DEX in the cultured endothelial cells completely blocked the glucocorticoid-induced ROS overproduction, indicating that the action of glucocorticoids on ROS production is mediated specifically through glucocorticoid receptors.
Together, these findings demonstrate that the increase in cortisol (i.e. a glucocorticoid)
that occurs with mental stress may impair endothelial function via a glucocorticoid-receptor-
mediated increased production of ROS, and that antioxidant administration may help to attenuate
this negative effect.

### 2.5.2.3 Cortisol and ET-1

While CRH has been shown to stimulate increased release of ET-1 from the endothelium
in vitro (Wilbert-Lampen et al. 2006b), cortisol has been shown to induce ET-1 release from
vascular smooth muscle cells (Kanse et al. 1991). Moreover, oxidative stress promotes the
release of ET-1 from the endothelium (Harris and Matthews 2004); thus in addition to the direct
effect of cortisol on inducing ET-1, cortisol may increase circulating ET-1 secondary to its effect
on oxidative stress. In support of these mechanisms, plasma ET-1 has indeed been shown to
increase with acute stress in healthy humans (Mangiafico et al. 2002; Treiber et al. 2002). Thus a
cortisol-induced increase in ET-1 may contribute to the impairment in endothelial function
observed with mental stress.

In support of the involvement of ET-1 in mental stress-mediated endothelial dysfunction,
Spiker and colleagues (2002) measured radial artery FMD before and after (10, 30 and 45
minutes) mental stress during intra-arterial infusion of an endothelin A (ET\textsubscript{A}) receptor antagonist
(BQ-123) or placebo (saline). They found that mental stress reduced FMD by approximately
50% 10 min post-stress, but that this blunting of FMD was completely abolished with the ET\textsubscript{A}
receptor antagonist (Table 2-1). This suggests that the mechanism underlying mental stress-
induced endothelial dysfunction involves activation of endothelin receptors, and a cortisol-
induced increase in ET-1 release (stimulating constriction and opposing the action of NO) may be
involved. However, in contrast with cortisol levels which peak 10 min or more post-stressor,
peak ET-1 levels post-stress have been reported as highest immediately post-stressor, falling to
baseline levels by 10 min post-stress (Mangiafico et al. 2002). This suggests that acute stress may also increase ET-1 via a cortisol-independent mechanism.

2.5.2.4 Metabolic effects of cortisol

Lastly, mental stress-induced elevations in cortisol may impair endothelial function secondary to effects on metabolism. Cortisol results in increased levels of free fatty acids (FFA) and glucose in the blood, via stimulation of triglyceride catabolism in adipose tissue, stimulation of gluconeogenesis in the liver, and inhibition of glucose uptake by many body cells (“insulin antagonism”) (Widmaier et al. 2006). Acute stress has been shown to elevate both blood lipids (Le et al. 1999) and glucose (Armario et al. 1996). Davda and colleagues (1995) showed that the FFA oleic acid reduces the activity of eNOS in vitro (cultured bovine pulmonary artery endothelial cells), which would contribute to endothelial dysfunction by attenuating the production of NO in response to a given stimulus. Additionally both elevated blood lipids and blood glucose have been shown to induce conduit and resistance vessel endothelial dysfunction (Williams et al. 1998; Steinberg et al. 1997; Williams et al. 1998; Kawano et al. 1999).

2.5.3 Pro-inflammatory cytokines

Pro-inflammatory cytokine levels may change acutely with mental stress (Steptoe et al. 2007; Huang et al. 2011; Aschbacher et al. 2012; Prather et al. 2009) and there is some indication that cortisol may influence these responses (Kunz-Ebrecht et al. 2003). Furthermore there is evidence suggesting that some cytokines impair endothelial function in vivo (Clapp et al. 2004; Hingorani et al. 2000; Kilickap et al. 2011; Erzen et al. 2007; Robinson et al. 2006) and in vitro work has demonstrated a negative impact of some cytokines on NO production (Goodwin et al. 2007; Verma et al. 2002; Hung et al. 2010) although this finding is not universal (Clapp et al. 2005; Rosenkranz-Weiss et al. 1994). As a whole, the evidence suggests that the interaction between acute inflammatory response activation, NO production and endothelial function is complex (Clapp et al. 2005; Bhagat and Vallance 1999; Antoniades et al. 2011). Recently,
Antoniades and colleagues (2011) reported that induction of an acute systemic inflammatory response (*Salmonella typhi* vaccine) that included increased interleukin 6 levels stimulated BH₄ production, and that this provided some protection from inflammation induced endothelial dysfunction. Further, their results indicate that the presence of this protective mechanism depends on genetic characteristics associated with the expression of the rate limiting enzyme in BH₄ production (GTP-cyclohydrolase). A positive impact of pro-inflammatory cytokines on BH₄ production has also been reported by others (Clapp et al. 2005; Rosenkranz-Weiss et al. 1994). More research is required to precisely define the role of pro-inflammatory cytokines in observations of endothelial dysfunction associated with acute mental stress specifically.

### 2.5.4 Sympathetic nervous system activity (SNA)

As with cortisol, several lines of evidence support a role for SNA in the mental stress-induced reduction in endothelial function, although opposing evidence also exists.

#### 2.5.4.1 Evidence for a contribution of SNA

As previously stated, several authors have reported evidence of endothelial dysfunction in humans during acute stress (Gottdiener et al. 2003; Lind et al. 2002; Eriksson et al. 2007; Sarabi and Lind 2001) and elevated SNA may be involved. The most direct evidence for the involvement of SNA in reducing endothelial function comes from a study by Hijmering and colleagues (2002) in which the investigators directly evoked an increase in SNA via a lower body negative pressure test and measured the effect on simultaneous brachial artery FMD with or without local α-adrenergic receptor blockade (phentolamine infusion). They found that FMD (but not endothelial independent dilation to infused nitroglycerine) was significantly reduced compared to baseline with elevated SNA (from 8.3 ± 3.4% to 3.6 ± 3.4%), but that α-adrenergic blockade completely restored the SNA-induced attenuation of FMD. This demonstrates that an increase in SNA with mental stress may contribute to a blunting of endothelial dependent dilation via activation of α-receptors.
With respect to mental stress directly, Eriksson and colleagues (2007) provided evidence for a β-receptor (and not α-receptor) mediated impairment in resistance vessel endothelial function (Table 2-1). In both primates (Skantze et al. 1998) and rabbits (Pettersson et al. 1990), manipulations to increase stress have been shown to result in endothelial injury that may be prevented with β-receptor blockade. Likewise in humans, β-blocker treatment has been shown to improve brachial artery FMD among patients with coronary artery disease (Matsuda et al. 2000).

Together, these studies indicate that acute elevation of SNA may contribute to endothelial dysfunction via direct stimulation of α- and β-adrenergic receptors. The particular mechanism by which SNA impairs endothelial function may depend on the method used to elevate SNA (Dyson et al. 2006) and the vascular bed being tested (e.g. conduit arteries or resistance vessels).

2.5.4.2 Evidence against a contribution of SNA

Support for a role of SNA in an acute mental stress-induced reduction in endothelial function is not unanimous. In the study by Dyson and colleagues (2006) mentioned previously, the effect of SNA on FMD was assessed using four different protocols to increase SNA: lower body negative pressure, cold pressor test, mental arithmetic task (i.e. mental stress), and activation of the muscle chemoreflex. It was found that the FMD response was dependent on the method used to elevate SNA, with no change in FMD relative to control in the mental arithmetic and lower body negative pressure tests, a reduction of FMD in the cold pressor test, and an improvement in FMD with activation of the muscle chemoreflex. It was concluded that blunted FMD is not a generalized response to an increase in SNA.

Similarly, Spieker and colleagues (2002) showed that while a norepinephrine infusion designed to simulate activation of the sympathetic nervous system resulted in a transient (~5 min) vasoconstriction, endothelial function as assessed by FMD was not affected 10 minutes later. This demonstrates that prior exposure to the primary physiological marker of SNA (i.e. norepinephrine) has no independent effect on acute endothelial function in the forearm.
experimental model utilized. In addition it has been observed that 1) a small decrease in muscle sympathetic nerve activity may occur with mental stress (Halliwill et al. 1997), and 2) a brief mental stress task can result in a lengthy impairment of endothelial function, long after systemic hemodynamic changes mediated by sympathetic activation are resolved (i.e. return of heart rate and mean arterial blood pressure to baseline levels) (Broadley et al. 2005; Ghiadoni et al. 2000). Taken together these findings indicate that while it is likely to play a role in some instances, SNA is not the sole mechanism mediating the deleterious effect of acute mental stress on endothelial function.

2.6 Perspective

In a recent meta-analysis, a 1% change in FMD (assessed via a standard reactive hyperemia protocol) was found to be associated with a 9% change in risk of cardiovascular events (Green et al. 2011). This suggests that the magnitude of transient mental stress-induced reductions in endothelial function is clinically relevant and could represent a key mechanistic link between psychosocial stress and cardiovascular disease. Repeated exposure to mental stress induced endothelial dysfunction might contribute to atherosclerosis progression via repeated attenuations in vasoprotection (Black and Garbutt 2002). In addition, acute stress-induced endothelial dysfunction could reduce perfusion and contribute to myocardial ischemia (Krantz et al. 1996; Rozanski et al. 1988; Gottdiener et al. 1994; Krantz et al. 2000; Sheps et al. 2002; Peix et al. 2006). Mitigating the impact of mental stress on endothelial function could therefore be important in terms of protecting cardiovascular health. Physical activity may represent a promising intervention in this regard, because it has been found to attenuate the negative impact of another acute stimulus (high fat meal consumption) on endothelial function (Johnson et al. 2011; Padilla et al. 2006; Tyldum et al. 2009), and because acute exercise and fitness have been shown to attenuate stress reactivity (Rejeski et al. 1992; Traustadottir et al. 2005; Spalding et al. 2004). Given that mental-stress-induced perturbations of endothelial function could contribute
importantly to the development of cardiovascular disease in the long term, further investigation of this and other possible interventions is warranted.

2.7 Summary and conclusions

In summary, acute experiences of mental stress evoke physiological responses involving increased HPA axis activity and SNA. These responses, while serving important functions, may also be maladaptive when experienced repeatedly or chronically, and specifically may increase cardiovascular disease risk (Krantz et al. 1996; Krantz et al. 2000; Rozanski et al. 1988; Gottdiener et al. 1994; Sheps et al. 2002). Stress induced impairments in endothelial function may be an important mechanism underlying the negative impact of stress on cardiovascular health. Acute exposure to mental stress that results in transient endothelial dysfunction could result in ischemia and/or a cumulative effect that is clinically important over the long term. Although individual and stressor-specific factors may influence the mechanism(s) involved, and the susceptibility to impairments, it appears that mental stress may impair endothelial function via several actions of CRH, cortisol, and SNA (summarized in Figure 2-2). Based on current evidence, it can be suggested that endothelial function should be evaluated during acute psychological stress to isolate the effects of CRH and SNA, and 20-40 minutes post-onset of stress to assess the effect of cortisol. Concurrent measurements of endocrine entities (CRH, cortisol), indicators of SNA (norepinephrine, heart rate, mean arterial blood pressure, muscle sympathetic nerve activity), and potential downstream contributors (NO$_2$/?NO$_3$, ET-1, lipemia, glycemia), with attention to the potential for vessel/vascular-bed-specific effects, should be undertaken to provide more refined mechanistic insight into observed responses. Elucidating the complexities of the relationship between psychological stress and endothelial function will aid in providing the foundation for the development of interventions to reduce the risk of cardiovascular disease.
2.8 References


Chapter 3 – LITERATURE REVIEW PART II:

The impact of acute fat consumption on vascular endothelial function:

Evidence, moderating factors, mechanisms and importance

This chapter is formatted for submission to the journal *Applied Physiology, Nutrition and Metabolism*. 
3.1 Abstract

The relationship between dietary fat and cardiovascular disease is well-established, and fat consumption-induced impairment in vascular endothelial function may be an important mechanistic link. Several studies have demonstrated that endothelial function is transiently impaired following a single high-fat meal, from as early as 1 h to up to 8 h post-meal. Since humans spend a considerable amount of time in a postprandial (fed) state, such transient declines in endothelial function could accumulate to become clinically relevant over the long term. The finding of acute fat-induced endothelial dysfunction is not wholly consistent however, and reconciling conflicting findings is critical to understanding the complexities of the relationship between dietary fat and cardiovascular disease. The present review will therefore: 1) integrate the current literature regarding the impact of acute fat consumption on endothelial function, and identify moderating factors that may explain disparate results; 2) identify potential mechanisms that may contribute to an interaction between fat consumption and endothelial function, and 3) provide recommendations to guide future advancement.
3.2 Introduction

Atherosclerotic cardiovascular disease (CVD) is the most common cause of disease-related disability and death in developed countries (Fuster 1999; Heart and Stroke Foundation 2012), and it has a complex etiology that includes lifestyle factors such as diet (Shekelle et al. 1981). Indeed, the relationship between excessive fat consumption, hyperlipidemia, and coronary artery disease is well established (Shekelle et al. 1981; Stone 1990), and although the mechanisms are not fully understood, diet modification (i.e. reducing intake of fat) is recommended in the prevention and management of CVD (Heart and Stroke Foundation 2012). While fasting blood lipid measurements are routinely performed to assess CVD risk (Austin et al. 1998), there is evidence for wide variability in the postprandial lipemia response to a fatty meal challenge (Schrezenmeir et al. 1992; Gaenzer et al. 2001), and recent studies have revealed that the postprandial lipid profile might be more relevant for determination of CVD risk (Simpson et al. 1990; Nordestgaard et al. 2007; Bansal et al. 2007; Ebenbichler et al. 1995).

An impairment in vascular endothelial cell function may represent a major mechanistic link between fat consumption and the development of CVD. The vascular endothelium, the innermost lining of the blood vessels, plays a crucial role in the regulation of platelet aggregation and adhesion, smooth muscle cell proliferation and migration, and vascular tone, in part through its production and release of nitric oxide (NO; see Figure 3-1 A) (Behrendt and Ganz 2002; Lockhart et al. 2006; Luscher and Noll 1995). Overall there is an abundance of evidence indicating that the proper functioning of the endothelial cells is essential for the maintenance of vascular health (Cunningham and Gotlieb 2005; Feletou and Vanhoutte 2006), and that a dysfunctional endothelium precedes and plays a role in the development of atherosclerosis (Celermajer et al. 1992; Drexler and Hornig 1999; Kawashima and Yokoyama 2004). Endothelial function is impaired in populations with cardiovascular risk factors (Pierce et al. 2008) and disease (Neunteufl et al. 1997) and endothelial dysfunction is a predictor of future
cardiovascular events in both healthy individuals (Shechter et al. 2009; Rossi et al. 2008) and those with established CVD (Patti et al. 2005; Gokce et al. 2003; Perticone et al. 2001).

In humans, endothelial function is assessed by measuring the increase in blood flow (an index of resistance vessel function) or conduit artery diameter elicited by stepwise infusions of endothelium-dependent vasodilator substances (such as acetylcholine; Ach), or by measuring the increase in arterial diameter following an increase in blood flow associated shear stress (endothelium-dependent flow mediated dilation; FMD) (Lockhart et al. 2006). The most common non-invasive technique used to increase shear stress for FMD assessment involves the release of a temporary limb occlusion (reactive hyperemia) (Celermajer et al. 1992; Thijssen et al. 2011), although exercise may also be used (Grzelak et al. 2010; Padilla et al. 2006a). These measurements assess the vasoregulatory ability of the endothelium, which is assumed to reflect concurrent alterations in its other vasoprotective functions (Landmesser et al. 2004). The close correlation between endothelial function in the brachial (upper arm) and coronary (heart) arteries indicates a systemic nature of endothelial dysfunction in atherosclerosis (Takase et al. 1998; Anderson et al. 1995) and has supported the use of brachial artery FMD as a surrogate for more invasive assessments of coronary artery endothelial function.

Using the described methods of endothelial function assessment, several investigators have found that acute fat consumption adversely impacts endothelial function (e.g. Vogel et al. 1997; Gaenzer et al. 2001), although this finding is not unanimous (e.g. Harris et al. 2012; Johnson et al. 2011) (see Table 3-1 for complete list). As humans spend a significant part of the day in the postprandial state (Sies et al. 2005; de Koning and Rabelink 2002; Hyson et al. 2003), such transient declines in the anti-atherogenic functions of the endothelium could accrue to become clinically relevant in the long term. Thus, the acute impact of fat consumption on endothelial function may be an important component of the chronic relationship between dietary fat and CVD.
With this as a foundation, this review is intended: 1) to integrate the current state of knowledge regarding the impact of acute fat consumption on endothelial function in humans, and to identify factors that may moderate this relationship and explain conflicting results, 2) to provide a brief synopsis of potential mechanisms that may contribute to an interaction between fat consumption and endothelial function, and 3) to provide recommendations to guide future advancement.
Table 3-1. A summary of studies that examined the effect of fat consumption (FC) on endothelial function (EF) in humans

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Meal: energy &amp; fat composition</th>
<th>Effect of FC on EF</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. 2001</td>
<td>T2D (7 M, 5 F)</td>
<td>1,480 kcal, 80 g SF</td>
<td>↓</td>
<td>GTN-mediated vasodilation not changed</td>
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<tr>
<td></td>
<td>HS (5 M, 7 F)</td>
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<tr>
<td>Anderson et al. 2006</td>
<td>T2D (14 M, 6 F)</td>
<td>1,480 kcal, 80 g SF</td>
<td>↓</td>
<td>Pre-treatment (2 days) with Vitamin C: ↑ baseline FMD and attenuated postprandial ↓</td>
</tr>
<tr>
<td>Ayer et al. 2010</td>
<td>Obese (7 M, 4 F)</td>
<td>1,000 kcal, 60 g fat</td>
<td>↔</td>
<td>FMD not different between obese and normal weight individuals pre- or post-meal</td>
</tr>
<tr>
<td>Bae et al. 2001</td>
<td>HS (10 M, 10 F)</td>
<td>803 kcal; 53.4 g fat</td>
<td>↓</td>
<td>ΔTG correlated negatively with ΔFMD ( r = -0.650 ), and positively with Δ oxidative stress production ( r = 0.798 ) which correlated negatively with ΔFMD ( r = -0.784 )</td>
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<tr>
<td>Bae et al. 2003</td>
<td>HS (10 M)</td>
<td>803 kcal, 53.4 g</td>
<td>↓</td>
<td>HFM + Vitamin E (800 IU), or LFM (802 kcal, 3 g fat): ↔ FMD Inverse correlation between ΔFMD and ΔTG at 2 h post-meal ( r = -0.54 )</td>
</tr>
<tr>
<td>Berry et al. 2008</td>
<td>HS (17 M)</td>
<td>853 kcal, 50 g of fat: 1) stearic acid (SA), 2) oleic acid (HO)</td>
<td>↓ HO ↔ SA</td>
<td>Postprandial ↑ in TG was lower after SA vs HO meal HO meal: oxidative stress ↑; SA meal: oxidative stress ↔</td>
</tr>
<tr>
<td>Borucki et al. 2009</td>
<td>HS (8 M, 7 F)</td>
<td>1g fat/ kg body weight</td>
<td>↓ with 2.5g L-arginine</td>
<td>Phenylalanine and leucine (same insulin response as arginine but without arginine substrate for NO synthesis) did not prevent ↓ in FMD post-HFM</td>
</tr>
<tr>
<td>Burton-Freeman et al. 2012</td>
<td>HS (13 M, 12 F)</td>
<td>848 kcal, 44 g fat</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Ceriello et al. 2002</td>
<td>T2D (22 M, 18 F)</td>
<td>700 kcal and 75 g fat/m² body surface area</td>
<td>↓ less with 40 mg/d simvastatin</td>
<td>5-7 d simvastatin: no effect on lipids, but attenuated ↓ FMD and ↑ nitrotyrosine (NT; used to infer production of peroxynitrite, a product of ROS-inactivated NO) 3 mo simvastatin: lower ↑ in TG and NT, and attenuated ↓ FMD</td>
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<td></td>
<td>HS (12 M, 8 F)</td>
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<tr>
<td>Ceriello et al. 2005</td>
<td>T2D (12 M, 8 F)</td>
<td>700 kcal and 75 g fat/m² body surface area</td>
<td>↓ less following atorvastatin, irbesartan or both</td>
<td>5-7 d atorvastatin (statin; 40 mg/d) treatment or irbesartan (ANG II receptor inhibitor; 300 mg/d), alone or in combination: no effect on lipid parameters or blood pressure, but ↑ basal FMD and attenuated postprandial ↓ in FMD and ↑ in NT and inflammatory markers</td>
</tr>
<tr>
<td>Cortes et al. 2006</td>
<td>Hypercholesterolemia (11 M, 1 F)</td>
<td>~1,200 kcal 80 g fat (with either 25 ml olive oil or 40 g walnuts)</td>
<td>↔ HS, ↑ hypercholesterolemic, walnut ↓ both groups, olive oil</td>
<td>Walnuts are a source of antioxidants and L-arginine Plasma ADMA concentrations were unchanged</td>
</tr>
<tr>
<td></td>
<td>HS (9 M, 3 F)</td>
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<tr>
<td>Djousse et al. 1999</td>
<td>HS (7 M, 6 F)</td>
<td>15 kcal and 0.8 g fat/kg body weight</td>
<td>↔</td>
<td>Non-significant trend for ↓ FMD following HFM with/without red wine (3 ml/kg)</td>
</tr>
<tr>
<td>Evans et al. 2000</td>
<td>T2D (11 M, 9 F)</td>
<td>80 g SF</td>
<td>↓</td>
<td>Ciprofibrate (100 mg/d, 3 mo): ↑ FMD at fasting and 4 h post-meal, ↓ TG, significant attenuation of ↑ in oxidative stress</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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<tr>
<td>Fahs et al. 2010</td>
<td>HS (10 M, 10 F)</td>
<td>~1,000 kcal, 51-54 g fat</td>
<td>↓ ↔ HFM + fish oil supplement</td>
<td>FMD normalized for shear rate Fish oil supplement: ~1 g omega 3 fatty acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)</td>
</tr>
<tr>
<td>Fard et al. 2000</td>
<td>T2D (34 M, 16 F)</td>
<td>1,265 kcal, 105 g fat</td>
<td>↓</td>
<td>↑ in plasma ADMA following HFM was significantly and inversely related to the ↓ in FMD (r = -0.37)</td>
</tr>
<tr>
<td>Funada et al. 2002</td>
<td>HS (14 M, 10 F; 11 with mild hypertension)</td>
<td>40 g cream / m² of body surface area (mean 24 g fat)</td>
<td>↓ ↔</td>
<td>Subjects divided into 2 groups: “high responders” [postprandial remnant-like particle cholesterol (RLP-C) of &gt;7.5 mg/dl, n = 8], and “normal responders” (postprandial RLP-C level ≤ 7.5 mg/dl, n = 16) FMD significantly lower (pre- and post-meal) in high responders than normal responders</td>
</tr>
<tr>
<td>Gaenzer et al. 2001</td>
<td>HS (17 M)</td>
<td>730 kcal and 65 g fat per m² body surface area</td>
<td>↓</td>
<td>FMD showed diurnal variation (↑ from morning to afternoon) Postprandial FMD correlated inversely with postprandial lipemia (r = -0.81 for 4 h and r = -0.57 for 8 h postprandial)</td>
</tr>
<tr>
<td>Giannattasio et al. 2005</td>
<td>Moderate dyslipidemia (10 M, 6 F) HS (7 M)</td>
<td>680 kcal/m² of body surface area with 83% fat</td>
<td>↓ dyslipidemic ↔ HS</td>
<td>↓ in FMD (expressed as absolute diameter change) correlated with ↑ in serum TG post-HFM (r = 0.49)</td>
</tr>
<tr>
<td>Gokce et al. 2001</td>
<td>HS (8 M, 6 F)</td>
<td>1,067 kcal, 56 g fat</td>
<td>↓</td>
<td>Baseline vessel size increased post-meal with no change in peak diameter</td>
</tr>
<tr>
<td>Gudmundsson et al. 2000</td>
<td>HS (11 M, 4 F)</td>
<td>~910 kcal, 50 g fat</td>
<td>↔</td>
<td>FMD comparison relative to LFM (~900 kcal, 4 g fat); no pre-meal comparison</td>
</tr>
<tr>
<td>Harris et al. 2012</td>
<td>HS (10 M, 15 F)</td>
<td>940 kcal, 48 g fat</td>
<td>↔ women ↓ men</td>
<td>Female postprandial FMD was unchanged from baseline throughout the menstrual cycle (tested at FO, L and ME phases)</td>
</tr>
<tr>
<td>Johnson et al. 2011</td>
<td>HS: 7 active (ACT), 7 inactive (INA) (4 M, 3 F per group)</td>
<td>940 kcal, 48 g fat</td>
<td>↓ INA ↔ ACT</td>
<td>ACT = physical activity 3+ times/week for ≥ 30 min at moderate+ intensity INA = &lt; 30 min moderate physical activity / week ↑ TG not different between groups; ACT had greater antioxidant activity than INA at baseline and following HFM</td>
</tr>
<tr>
<td>Koulouris et al. 2010</td>
<td>HS (18 M, 3 F)</td>
<td>1,000 kcal and 50 g fat</td>
<td>↓ SF ↔ MUFA &amp; PUF</td>
<td>All 4 meals significantly raised plasma TG SF + red wine (250 ml): baseline artery diameter increased; peak diameter not different from pre-meal</td>
</tr>
<tr>
<td>Lee et al. 2002</td>
<td>HS (12)</td>
<td>?</td>
<td>↓ ↔ HFM + vitamin E</td>
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<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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<tr>
<td>Lin et al. 2008</td>
<td>HS (14 M), divided into 2 groups</td>
<td>900 kcal, 50 g fat (with/without 15 g L-arginine)</td>
<td>↓ less with L-arginine</td>
<td>Glutathione peroxidase (GSH-Px; antioxidant enzyme) ↓ after HFM but ↔ with addition of L-arginine Significant ↑ in P-selectin (cell adhesion molecule on surface of activated endothelial cells) and vWF (procoagulant molecule that increases in response to endothelial cell damage) after HFM in both groups</td>
</tr>
<tr>
<td>Liu et al. 2002</td>
<td>CAD (74) HS (50) (overall, 100 M, 24 F; 64 with hypertension, 54 smokers)</td>
<td>800 kcal, 50 g fat</td>
<td>↓ ↔ HFM + Vitamin C</td>
<td>HFM + Vitamin C: mild tendency toward ↑ and ↓ in FMD in CAD and HS groups respectively Decrement of postprandial FMD correlated positively with increment of 2 h TG concentration in patients without Vitamin C (n = 62, r = 0.545)</td>
</tr>
<tr>
<td>Maggi et al. 2004</td>
<td>Moderate dyslipidemia (15 M)</td>
<td>693 kcal (85% fat) per m² body surface</td>
<td>↓</td>
<td>↓ in FMD at 6 h correlated significantly with the AUC of TG (r = 0.53)</td>
</tr>
<tr>
<td>Marchesi et al. 2000</td>
<td>HS (10 M)</td>
<td>700 kcal, 65 g fat per m² body surface</td>
<td>↓</td>
<td>Strong inverse correlation between AUC postprandial TG (i.e. after subtraction of baseline TG) and AUC of ∆FMD post-HFM (r = -0.70)</td>
</tr>
<tr>
<td>Marchesi et al. 2003</td>
<td>Dyslipidemia (7 M, 3 F)</td>
<td>700 kcal, 65 g fat per m² body surface</td>
<td>↓</td>
<td>ICAM and VCAM increased from baseline at 2, 4 and 6 h postprandial</td>
</tr>
<tr>
<td>Muntwyler et al. 2001</td>
<td>HS (12 M)</td>
<td>700 kcal/m² body surface area (83% fat)</td>
<td>↔</td>
<td>No significant correlation between ↑ in postprandial TG-rich lipoproteins and ∆ in Ach-induced vasodilation</td>
</tr>
<tr>
<td>Nagashima and Endo 2011</td>
<td>Obese (24 M)</td>
<td>240 kcal ~25 g fat</td>
<td>↓ ↔ pitavastatin</td>
<td>Pitavastatin (2 mg/day, 2 wk): attenuated ↑ in postprandial TG and abolished ↓ FMD Correlation between postprandial ∆TG and ∆FMD after 2 weeks of statin treatment (r = -0.737)</td>
</tr>
<tr>
<td>Ng et al. 2001</td>
<td>HS (10 M)</td>
<td>?</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Nicholls et al. 2006</td>
<td>HS (8 M, 6 F)</td>
<td>1 g of fat/kg body weight</td>
<td>↓ SF ↔ PUFA (trend for ↓)</td>
<td>Similar ↑ in plasma TG, insulin, and nonesterified fatty acids after both meals Hyperemic flow &gt; post- vs pre-meals</td>
</tr>
<tr>
<td>Ong et al. 1999</td>
<td>HS (16 M)</td>
<td>765 kcal, 50 g fat</td>
<td>↓ vs baseline ↓ vs LFM</td>
<td>LFM: 765 kcal, 5 g fat</td>
</tr>
<tr>
<td>Padilla et al. 2006a</td>
<td>HS (5 M, 3 F)</td>
<td>900 kcal, 50 g (HFM) or 0 g (LFM) fat</td>
<td>↓ RH vs LFM ↔ AH vs LFM</td>
<td>Impact of HFM depended on method of achieving shear stress stimulus [reactive hyperemia (RH) or active hyperemia (AH; 5 min handgrip exercise)]</td>
</tr>
<tr>
<td>Padilla et al. 2006b</td>
<td>HS (5 M, 3 F)</td>
<td>945 kcal, 48 g (HFM) or 0 g (LFM) fat</td>
<td>↔ vs baseline ↓ vs LFM ▶ in HFM+EX</td>
<td>EX: 45-min continuous treadmill walking at 60% VO₂peak, 2 h after HFM</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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<tr>
<td>Plotnick et al. 1997</td>
<td>HS (7 M, 13 F)</td>
<td>900 kcal, 50 g (HFM) or 0 g (LFM) fat, with/without 1 g Vitamin C, 800 IU Vitamin E</td>
<td>↓ ↔ in LFM, HFM + vitamins, or LFM + vitamins</td>
<td>ΔFMD after LFM and HFMs correlated inversely with 2 h postprandial ATG ($r = -0.54$)</td>
</tr>
<tr>
<td>Plotnick et al. 2003</td>
<td>HS (14 M, 24 F)</td>
<td>900 kcal, 50 g fat</td>
<td>↓ ↔ vs baseline after 4 weeks of JP or JP+V (trend for ↓)</td>
<td>JP = Juice Plus (powdered fruit and vegetable juice concentrate), V = Vineyard (antioxidant and herbal extract supplement) In subjects treated with supplements, concentrations of serum nitrate/nitrite increased from 78 ± 39 to 114 ± 62 μm/L</td>
</tr>
<tr>
<td>Raitakari et al. 2000</td>
<td>HS (7 M, 5 F)</td>
<td>1,030 kcal, 61 g fat (48% SF, 40% MUFA, 7.4% PUFA, 4.6% trans; OR 10, 85, 5, 0%)</td>
<td>↔</td>
<td>Trend for ↓ FMD after both HFMs Post-ischemic hyperemia greater after both HFMs (i.e. &gt; shear stimulus)</td>
</tr>
<tr>
<td>Rudolph et al. 2007</td>
<td>HS (10 M, 14 F)</td>
<td>~1,250 kcal, 31-49 g fat</td>
<td>↓</td>
<td>Postprandial baseline diameter increased vs fasting Postprandial ATG did not correlate with Δ baseline diameter or FMD ADMA concentrations did not change postprandially</td>
</tr>
<tr>
<td>Rueda-Clausen et al. 2007</td>
<td>HS (10 M)</td>
<td>600 kcal, 60 ml oil (olive, soybean, palm) either fresh or at one of 2 deep-fry levels (80 and 160 min)</td>
<td>↓</td>
<td>Similar ↓ FMD and ↑ TG with all meals independent of type of oil and its deep-fry level No correlation between ↓ in FMD and postprandial TG ↑</td>
</tr>
<tr>
<td>Sarabi et al. 2001</td>
<td>HS (5 M, 5 F)</td>
<td>900 kcal (for M), 600 kcal (for F); 34 g or 23 g fat</td>
<td>↓</td>
<td>At 1 h post-meal the serum FFA level was inversely related to endothelium-dependent vasodilation (MCh infusion; $r = -0.74$), although no significant net changes in FFA levels were seen</td>
</tr>
<tr>
<td>Schillaci et al. 2001</td>
<td>HS (10 F)</td>
<td>700 kcal, 65 g fat per m² body surface</td>
<td>↔ F ↓ M</td>
<td>compared to M in Marchesi et al., 2000</td>
</tr>
<tr>
<td>Schinkovitz et al. 2001</td>
<td>HS (6 M, 5 F)</td>
<td>1,200 kcal, 90 g fat</td>
<td>↓</td>
<td>HFM did not influence total plasma antioxidant capacity or plasma peroxides Peak RH transiently reduced at 2 h post-HFM No correlation between peak RH and peak TG or AUC TG</td>
</tr>
<tr>
<td>Sejda et al. 2002</td>
<td>HS (6 M, 5 F)</td>
<td>14 kcal/kg, 44.8% fat acutely, after 4-week HF (44.5%) or LF (26%) diet</td>
<td>↔</td>
<td>Greater baseline FMD after high-fat diet than low-fat diet (~5.3% vs 3.1%) Non-significant trend for ↑ FMD postprandially after both diet regimens, possibly reflecting diurnal variation</td>
</tr>
<tr>
<td>Shimabukuro et al. 2006</td>
<td>HS (6 M, 6 F)</td>
<td>30 g fat/m², 35% fat</td>
<td>↓</td>
<td>Decreases in peak and total RH were correlated with the increase in plasma FFA ($r = -0.535$ and $r = -0.486$, respectively)</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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<tr>
<td>Silvestre et al. 2008</td>
<td>HS (12 M)</td>
<td>13 kcal, 1.4 g of fat/kg</td>
<td>↔ NoEX, Ex-16, Ex-4</td>
<td>EX: 6 resistance exercises and 30 min of treadmill running, 16 h (EX-16) or 4 h (EX-4) before meal TG AUC was significantly lower in EX-16 and EX-4 vs NoEx All FMDs (pre- and post-prandial) were small (range: 1.4% to 2.8%)</td>
</tr>
<tr>
<td>Sodre et al. 2011</td>
<td>HS (17 M)</td>
<td>40 g fat / m² body surface</td>
<td>↓</td>
<td>ROS generation reduced in postprandial period</td>
</tr>
<tr>
<td>Steer et al. 2003</td>
<td>HS (26 M and F)</td>
<td>700 and 900 kcal for subjects &lt; and &gt; 70 kg respectively (34, 20, or 3% fat)</td>
<td>↓ 34% fat ↔ 20% fat ↑ 3% fat</td>
<td>Resistance vessel endothelial function (Mch infusion) No correlation between ΔMch-FBF and ΔTG Between-subjects design</td>
</tr>
<tr>
<td>Strey et al. 2004</td>
<td>HS (10 F), T2D (19 F)</td>
<td>660 kcal; 41 g fat</td>
<td>↔</td>
<td>Resistance vessel endothelial function (Ach infusion) Resting FBF increased in both groups post-HFM</td>
</tr>
<tr>
<td>Tentolouris et al. 2008</td>
<td>T2D (21 M, 12 F)</td>
<td>~560 kcal, 36 g fat</td>
<td>↓ SF ↔ MUFA</td>
<td>Similar changes in plasma glucose, insulin, lipid concentrations, and total plasma antioxidant capacity following both meals</td>
</tr>
<tr>
<td>Tsai et al. 2004</td>
<td>HS (16 M)</td>
<td>900 kcal, 50 g fat</td>
<td>↓</td>
<td>GSH-Px (antioxidant enzyme) 2 h after HFM 8-PGF2α (marker of oxidative stress) 4 h after HFM</td>
</tr>
<tr>
<td>Tushuizen et al. 2006</td>
<td>HS (17 M)</td>
<td>2 HFMs 4 h apart, each ~800 kcal, 50 g fat</td>
<td>↓ significant after 2nd HFM</td>
<td>↑ oxidized LDL (oxLDL)/LDL cholesterol ratio and MDA concentrations after the second meal</td>
</tr>
<tr>
<td>Tyldum et al. 2009</td>
<td>HS (8 M)</td>
<td>~910 kcal, 48 g fat</td>
<td>↓  less if preceded by CME ↔ if preceded by HIIE Continuous moderate intensity exercise (CME): 47 min treadmill walking at 60-70% HRmax High intensity interval exercise (HIIE): 10 min warm-up, 4 x 4 min at 85-95% HRmax (separated by 3 min active recovery), cool-down (5 min) CME and HIIE were isocaloric and preceded the HFM by 16-18 h Prior to HFM, both CME and HIIE improved FMD measured 16-18 h post-exercise Antioxidant status strongly correlated with FMD (r = 0.9)</td>
<td></td>
</tr>
<tr>
<td>Vogel et al. 1997</td>
<td>HS (5 M, 5 F)</td>
<td>~900 kcal, 50 g fat</td>
<td>↓</td>
<td>Mean change in postprandial FMD at 2, 3, and 4 h correlated with change in 2 h serum TG (r = -0.51)</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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</tr>
<tr>
<td>Vogel et al. 2000</td>
<td>HS (5 M, 5 F)</td>
<td>1 of 5 meals, 900 kcal and 50 g fat</td>
<td>Depends on content of meal</td>
<td>Meals chosen to have different fat sources and antioxidants: 1) olive oil and bread, 2) olive oil, bread, vitamins C and E, 3) olive oil, bread, foods containing antioxidants (balsamic vinegar, salad), 4) canola oil and bread, 5) salmon and crackers. Meal 1: reduced FMD (~14.3 to 9.9%), and inverse correlation between postprandial changes in serum TG and FMD ($r = -0.47$); no change in FMD following all other meals.</td>
</tr>
<tr>
<td>Volek et al. 2008</td>
<td>HS (16 M, 14 F)</td>
<td>908 kcal, 85 g fat, with/without 2 g L-carnitine (3 weeks supplementation)</td>
<td>↓ placebo vs carnitine (not vs baseline)</td>
<td>No significant postprandial Δ or effect of carnitine on TNF-alpha (inflammatory marker) and MDA (oxidative response). No association between TG (expressed as AUC or values at any time point) and FMD. Plasma IL-6 increased post-HFM; no effect of carnitine.</td>
</tr>
<tr>
<td>West et al. 2005</td>
<td>T2D (13 M, 5 F)</td>
<td>~625 kcal, 50 g fat with: 1) MUFA, 2) ALA+MUFA, with 3.3 g of ALA, 3) DHA+MUFA, with 2.8 g EPA, 1.2 g DHA, 0.2 g ALA</td>
<td>↔ those with low fasting TG, ↑ ALA+MUFA and DHA+MUFA in those with high fasting TG</td>
<td>After the MUFA meal, there was an inverse relationship between TG level and FMD ($r = -0.50$); this relationship was reversed following meals with omega-3 fatty acids ($r = 0.49$ for each of ALA+MUFA and DHA+MUFA meals).</td>
</tr>
<tr>
<td>Westphal et al. 2006</td>
<td>HS (8 M, 8 F)</td>
<td>1 g fat / kg body weight, with/without addition of 50 g sodium caseinate or soy protein</td>
<td>↓ HFM ↔ HFM + protein</td>
<td>Addition of protein to HFM resulted in: ↓ TG and FFA, ↑ insulin, and ↑ arginine/ADMA ratio.</td>
</tr>
<tr>
<td>Westphal and Luley 2011</td>
<td>HS (2 M, 16 F)</td>
<td>1 g fat / kg body weight: rich (918 mg) or poor (15 mg) in flavanols</td>
<td>↓ flavanol-poor HFM ↓ less flavanol-rich HFM</td>
<td>Postprandial TG and FFA not different between flavanol-rich and flavanol-poor HFMs</td>
</tr>
<tr>
<td>Williams et al. 1999</td>
<td>HS (10 M)</td>
<td>~900 kcal and: 1) 64 g fat that had been used for deep frying in a fast food restaurant, 2) 64 g unused cooking fat, or 3) a LFM (480 kcal; 18.4 g)</td>
<td>↓ used cooking fat ↔ unused cooking fat or LFM</td>
<td>↑ TG after all meals with greatest increase after unused fat meal. Postprandial ↑ TG not associated with Δ in FMD.</td>
</tr>
<tr>
<td>Williams et al. 2001</td>
<td>14 subjects</td>
<td>?</td>
<td>↔</td>
<td>Meals rich in olive oil and safflower oil used hourly for deep-frying for 8 h and containing high levels of lipid oxidation products.</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
<td>Meal: energy &amp; fat composition</td>
<td>Effect of FC on EF</td>
<td>Comments</td>
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<tr>
<td>Wilmink et al. 2000</td>
<td>HS (10 M, 10 F)</td>
<td>50 g fat/m² body surface area; after 2 weeks folic acid (10 mg/day) or placebo</td>
<td>↓ placebo</td>
<td>MDA ↑ after HFM in placebo condition; ↔ in folic acid</td>
</tr>
<tr>
<td>Yunoki et al. 2011</td>
<td>17 M, 3 F (9 with dyslipidemia)</td>
<td>30 g fat/m² body surface area</td>
<td>↓ pre-treatment</td>
<td>Ezetimibe: lipid-lowering drug (selectively inhibits cholesterol absorption); suppressed postprandial ↑ TG (AUC) and remnant lipoprotein cholesterol</td>
</tr>
</tbody>
</table>

Abbreviations: Ach = acetylcholine; ACT = active; ADMA = asymmetric dimethylarginine; AH = active hyperemia; ALA = α-linolenic acid; ANG II = angiotensin II; AUC = area under the curve; BMI = body mass index; CAD = coronary artery disease; CHO = carbohydrates; CME = continuous moderate exercise; DHA = docosahexaenoic acid; EF = endothelial function; EFI = endothelial function index; EPA = eicosapentaenoic acid; EX = exercise; F = female; FBF = forearm blood flow; FFA = free fatty acid; FMD = flow mediated dilation (measured in brachial artery unless otherwise stated); FO = follicular; GSH-Px = glutathione peroxidase; GTN = glyceryl trinitrate; HDL = high density lipoprotein; HFM = high-fat meal; HIIE = high intensity interval exercise; HO = high oleic sunflower oil; HRmax = maximum heart rate; HS = healthy subjects; ICAM = intercellular adhesion molecule; INA = inactive; IL-6 = interleukin-6; L = luteal; LBF = leg blood flow; LDL = low density lipoprotein; LFM = low-fat meal; M = male; Mch = methacholine chloride; MDA = malondialdehydes; ME = menses; MUFA = monounsaturated fatty acids; NO = nitric oxide; NT = nitrotyrosine; OGTT = oral glucose tolerance test; oXLDL = oxidized low density lipoprotein; PUFA = polyunsaturated fatty acids; RH = reactive hyperemia; RLP-C = remnant-like particle cholesterol; ROS = reactive oxygen species; SA = stearic acid; SF = saturated fat; SNP = sodium nitroprusside; SOD = superoxide dismutase; TBARS = thiobarbituric reactive substances; TG = triglyceride; T2D = type 2 diabetes; VCAM = vascular cellular adhesion molecule; VLDL = very low density lipoprotein; vWF = von Willebrand factor; ↓ = decrease; ↑ = increase; ↔ = no change.

Please see Appendix B for a more comprehensive version of this table, including specific meal contents and protocol details.
3.3 The acute impact of fat consumption on endothelial function

There is considerable variability in the literature regarding the influence of acute ingestion of fat on endothelial function. Indeed, while several studies have identified a negative impact of acute fat consumption on endothelial function in both resistance and conduit arteries (e.g. Vogel et al. 1997; Gaenzer et al. 2001; Schinkovitz et al. 2001), others have shown no effect (e.g. Harris et al. 2012; Johnson et al. 2011; Muntwyler et al. 2001), or divergent effects depending on the particular fat composition (e.g. Berry et al. 2008, Steer et al. 2003) (see Table 3-1 for comprehensive summary).

Vogel and colleagues (1997) were the first to test the hypothesis that consumption of a single high fat meal would acutely impair endothelial function in healthy subjects. They assessed FMD before and hourly for 6 h post-consumption of single isocaloric high- and low-fat meals (900 calories, 50 g and 0 g of fat respectively). They found that FMD was significantly impaired at 2 through 4 h following consumption of the high fat meal (HFM), with the peak impairment occurring at 4 h postprandial (~21% at baseline to 10% at 4 h). In contrast, no changes from baseline were observed after the low fat meal (LFM).

Similar findings of impaired endothelial function following consumption of a HFM have been demonstrated by others (Table 3-1), with impairment being observed as early as 1 h post-meal consumption (Ceriello et al. 2002; Sarabi et al. 2001; Steer et al. 2003), often peaking at 4 h postprandial (Plotnick et al. 1997; Yunoki et al. 2011; Anderson et al. 2001; Tentolouris et al. 2008; Vogel et al. 1997), and lasting as long as 8 h (Anderson et al. 2006; Sodre et al. 2011).

In contrast, Djousse et al. (1999) found no impairment in FMD following consumption of a HFM, with or without red wine (added for its antioxidant polyphenols), in young, healthy men and women (n = 13). There was a trend for reduced FMD however, with FMD of 9.5 ± 5.0% at baseline and 6.8 ± 3.6% at 4 h postprandial, so it is possible that the study was underpowered to detect a true effect.
In a larger sample \((n = 24)\), Funada and colleagues (2002) also found no impairment in FMD following consumption of a HFM in middle-aged normolipidemic men and women. In their study, they divided subjects into “high responders” and “normal responders” based on levels of postprandial remnant-like particle cholesterol [RLP-C; a lipid subfraction, considered highly atherogenic, that correlates well with plasma triglycerides (van Hees et al. 2008); high responders had RLP-C > 7.5 mg/dl, normal responders ≤ 7.5 mg/dl]. They identified that while FMD did not change postprandially in either group, high responders had significantly lower FMD both at baseline and postprandial than normal responders (baseline vs 4 h FMD for high and normal responders respectively: ~4.3 vs 4.2% and 8.3 vs 8.6%) (Funada et al. 2002). Of the test meals in all the studies reviewed, the HFM in this study contained the least amount of fat (average of 24 g), possibly explaining the lack of a demonstrated effect. While no acute decline in FMD was observed, the findings do suggest an association between magnitude of postprandial lipemia and existing endothelial function, and support the assertion that endothelial dysfunction may be a component of the chronic interaction between fat consumption and CVD.

Several other authors have reported preserved endothelial function following acute consumption of a fatty meal challenge (Djousse et al. 1999; Funada et al. 2002; Giannattasio et al. 2005; Gudmundsson et al. 2000; Harris et al. 2012; Johnson et al. 2011; Muntwyler et al. 2001; Padilla et al. 2006a; Raitakari et al. 2000; Schillaci et al. 2001; Sejda et al. 2002; Silvestre et al. 2008; Strey et al. 2004; Gokce et al. 2001; Williams et al. 2001). These disparate findings demonstrate that the relationship between fat consumption and endothelial function is not straightforward. Indeed, there is evidence that several factors may moderate the relationship between fat consumption and endothelial function, including: meal composition, magnitude of postprandial lipemia, sex, physical activity, underlying health status, and the vasodilatory effect of meal consumption. These factors will be discussed in the following sections.
3.4 Factors that may moderate the relationship between fat consumption and endothelial function

3.4.1 Meal composition

Several research groups have found that the impact of a HFM on endothelial function depends on the specific composition of the meal (Berry et al. 2008; Cortes et al. 2006; Koulouris et al. 2010; Nicholls et al. 2006; Steer et al. 2003; Tentolouris et al. 2008; Vogel et al. 2000; Williams et al. 1999; Tushuizen et al. 2006; Westphal et al. 2006; Westphal and Luley 2011; Lin et al. 2008; Borucki et al. 2009; Wilminck et al. 2000; Anderson et al. 2006; Bae et al. 2003; Lee et al. 2002; Liu et al. 2002; Plotnick et al. 1997; Plotnick et al. 2003). Meal composition factors that may be important include: the total amount of fat, the particular type of fat (e.g. saturated versus mono- or polyunsaturated), and other components of the meal including protein, L-arginine or antioxidants.

3.4.1.1 Total amount of fat

First, as is intuitive, the total amount of fat seems to influence the impact of a HFM on endothelial function. Steer et al. (2003) assessed endothelium-dependent and –independent resistance vessel function [local arterial infusions of methacholine chloride (MCh) and sodium nitroprusside (SNP) respectively] before and 1 and 2 h after an ordinary mixed meal (34% energy from fat), or isocaloric meals with low-fat (20%) or minimal fat (3%) content. Compared to a fasting baseline, they found that the MCh-induced forearm blood flow was reduced following the 34% fat meal, unchanged after the 20% fat meal, and increased after the 3% fat meal (responses to SNP infusion were unchanged following all meals), signifying that the amount of fat determined the nature of the effect on the vascular endothelium of resistance vessels.

Similarly, Tushuizen et al. (2006) found that brachial artery FMD was preserved after a single HFM, but became impaired following a second HFM consumed 4 h after the first (each containing 50 g fat). This suggests that a particular absolute amount of fat may be required for
impairment to be manifest, and consecutive HFMs may be additive in terms of their effect on the endothelium.

Together, these findings suggest that the total amount of fat is an important moderating factor. There may be a threshold for fat consumption, under which endothelial function remains intact, and above which endothelial function is compromised.

3.4.1.2 Type of fat

The fatty acid composition of a meal determines the composition of postprandial triglyceride particles (Williams 1997) and for a given amount of fat, the type of fat appears to influence the nature of the interaction with endothelial function. For instance, Nicholls and colleagues (2006) demonstrated that a meal rich in saturated fat decreased endothelial function in young healthy men and women, while a meal rich in polyunsaturated fat had no impact, and Koulouris et al. (2010) observed preserved endothelial function following meals rich in mono- or polyunsaturated fats but depressed FMD following consumption of a saturated fat-rich meal. Tentolouris et al. (2008) demonstrated similar results in middle-aged persons with type 2 diabetes, and West et al. (2005) demonstrated that meals rich in monounsaturated fats (50 g) containing 3-5 g of omega-3 fatty acids (polyunsaturated) did not alter or significantly improved endothelial function in persons with type 2 diabetes and low and high fasting triglyceride levels respectively. Similarly, acute fish oil supplementation (high in omega-3 fatty acids) has been shown to protect against impaired endothelial function following a high-fat meal (Fahs et al. 2010). Vogel et al. (2000) assessed the impact of 5 different meals on subjects’ postprandial FMD [all with 900 kcal and 50 g fat from different sources: olive oil, canola oil (with 5 g of omega-3 fatty acids), or salmon (with 6 g omega-3), with two olive oil meals also containing antioxidant vitamins (C and E) or foods (balsamic vinegar and salad)]. They found that only one of the meals (consisting of olive oil and bread) negatively impacted postprandial FMD, despite similar increases in serum triglycerides across all meals.
Overall, findings are suggestive of a negative impact of saturated fat (e.g. Nicholls et al. 2006; Koulouris et al. 2010), with variability in the impact of unsaturated fats [increase (West et al. 2005), decrease (Ong et al. 1999; Rueda-Clausen et al. 2007) or no change (Tentolouris et al. 2008; Nicholls et al. 2006) in FMD relative to preprandial levels]. These observations may help to explain the evidence that consumption of saturated fat promotes atherogenesis, whereas mono- and polyunsaturated fats are potentially protective (Hu and Willett 2002; Kushi et al. 1985).

With respect to heat-modified fat, Williams et al. (1999) found that FMD decreased from fasting to 4 h following a meal with ~65 g of cooking fat that had been used for deep-frying, but was unchanged following a meal with an equivalent amount of unused cooking fat. This shows that degradation products of heated fat may contribute to endothelial dysfunction, and strengthens the assertion that it is not only the amount of fat that is important but its particular composition. Note that other findings are contradictory (Rueda-Clausen et al. 2007; Williams et al. 2001), with similar impairments following intake of either fresh or heated oil, however the total durations of deep-frying [80 or 160 min (Rueda-Clausen et al. 2007) or 8 h (Williams et al. 2001)] were substantially less than that in the study by Williams and colleagues (used for 1 week in a fast-food restaurant) (Williams et al. 1999).

### 3.4.1.3 Other meal components

Aside from the amount of fat and its composition, other meal components have been found to have moderating influences. For instance, the addition of protein (50 g caseinate or 50 g soy protein) neutralized the impact of a HFM (1 g fat / kg body weight) on FMD (Westphal et al. 2006), while the addition of flavanol to a fatty cocoa beverage (1 g fat / kg body weight) attenuated the reduction in postprandial FMD relative to an identical fatty beverage that was depleted of flavanols (Westphal and Luley 2011). Lin et al. (2008) and Borucki et al. (2009) found that the addition of L-arginine, the substrate for NO production, to a HFM attenuated or abolished the postprandial reduction in FMD, while Cortes et al. (2006) found that a HFM rich in
walnuts (a source of L-arginine) had no impact on postprandial FMD in healthy participants but actually improved it in those with hypercholesterolemia. In a placebo-controlled trial, Wilmink et al. (2000) found that 2 weeks of folic acid treatment (10 mg/day) prevented the acute decline in FMD following consumption of a HFM.

Similarly, several investigators have observed that pre-treatment with antioxidants (Anderson et al. 2006; Plotnick et al. 2003) or co-administration of antioxidants with the HFM (Bae et al. 2001; Lee et al. 2002; Liu et al. 2002; Plotnick et al. 1997; Bae et al. 2003) blunted or eliminated the postprandial FMD decline. Antioxidants naturally occurring in fruits and vegetables however may not be sufficient to offset the negative impact of a HFM (Rudolph et al. 2007), and inclusion of red wine (which contains several polyphenolic compounds that have antioxidant properties) has also had mixed results with no effect (Djousse et al. 1999) or a delayed counteracting effect (no impact at 2 h but attenuated impairment at 4 h) (Koulouris et al. 2010).

3.4.1.4 Summary of moderating impact of meal composition

Therefore there is evidence that the impact of a HFM on endothelial function is moderated by the total amount and type of fat, and the other elements that comprise the remainder of the meal (e.g. protein, flavanols, L-arginine, folic acid, antioxidants). Some of these meal components are thought to exert their protective effect by attenuating the rise in lipemia post-meal consumption (e.g. protein; Westphal et al. 2006), the importance of which is discussed below (“Magnitude of Postprandial Lipemia”), while others are thought to act on downstream pathways that are affected by fat consumption [e.g. by providing substrate for NO production (Cortes et al. 2006), or by reducing fat-induced oxidative stress (Plotnick et al. 1997)]. This highlights the complexity of diet-endothelial function interactions, the difficulty of isolating an impact of fat per se, and the importance of studying its impact both in isolation and in the context of ecologically valid meals.
3.4.2 Magnitude of postprandial lipemia

In addition to the fat content and type, the resulting magnitude of lipemia for a given fat challenge may influence the interaction between fat consumption and endothelial function. Gaenzer et al. (2001) measured subjects’ FMD before and at 4 and 8 h after ingestion of a HFM (730 kcal, 65 g fat / m² body surface area) or in a continued fasting state. They found that postprandial FMD was lower than FMD in the continued fasting condition at the 4 h time point. At this time point, there was wide inter-subject variability in plasma triglyceride levels (range ~0.79 to 4.78 mmol/L, mean ~2.5 mmol/L), and the subjects’ FMD correlated inversely with the magnitude of postprandial lipemia [expressed as the 8 h area under the curve (AUC) of triglyceride; \( r = -0.81 \)], while there was no correlation between postprandial FMD and fasting triglyceride levels. Furthermore, when subjects were divided into low and high postprandial lipemia responders (based on magnitude of 8 h triglyceride AUC), two clear patterns of responses were identified. In the low postprandial lipemia group, FMD was unaffected by the HFM, with similar values for postprandial and continued fasting FMD at both the 4 and 8 h time points. In contrast, in the high responder group FMD was significantly lower at 4 h following the HFM versus continued fasting, with a tendency to remain depressed at 8 h. Thus for a given fatty-meal challenge, subjects with small changes in blood lipid levels were protected from a deleterious impact on the endothelium, and on average the larger the individual excursion in lipemia from fasting levels the greater the transient impact on endothelial function.

Similarly, Berry et al. (2008) tested the hypothesis that ingestion of dietary fats that evoke different degrees of postprandial lipemia would differentially impact FMD. Subjects’ FMD was assessed before and 3 h after consumption of a meal with 50 g fat from either stearic acid (known to result in blunted postprandial triglyceride increases) or oleic acid. They found that the postprandial increase in triglyceride was indeed lower following the meal rich in stearic acid than the oleic acid meal, and FMD was unchanged in the former and reduced in the latter. Several other studies have identified significant correlations between the magnitude of lipemia
and the postprandial reduction in endothelial function (Bae et al. 2001; Bae et al. 2003; Gaenzer et al. 2001; Giannattasio et al. 2005; Gokce et al. 2001; Liu et al. 2002; Maggi et al. 2004; Marchesi et al. 2000; Plotnick et al. 1997; Sarabi et al. 2001; Shimabukuro et al. 2006; Vogel et al. 1997). Together, these studies provide evidence that the increase in blood lipids, as contingent on individual differences and the particular type of fat, is an important component of the interaction between fat consumption and endothelial function.

In further support of the importance of the magnitude of lipemia, several studies have demonstrated that treatment with lipid-lowering drugs (statins, fibrates, and ezetimibe) mitigates the postprandial rise in triglycerides and attenuates or abolishes the reduction in FMD following a HFM in persons with type 2 diabetes, obesity, or dyslipidemia (Ceriello et al. 2002; Ceriello et al. 2005; Evans et al. 2000; Nagashima and Endo 2011; Yunoki et al. 2011).

In contrast, in the study by Steer et al. (2003) mentioned earlier, serum triglyceride levels increased more after the 20% fat meal than following the 34% fat meal, but endothelium-dependent vasodilation was only impaired after the 34% fat meal. This suggests that it is unlikely that the triglyceride level per se determined the endothelial vasodilatory function, however since this study utilized a between-groups design one must be careful in drawing this conclusion. Similarly, in the previously mentioned study by Williams et al. (1999), the increase in plasma triglycerides was greater after the unused fat meal than the used fat meal, while FMD was unaffected, a finding that was attributed to differences in fatty acid composition and oxidation products of the heated fat versus total content. Thus in some instances, the magnitude of postprandial lipemia does not appear to be closely related to the resultant impairment in endothelial function, and in a number of studies a postprandial impairment in endothelial function was observed to have no correlation with the magnitude of lipemia (Anderson et al. 2001; Berry et al. 2008; Johnson et al. 2011; Muntwyler et al. 2001; Rudolph et al. 2007; Rueda-Clausen et al.)
It has been suggested that these discrepant findings may be due in part to differences in the way data are quantified across studies (e.g. single measure versus AUC, or absolute versus relative changes) (Johnson et al. 2011; Wallace et al. 2010), and differences in study design including variations in lipid load (Wallace et al. 2010). Given the available evidence, it appears that the degree of lipemia response contributes to the ultimate impact of a HFM on endothelial function, but is unlikely to be the only important factor.

### 3.4.3 Sex

Few studies have evaluated the impact of a HFM on female subjects’ endothelial function in isolation, but there is evidence that women may be protected from this vascular insult. Schillaci et al. (2001) and Marchesi et al. (2000) assessed brachial artery FMD before and 2, 4, 6 and 8 h after consumption of a HFM (65 g fat and 700 kcal/m² body surface area) in young women (premenopausal) and men respectively. It was found that in men (Marchesi et al. 2000), FMD fell significantly from ~14.5% during fasting to ~3.5 and 4% at 2 and 4 h postprandial and returned to levels not significantly different from baseline thereafter, whereas in women (Schillaci et al. 2001) FMD was unchanged from ~14-15% at all time points.

Similarly, Harris and colleagues (2012) assessed FMD before and at 4 h following consumption of a HFM (940 kcal, 48 g fat) in young women during the menses (ME), follicular (FO) and luteal (L) phases of the menstrual cycle, and in young men. They found that while FMD was elevated in the FO and L phases (~12 and 11% respectively, versus 8% in ME), postprandial FMD was unaltered from fasting levels regardless of menstrual cycle phase. In contrast, men exhibited a ~50% reduction in FMD (~6% to 3%) despite having similar 17β-estradiol concentrations as women during menses. Even post-menopause, women with type 2
diabetes and healthy control women have been found to have preserved resistance vessel endothelial function following a HFM (660 kcal, 41 g fat) (Strey et al. 2004).

Thus there is evidence that women are protected from the vascular insult of a HFM. This may perhaps explain the inability to detect a significant relationship between fat consumption and endothelial function in some studies in which both men and women are included as subjects and the sample size is small (e.g. Djousse et al. 1999), and the apparent vascular protection in women may help to explain sex differences in CVD risk (Isles et al. 1992).

3.4.4 Physical activity

Both habitual physical activity levels and acute bouts of exercise may also moderate the relationship between fat consumption and endothelial function. Johnson et al. (2011) evaluated the effect of a HFM (940 kcal, 48 g fat) on endothelial function in active and inactive volunteers (where “active” was defined as at least 30 min of activity 3 times per week at moderate intensity, and “inactive” as less than 30 min of moderate physical activity per week). They found that FMD at 4 h postprandial was significantly reduced in inactive subjects, but was unchanged from baseline in those who were habitually active.

In terms of an acute effect of exercise, Padilla et al. (2006b) demonstrated that a session of aerobic exercise (45 min walking at 60% \( \dot{V}\text{O}_2\text{peak} \)) presented 2 h after ingesting a HFM actually elevated FMD relative to preprandial values. Tyldum et al. (2009) demonstrated an intensity-dependent protective effect of acute exercise. In their study, the decrement in postprandial FMD following a HFM was attenuated or completely abolished if the meal was preceded (16-18 h) by a bout of continuous moderate intensity exercise or isocaloric high intensity interval exercise respectively.

In contrast, Silvestre et al. (2008) found that FMD was preserved postprandially regardless of whether it was preceded by no exercise or exercise (resistance exercise and 30 min of treadmill running) performed 16 or 4 h before consumption of the HFM. Baseline artery
diameters were large in their group of subjects however (range 4.3 to 4.5 mm), and calculated
FMDs (pre- and post-prandial) were small (range ~1.4 to 2.8%). Therefore, perhaps there was
not enough resolution for differences between conditions to emerge.

Thus as a whole, there is evidence that both acute and chronic physical activity help to
protect against the vascular insult of a HFM. Additional work will be required to establish the
underlying mechanisms, the most advantageous timing of exercise in relation to fat ingestion, and
the optimal duration and intensity of activity.

3.4.5 Underlying health status

The interaction between fat consumption and endothelial function has been examined in
several populations, and there is some evidence that underlying health status may contribute to
the nature of the interaction. Giannattasio and colleagues (2005) found that an oral fat load
administered indexed to body surface area (680 kcal/m², 83% fat) reduced radial artery FMD in
middle-aged subjects with moderate hypertriglyceridemia and hypercholesterolemia (~12 to 5%
for baseline versus 6 h postprandial), but had no impact on FMD in healthy control subjects. This
suggests that health status may be important and that in some cases underlying cardiovascular risk
factors must be present in order for acute impairment in endothelial function to manifest
following consumption of a single HFM.

Moreover, without exception, FMD was found to be reduced following consumption of a
HFM in those studies whose subject populations had underlying cardiovascular risk factors
[including type 2 diabetes (Anderson et al. 2001; Anderson et al. 2006; Ceriello et al. 2002;
Evans et al. 2000; Fard et al. 2000; Lee et al. 2002; Tentolouris et al. 2008), dyslipidemia
(Yunoki et al. 2011; Maggi et al. 2004; Giannattasio et al. 2005; Marchesi et al. 2003), or obesity
(Nagashima and Endo 2011)] or established coronary artery disease (Liu et al. 2002). Thus
persons with compromised health status may be more vulnerable to the vascular insult of a HFM.
However, endothelial dysfunction following fat consumption has been observed in young, healthy
individuals without cardiovascular risk factors (e.g. Bae et al. 2003; Westphal et al. 2006), signifying that although underlying risk factors may increase susceptibility, healthy persons may still be at risk.

3.4.6 Vasodilatory effect of meal consumption

Lastly, the relationship between fat consumption and endothelial function is further complicated by instances when meal consumption evokes peripheral vasodilation and increased basal blood flow (i.e. altered vascular tone at rest complicates interpretation of changes in vascular reactivity). Gokce and colleagues (2001) demonstrated that FMD declined from ~14.7% during fasting to 10.6% at 2 h post-consumption of a HFM (~1,060 kcal, 56 g fat), however this decline was associated with a 6% increase in baseline brachial artery diameter (~3.5 to 3.7 mm), and the same peak diameter was achieved during reactive hyperemia (Gokce et al. 2001). In other words, the reduction in calculated FMD in this study can be attributed to basal vasodilation, as a similar peak diameter leads to a reduced FMD when expressed as a percentage of a larger baseline.

Other investigators have identified similar postprandial increases in artery diameter (Rudolph et al. 2007; Raitakari et al. 2000; Koulouris et al. 2010), and in these cases it is possible that the observed decrements in FMD are mathematical artifacts due to changes in baseline diameter. The functional significance of the smaller calculated FMD response following fat consumption in these cases is difficult to interpret. Notably, others have observed impaired FMD following fat consumption in the absence of a change in basal artery diameter (e.g. Vogel et al. 2007; Nicholls et al. 2006), and thus while the vasodilatory effect of meal consumption may complicate interpretation in some instances it cannot always account for the observed postprandial impairment in endothelial function.
In summary, several aspects of meal composition have been found to be physiologically relevant with respect to interaction with endothelial function, and therefore differences in meal composition complicate comparisons between studies (e.g. liquid test meal versus publicly available food; differences in total amount of fat; fat content administered as absolute quantity versus indexed to subject weight or body surface area; different proportions of saturated, monounsaturated, and polyunsaturated fat; dissimilar macronutrient composition and/or caloric content; additional components of meal). In addition several meal-independent factors that may moderate the interaction between fat consumption and endothelial function have been identified, and may play a role in generating disparate findings. A summary of moderating factors and the expected directionality of their influence is provided in Table 3-2. It is acknowledged that there are likely other factors in addition to these that remain to be established.
Table 3-2. Summary of factors that may influence the acute interaction between fat consumption and endothelial function

<table>
<thead>
<tr>
<th>Moderating factor</th>
<th>General impact on fat-consumption-induced endothelial function impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal fat content</td>
<td>&gt; fat content (absolute or %) = &gt; impairment; there may be a threshold that must be reached for impairment to manifest</td>
</tr>
<tr>
<td>Fatty acid composition</td>
<td>saturated fat detrimental in most cases; impact of mono- and polyunsaturated fats more controversial (may have no impact, increase or decrease endothelial function)</td>
</tr>
<tr>
<td>Other meal components</td>
<td>protein, flavanols, L-arginine and folic acid attenuate or protect against impairment</td>
</tr>
<tr>
<td>Addition of antioxidants to meal, or pre-treatment with antioxidant phytochemicals</td>
<td>blunt or eliminate postprandial decrease in endothelial function</td>
</tr>
<tr>
<td>Magnitude of postprandial lipemia (influenced by individual differences and type of fat)</td>
<td>&gt; lipemia = &gt; impairment</td>
</tr>
<tr>
<td>Sex</td>
<td>&gt; impairment in men vs women</td>
</tr>
<tr>
<td>Habitual physical activity</td>
<td>protects against decline in endothelial function post-HFM</td>
</tr>
<tr>
<td>Acute exercise in proximity to meal consumption (pre- or post-consumption)</td>
<td>attenuates or abolishes post-HFM decline, or augments endothelial function versus pre-meal values</td>
</tr>
<tr>
<td>Underlying health status (e.g. healthy versus presence of type 2 diabetes, dyslipidemia, obesity, coronary artery disease)</td>
<td>&gt; propensity for impairment with underlying risk factors or pathologies</td>
</tr>
<tr>
<td>Influence of meal consumption on baseline vessel diameter and blood flow</td>
<td>complicates interpretation; same peak diameter is a smaller % change from a larger baseline diameter</td>
</tr>
</tbody>
</table>

See text for details and references. HFM = high-fat meal.
3.5 Putative mechanisms underlying the impact of acute fat consumption on endothelial function

The mechanisms by which consumption of a HFM may result in an impairment of vascular endothelial function are likely multifactorial and are not fully understood (Gill et al. 2003) although the postprandial increase in oxidative stress seems to be the most important factor (Vigna et al. 2004). Other possible contributing mechanisms include: a postprandial rise in endogenous NO inhibitors/antagonists (Fard et al. 2000; Westphal et al. 2006), and/or direct cytotoxicity of triglyceride-rich lipoproteins to endothelial cells (Chung et al. 1998; Hennig et al. 1992; Speidel et al. 1990). These mechanisms will be discussed in brief here.

3.5.1 Increased oxidative stress

“Oxidative stress” denotes a disturbance of the redox state, characterized by an imbalance between the prooxidant load and antioxidant defense that can potentially lead to molecular damage (Sies et al. 2005). Extensive research has demonstrated that the postprandial increase in lipid levels following consumption of a high fat meal leads to increased oxidative stress (Sies et al. 2005), which subsequently results in an impairment in endothelial function (reviewed in Wallace et al. 2010). The pathways by which this is proposed to occur are illustrated in Figure 3-1B.

In brief, the postprandial increase in blood lipids uncouples oxidative phosphorylation and results in superoxide (O$_2^-$) production by the mitochondria (Woodman et al. 2005; Brownlee 2005). Superoxide degrades NO directly, resulting in formation of peroxynitrite (ONOO$^-$), which is itself a potent oxidant (Beckman and Koppenol 1996). Peroxynitrite oxidizes BH$_4$, a critical cofactor for endothelial nitric oxide synthase (eNOS), resulting in eNOS uncoupling (i.e. in the absence of sufficient BH$_4$ cofactor, O$_2$ becomes the substrate for eNOS instead of L-arginine,
Figure 3-1. Pathways of endothelial nitric oxide (NO) production and endothelial dysfunction during postprandial lipemia.

(A) Pathways of endothelial NO production. NO is synthesized from its precursor, L-arginine, along with electrons carried by NADPH and molecular oxygen ($O_2$), with the help of the enzyme endothelial nitric oxide synthase (eNOS) and other cofactors such as tetrahydrobiopterin (BH$_4$). eNOS can be activated by shear stress from arterial blood flow, insulin, and small molecule agonists such as Acetylcholine (Ach).

(B) During postprandial lipemia, there is an increased influx of free fatty acids (FFAs) into the muscle, adipose and hepatic tissue (Goldberg et al. 2009), and the vascular endothelial cells (Brownlee 2005), resulting in FFA oxidation in the mitochondria. The increased flux of FFAs through β-oxidation and FFA-derived acetyl CoA through the tricarboxylic acid cycle (TCA) results in overproduction of electron donors (NADH and FADH$_2$) at the electron transport chain (inset). As a result, the voltage gradient across the mitochondrial membrane increases to a point where electron ($e^-$) transfer inside complex III is blocked (Korshunov et al. 1997). This causes electrons to be backed up to coenzyme Q where they are donated to molecular oxygen and superoxide ($O_2^-$) is formed (Brownlee 2005). $O_2^-$ scavenges NO, resulting in the formation of peroxynitrite (ONOO$^-$), itself a potent oxidant (Beckman and Koppenol 1996). Peroxynitrite and $O_2^-$ can oxidize BH$_4$, which leads to eNOS uncoupling (i.e. production of $O_2^-$ instead of NO by eNOS) (Forstermann and Munzel 2006a; Forstermann 2006). Thus oxidative stress results in reduced NO bioavailability, via both a reduced production (due to uncoupled eNOS) and enhanced degradation (via $O_2^-$), and subsequent loss of its vasoprotective properties. FAD, FADH$_2$ = oxidized and reduced flavin adenine dinucleotide; NAD+, NADH = oxidized and reduced nicotinamide adenine dinucleotide; NADPH = nicotinamide adenine dinucleotide phosphate. Adapted with permission from (Wallace et al. 2010).
resulting in the production of $O_2^-$ in lieu of NO) (Forstermann 2006; Forstermann and Munzel 2006b). This creates an amplification pathway, whereby the initial increase in oxidative stress ($O_2^-$) potentiates increasing oxidative disruptions (secondary to ONOO'). Thus increased postprandial oxidative stress results in impaired endothelial function via both a reduced production of NO, and an increase in its breakdown, resulting in an attenuation of its vasoprotective and vasodilatory properties.

In support of this proposed mechanistic link, several investigators have observed simultaneous increases in oxidative stress and reductions in FMD following consumption of a single HFM (Bae et al. 2001; Ceriello et al. 2002; Anderson et al. 2001; Anderson et al. 2006; Tsai et al. 2004), that are attenuated (Ceriello et al. 2002; Anderson et al. 2006) or abolished (Bae et al. 2003; Lee et al. 2002; Liu et al. 2002; Plotnick et al. 1997; Plotnick et al. 2003) with pretreatment with antioxidants or co-administration of antioxidants with the HFM. In addition, the role of oxidative stress in impaired FMD following a HFM may explain the moderating impacts of physical activity and underlying disease status, because these factors are known to influence antioxidant defenses (Tyldum et al. 2009; Chehade et al. 2013). Longer-term intervention studies are required to determine whether regular antioxidant supplementation may combat recurring postprandial increases in oxidative stress and subsequent endothelial dysfunction.

### 3.5.2 Increased endogenous NO inhibitors or antagonists

A second mechanism by which fat consumption may impair endothelial function is via a postprandial increase in endogenous NO inhibitors (e.g. asymmetric dimethylarginine; ADMA) or antagonists (e.g. endothelin-1; ET-1).

#### 3.5.2.1 Asymmetric dimethylarginine

ADMA is a product of dimethylation of proteins and acts as an endogenous eNOS inhibitor that is associated with endothelial dysfunction (Boger 2003). Fard and colleagues (2000) measured plasma ADMA and FMD before and 5 h after ingestion of a HFM (1,265 kcal,
105 g fat) or an isocaloric meal with 0 g fat in subjects with type 2 diabetes. They found that the preprandial ADMA correlated inversely with FMD \((r = -0.32)\), such that the greater the ambient ADMA the smaller the basal vascular endothelial function. Moreover, following the HFM, the FMD was significantly reduced (~7% to 1%), and the reduction in FMD was inversely related to the increase in ADMA \((r = -0.37)\). No changes in AMDA or FMD were observed following consumption of the fat-free meal. These data suggest that changes in ADMA levels in response to a high-fat load may contribute to endothelial dysfunction.

This increase in ADMA following fat consumption is not always shown however (Cortes et al. 2006; Rudolph et al. 2007; Westphal et al. 2006). For instance, in healthy individuals, Westphal and colleagues (2006) measured FMD and the ratio of L-arginine/ADMA before and hourly for 8 h after consumption of a HFM alone (1 g fat / kg body weight) or a HFM plus 50 g of caseinate or soy protein. They found that FMD was significantly reduced following the HFM alone, but that the L-arginine/ADMA ratio was unchanged. In contrast, the addition of protein to the HFM attenuated the increase in postprandial triglycerides, increased the L-arginine/ADMA ratio, and abolished the impairment in FMD. Thus while the L-arginine/ADMA ratio was unchanged following the HFM alone, this was unfavourable relative to the increase in L-arginine/ADMA following the HFM plus protein, and may still be important as the ratio of L-arginine/ADMA correlates with endothelial function in healthy persons (Bode-Boger et al. 2003).

### 3.5.2.2 Endothelin-1

Indirect evidence suggests that elevated ET-1, a potent vasoconstrictor, may also contribute to the reduction in FMD following a HFM. In rats, plasma ET-1 levels were found to increase after 4 weeks on a high-cholesterol diet, and ET-1 levels correlated with plasma total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) concentrations (Horio et al. 1991), although it is unclear whether chronic diet modification was essential for these effects. In cultured human endothelial cells, lipoproteins have been found to
stimulate release of ET-1 in a time- and dose-dependent manner (Horio et al. 1993), and elevated ET-1 has been found in the circulation of hypercholesterolemic humans (Mangiafico et al. 1996). While these data suggest a possible role for elevated ET-1, to our knowledge ET-1 has never been measured in conjunction with FMD post-fat consumption.

3.5.3 Cytotoxicity of triglyceride-rich lipoproteins to endothelial cells

Lastly, in cell culture studies, triglyceride-rich lipoproteins have been shown to be directly cytotoxic to endothelial cells. For instance, Speidel et al. (1990) found that hypertriglyceridemic serum and triglyceride-rich lipoproteins that were lipolyzed in vitro with lipoprotein lipase were cytotoxic to cultured human umbilical vein endothelial cells, as evidenced by their detachment from the culture dish (Speidel et al. 1990). This cell injury was inhibited when high density lipoprotein (HDL) was added to the culture medium in an equivalent concentration to the offending lipids. Others have similarly demonstrated cytotoxicity of lipids (Hennig et al. 1992; Chung et al. 1998), with the degree of endothelial cell injury depending on the dose and duration of exposure (Hennig et al. 1992).

These observations may partly explain the moderating influences of the type of fat (i.e. some fatty acid families more cytotoxic than others) and the magnitude of postprandial lipemia (i.e. relevance of amount of endothelial exposure). Thus in addition to the functional impairments imposed by increases in oxidative stress and endogenous NO inhibitors, the postprandial increment in lipemia may reduce the structural integrity of the endothelium via direct cytotoxicity to endothelial cells.

3.6 Future directions

Further research will be required to: 1) elaborate on the mechanisms by which fat consumption interacts with endothelial function; 2) to isolate the effects of fat per se from other dietary components, and to identify how fat consumption in combination with other nutrients
alters the nature of the interaction with the vascular endothelium; and 3) to establish the optimal dose and combination of foods with respect to cardiovascular health.

Given what is currently known about the factors that may moderate the relationship between fat consumption and endothelial function (Table 3-2), a number of recommendations to guide future research can be suggested. First, future studies should carefully quantify the amount and type of fat administered, and possibly control for inter-individual differences in lipid absorption and metabolism (either by indexing responses to plasma lipid levels, or by identifying individuals as fat “responders” and “non-responders” and performing separate analyses). Since it is currently unknown how long an acute bout of exercise may interact with the impact of fat consumption, it can conservatively be recommended that testing should commence at least 24-48 h post-acute physical activity, and subjects’ fitness levels should be documented. Data for male and female participants should be examined separately, and premenopausal women should be tested in days 1-7 of the menstrual cycle as consistent with FMD assessment guidelines (Thijssen et al. 2011). Subject antioxidant activity and oxidative stress should also be quantified where possible to gain mechanistic insight, although the most appropriate biomarkers for oxidative stress also remain to be established (Wallace et al. 2010). Lastly, use of standardized lipid loads [i.e. oral fat tolerance tests (Dekker et al. 2007; Maraki et al. 2011)] may be useful for isolating the specific impact of different fatty acid families, although fat should also be studied in the context of ecologically valid meals in order to reflect real-life manifestation of fat intake.

3.7 Summary and conclusions

A well-functioning vascular endothelium exerts an anti-atherogenic influence and is critical for the maintenance of vascular health. Lifestyle factors such as a high fat diet can acutely impair the function of the endothelium, and cumulatively may be relevant in the long term with respect to CVD risk. Chronic consumption of a high fat diet has been linked with CVD, and acute impairment of endothelial function following consumption of a fatty meal may be an
important mechanistic link. The relationship between fat consumption and endothelial function is complex and appears to be modulated by many factors (see Table 3-2 for summary). Although the mechanisms underlying this relationship are likely multifactorial, evidence suggests that fat consumption impairs endothelial function primarily via an increase in oxidative stress, and that an increase in endogenous NO inhibitors/antagonists and/or direct cytotoxicity of postprandial lipemia to the endothelial cells may also contribute. Future research is required to elucidate the complexities of the relationship between acute fat consumption and endothelial function and will help to inform public health recommendations aimed at reducing the risk of cardiovascular disease.
3.8 References


Chapter 4 – Literature Review Part III:

Exercise intolerance in type 2 diabetes: is there a cardiovascular contribution?

Under review in the Diabetes journal with the following authors:

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4.1 Abstract

Physical activity is critically important for type 2 diabetes management, yet adherence levels are poor. This might be partly due to disproportionate exercise intolerance. Submaximal exercise tolerance is highly sensitive to muscle oxygenation; impairments in exercising muscle oxygen delivery may contribute to exercise intolerance in type 2 diabetes since there is considerable evidence for the existence of both cardiac and peripheral vascular dysfunction. While uncompromised cardiac output during submaximal exercise is consistently observed in type 2 diabetes, it remains to be determined whether an elevated cardiac sympathetic afferent reflex could sympathetically restrain exercising muscle blood flow. Furthermore, while deficits in endothelial function are common in type 2 diabetes and are often cited as impairing exercising muscle oxygen delivery, no direct evidence in exercise exists, and there are several other vasoregulatory mechanisms whose dysfunction could contribute. Finally, while there are findings of impaired oxygen delivery, conflicting evidence also exists. A definitive conclusion that type 2 diabetes compromises exercising muscle oxygen delivery remains premature. We review these potentially dysfunctional mechanisms in terms of how they could impair oxygen delivery in exercise, evaluate the current literature on whether an oxygen delivery deficit is actually manifest, and correspondingly identify key directions for future research.
4.2 Introduction

Exercise is a cornerstone of diabetes management, benefiting glycemia and insulin sensitivity and having a protective influence on cardiovascular health (1, 2). In addition, cardiovascular fitness is a strong independent predictor of mortality in persons with type 2 diabetes (3). Unfortunately however, persons with type 2 diabetes suffer from disproportionate levels of exercise intolerance, which affects the ability or willingness of persons with this disease to participate in physical activity (4). This exercise intolerance is manifested as chronic fatigue and weakness (5), together with slowed oxygen uptake kinetics and reduced maximal oxygen consumption capacity (~20% reduction versus controls matched for age, BMI, and questionnaire-based habitual activity level) (6-9). There is also preliminary evidence for increased perceived exertion during submaximal exercise even after adjusting for relative work intensity (10). Disproportionately low exercise tolerance (4) may be one of the principal barriers to a physically active lifestyle (10, 11), and indeed adherence to physical activity in persons with this disease is quite poor (11-13). Understanding and combating the causes of exercise intolerance will be critical in addressing this problem. Importantly, submaximal exercise intensity (not peak exercise intensity) is most relevant for assessment of exercise intolerance related to participation in physical activity (14).

Compromised convective muscle oxygen delivery (mO$_{2}$del) can increase fatigue progression during submaximal exercise in healthy persons (15, 16), thereby impairing exercise tolerance. Initial investigations into whether impairment in mO$_{2}$del is inherent in persons with type 2 diabetes took place in the early 1990’s (17, 18). In a recent review of the literature, Reusch et al. (4) concluded that “As such, nutritive blood flow appears to be an important contributor to the impaired response of the skeletal muscle to exercise in people with T2D [type 2 diabetes].”
However, while some studies report evidence that either the dynamic increase or the steady state of \( \text{mO}_2 \text{del} \) to exercising muscle is compromised as a result of type 2 diabetes in humans (7, 17-21) these findings are not universal (22-26). Furthermore, the degree to which compromised oxygen supply might explain disproportionate exercise intolerance in type 2 diabetes is currently unknown. Therefore the purpose of this perspectives paper is: 1) to provide a framework for understanding how muscle oxygenation (PmyoO\(_2\)) is established by the cardiovascular system and how PmyoO\(_2\) influences muscle metabolism and processes related to fatigue, 2) to critically assess the current understanding of convective O\(_2\) delivery (mO\(_2\)del) and diffusive O\(_2\) flux to exercising muscle in type 2 diabetes and their relationship to exercise intolerance, and 3) based on this assessment, to identify key research questions to guide future advancement in the field.

### 4.3 Cardiovascular determination of skeletal muscle oxygenation (PmyoO\(_2\)) (see Figure 4-1)

A primary role of the cardiovascular system during exercise is to provide the muscle with adequate O\(_2\) to support aerobic metabolism. The effectiveness of this role is ultimately reflected by the partial pressure of oxygen within skeletal muscle fibers (PmyoO\(_2\)) at a given oxygen consumption (\( \dot{V}O_2 \)) which is a key determinant of submaximal exercise skeletal muscle contractile and metabolic function (27). Of particular importance is the fact that PmyoO\(_2\) can vary at the same \( \dot{V}O_2 \) depending on the rate of oxygen delivery from the lungs to the skeletal muscle capillaries (i.e. mO\(_2\)del), and the diffusive conductance of the muscle for oxygen (mDKO\(_2\)).

The Fick Principle describes the relationship between muscle \( \dot{V}O_2 \), oxygen delivery (mO\(_2\)del) and oxygen extraction (arterio-venous O\(_2\) difference). Muscle oxygen delivery is the product of muscle blood flow (MBF) and arterial oxygen content (CaO\(_2\)) and it establishes the
Figure 4-1. Cardiovascular pathway for oxygen delivery to the mitochondria.

The “flow” of oxygen has a convective component delivering oxygen to the capillaries, where a diffusive component determines the delivery from the capillaries into the myocytes. (A) The Fick Principle identifies the role of vasodilation and the arterio-venous pressure gradient in increasing convective oxygen delivery ($mO_2\text{del}$) via increasing muscle blood flow (MBF). Arterial pressure is ultimately determined by the cardiac supply of blood to the arteries (cardiac output; CO) in balance with total systemic vascular tone (total vascular conductance; TVK). Heart rate (HR) and stroke volume (SV) responses determine CO. (B) Fick’s Law identifies the role of diffusive conductance ($mDKO_2$) and the capillary plasma oxygen level ($P\text{cap}O_2$) in determining the diffusion of oxygen into the myocyte. A combination of increased MBF supplying oxygen to the capillaries and reduced hemoglobin (Hb) affinity for oxygen determine $P\text{cap}O_2$ during exercise. If T2D impairs oxygen delivery to the mitochondria, it would be as a result of problems with one or a number of these determinants along the cardiovascular pathway. Note that myocellular oxygenation ($P\text{myo}O_2$), which plays a key role in determining muscle metabolic and contractile function, can be different at the same $\dot{V}O_2$; changes in ADP and Pi can compensate for reductions in $P\text{myo}O_2$ (due to impaired convective and/or diffusive oxygen flow) to maintain $\dot{V}O_2$. $CaO_2 =$ arterial oxygen content, $CHO =$ carbohydrate, $H^+ =$ hydrogen ion associated with lactate formation, $La^- =$ lactate, $Pyr =$ pyruvate.
partial pressure of oxygen in the capillaries (PcapO₂; the driving pressure for diffusion of O₂ from the capillaries to the inside of the myocytes). Therefore, it interacts with mDKO₂ to determine the diffusive flux of oxygen into the myocyte according to Fick’s Law and thereby establishes PmyoO₂ at a given VO₂.

For example, at a fixed submaximal VO₂, and a fixed diffusive conductance, a reduction in convective delivery of O₂ to the capillary space relative to its removal via diffusion into the myocyte leads to a decrease in PcapO₂. This reduces the pressure gradient for the diffusion of O₂ into the myocytes such that the influx of O₂ into the muscle fibers is less than its consumption by the mitochondria. As a result PmyoO₂ will decrease until the pressure gradient for diffusive flux is restored, and diffusion into the myocyte once again equals VO₂, but at a lower PmyoO₂. Thus, all other things being equal, any factor compromising the convective O₂ delivery [in terms of rate (kinetics) and magnitude (at steady state)] or the diffusive capacity of the muscle for O₂ will result in reduced PmyoO₂.

4.4 How does PmyoO₂ determine exercise tolerance?

Even slight reductions in exercising muscle PmyoO₂ can have a significant impact on the muscle’s metabolic environment and force production capability, making exercise feel more difficult [for review see (27); Figure 4-2]. For example, a decrease in PmyoO₂ results in reduced force production at a given motor neuron activation level independent of a fatigue effect (28). This translates to: a) an obligatory reduction in exercise intensity, or b) greater motor drive being required to maintain the workload, which would be experienced as increased perceived effort (i.e. exercise feels more difficult). In addition, with reduced PmyoO₂ greater changes in muscle fiber ADP and inorganic phosphate (Pi) levels are required to maintain the same aerobic supply of ATP (29). Elevated levels of Pi evoke muscle fatigue (30), while elevated
Figure 4-2. Effect of PmyoO$_2$ on muscle metabolism and contractile function.

(A) Schematic representation of the factors influencing muscle force production (i.e. ADP + Pi, PmyoO$_2$, motor drive). Perceived effort is a function of the degree of motor drive for a given force production. (B) A reduced PmyoO$_2$ necessitates both an increase in ADP + Pi levels and an increase in motor drive to maintain the given aerobic supply of ATP and exercise intensity. This would be experienced as increased perceived effort. PmyoO$_2$ = partial pressure of oxygen in myocytes. Adapted from Tschakovsky and Pyke (27).
levels of ADP stimulate glycolysis out of proportion to oxidative phosphorylation, leading to metabolic acidosis (31). Muscle fatigue would require further increases in motor drive to maintain force production (16), and thereby increase perceived effort. Clearly, in the presence of reduced O₂ supply one’s ability and willingness to sustain a given level of physical activity would be compromised.

4.5 Cardiovascular contributions to exercise intolerance in type 2 diabetes

Limitations to exercise tolerance that are due to the cardiovascular system are thus due to the muscle oxygenation environment (PmyoO₂) that is established by the convective delivery of O₂ from the lungs to the skeletal muscle capillaries, and the diffusive capacity for the movement of O₂ from the capillaries to the inside of the myocytes (27). In type 2 diabetes, there is evidence for impairments that would predict compromised exercising O₂ delivery to exercising muscle, including: 1) a central (cardiac) contribution, and 2) a peripheral (vascular) contribution.

4.6 Convective muscle O₂ delivery: potential mechanisms of impairment

The existence of cardiac and vascular dysfunction form the basis for hypothesizing compromised mO₂del in exercise. Diabetic cardiomyopathy, defined as abnormal cardiac structure and function in the absence of hypertension or coronary artery disease, is common in type 2 diabetes (32, 33), and is likely influenced by the duration and complications of the disease (33). In terms of the vasculature, a recent systematic review and meta-analysis of 27 observational reports (1,042 individuals with type 2 diabetes and 601 control subjects) identified both macro- and micro-vessel endothelial (conduit artery flow mediated dilation, acetylcholine infusion) and vascular smooth muscle (oral nitroglycerine, sodium nitroprusside infusion) dysfunction as characteristic of persons with type 2 diabetes (34). These impairments would predict compromised mO₂del in exercise.
4.6.1 Impaired MBF: Is there a central (cardiac) contribution?

Arterial blood pressure is a function of the balance between cardiac output and total vascular conductance. Since the overwhelming majority of vascular conductance available for blood pressure regulation during whole body exercise resides in the exercising muscle, a cardiac contribution to impaired mO$_2$del in exercise would manifest as either reduced arterial pressure due to blunted cardiac output and no vasoregulatory compensation, or compensatory reduced exercising muscle vascular conductance, or both (35). Since persons with type 2 diabetes are not hypotensive during exercise (and indeed may have exaggerated blood pressure responses (36)), compromised mO$_2$del would have to be due to blunted exercising muscle vasodilation, most likely as a result of increased sympathetic vasoconstriction.

Functional deficits of diabetic cardiomyopathy include left ventricular diastolic (LVDD) (32, 33) and systolic (LVSD) (37) dysfunction and might only present with an exercise challenge (37, 38). Mechanisms thought to be responsible include impaired Ca$^{2+}$ handling in cardiac myocytes, increased renin angiotensin system activation leading to increased oxidative damage and interstitial fibrosis, increased reactive oxygen species (ROS) production, and reduced mitochondrial efficiency effecting greater disturbance to myocyte cellular homeostasis [for review see Boudina and Abel (39)]. Recently, Reusch et al. (4) concluded that “there are impairments in muscle substrate delivery and utilization presumed secondary to decreased cardiac function.” This conclusion was based on findings of an association between resting LVDD and reduced maximal exercise capacity (40, 41), evidence for LVDD at peak exercise (38), potentially compromised cardiac perfusion in exercise (38, 42) and the above mentioned mechanisms of cardiomyopathy. While the associations cited suggest the possibility of cardiac dysfunction as a contributor to compromised maximal exercising mO$_2$del, a closer look at the literature reveals that studies have so far been consistent in their findings of normal steady state (6, 7, 43, 44) and dynamic adjustments (43, 45) of cardiac output. These findings suggest that adequate cardiac output is available for distribution to exercising muscle and do not support a role for impaired
cardiac pump function which would necessitate sympathetic vasoconstriction of exercising muscle to regulate blood pressure.

In contrast, the hypothesis that heightened cardiac sympathetic afferent reflex (CSAR)-mediated activation might evoke increased sympathetic vasoconstriction (46, 47) in exercising muscle represents a heretofore unconsidered mechanism. The CSAR is a positive-feedback sympathoexcitatory reflex (48) that leads to increases in blood pressure, heart rate, and myocardial contractile function (49). It is sensitive to a reduced perfusion environment and increases in endothelin-1 (48), ROS, protons and adenosine (46) all of which could be elevated in the diabetic heart. CSAR augmentation in diabetes has recently been demonstrated in a rat model of streptozotocin induced diabetes (47). A disproportionately large increase in blood pressure during exercise in persons with type 2 diabetes (17, 20), together with elevated catecholamines (17), is consistent with this in humans. It remains to be determined if this reflex results in restraint of exercising skeletal muscle vasodilation and therefore mO$_2$del.

4.6.2 Impaired MBF: Is there a peripheral (vascular) contribution?

Virtually all studies examining vasoregulatory mechanisms in type 2 diabetes have been done in resting skeletal muscle, which lacks validity for drawing conclusions about exercise hyperemia. Mechanisms of vasodilation in exercising muscle are numerous and interdependent, and their complex integrated function to match mO$_2$del to demand remains poorly understood (50).

4.6.2.1 Feed-forward mechanisms

Skeletal muscle resistance vessels dilate immediately with the first increase in contraction intensity, resulting in an immediate but incomplete increase in MBF that reaches a plateau within ~5-7 seconds (51). Recent work by Crecelius et al. (52) and Ross et al. (53) have identified K$^+$, nitric oxide, vasodilating prostaglandins and adenosine as vasodilatory mechanisms that together account for virtually all of the rapid vasodilation in healthy microcirculation.
At present we do not know how these mechanisms might be compromised by type 2 diabetes. Observations of reduced partial pressure of oxygen in the microvasculature (PcapO\(_2\)) or increased hemoglobin/myoglobin deoxygenation ([HHb]) at the initiation of exercise in type 2 diabetic vs healthy skeletal muscle (22, 54, 55) would suggest that there is a MBF deficit at the onset of exercise. Similarly, MacAnaney et al (23) quantified the initial increase in calf vascular conductance (inferring blood flow) with the onset of moderate intensity rhythmic contractions and found that the mean response time was significantly slowed in persons with type 2 diabetes versus lean or heavy controls. However, Slade et al. (56) used blood oxygen level dependent (BOLD) signal obtained with functional magnetic resonance imaging (fMRI) as an indirect measure of calf MBF in subjects with short duration of diagnosed diabetes (average of 6.6 yr), and found no impairment.

In summary, in humans there is evidence for (22, 23) and against (56), and in rat models there is evidence for (54, 55) an impairment in the initial increase in mO\(_2\)del during exercise. Future studies are required to determine if, and which, rapid-acting initial mechanisms are impaired in persons with type 2 diabetes and whether disease duration plays a role.

4.6.2.2 Feedback mechanisms

Within ~15-20 seconds of the increase in contraction intensity a second slower feedback mediated increase in MBF is initiated that adjusts MBF to steady state (51). Feedback control is thought to come from vasodilatory factors in proportion to metabolic demand and from the deoxygenation state of hemoglobin (Hb) which indicates the mismatch between O\(_2\) demand and delivery. At present, nothing is known about the function of exercising muscle metabolic feedback mechanisms in persons with type 2 diabetes; future studies are required to determine whether these mechanisms are impaired. In contrast, there is evidence for impairment in the following deoxygenation-related feedback mechanisms.
4.6.2.2.1 Red blood cell (RBC)-O$_2$ sensor

Increases in skeletal muscle O$_2$ demand results in localized red blood cell (RBC) Hb desaturation which triggers proportional ATP release from the RBC (57). This ATP binds to endothelial purinergic (P$_{2Y}$) receptors, and evokes nitric oxide (NO) and non-NO dependent vasodilation that ascends the arteriolar tree (conducted vasodilation) and increases MBF (57, 58). In this way, O$_2$ supply may be spatially matched to O$_2$ demand.

A signal transduction pathway for the release of ATP from RBCs has been proposed [for review see (59)]. This pathway contains the heterotrimeric G protein G$_i$ (60) which activates adenylyl cyclase, a transmembrane protein that catalyzes the conversion of ATP to cyclic AMP (cAMP). Accumulation of cAMP leads to an increase in ATP efflux from the RBC (61). RBCs of persons with type 2 diabetes have a diminished ATP release in response to Hb desaturation, potentially due to a reduced expression of the membrane-bound G$_i$ protein (62). In addition, reduced RBC deformability due to increased glycosylation may also contribute since mechanical deformation of RBCs, as might occur in contracting muscle where O$_2$ demand has increased, is also a stimulus for the release of ATP (61). Finally, recent evidence indicates that the function of endothelial purinergic receptors for ATP is attenuated in type 2 diabetes (63). However, no studies to date have examined the functioning of the RBC-O$_2$ sensor mechanism in vivo and its contribution to any impairment in MBF and mO$_2$del in persons with type 2 diabetes has yet to be determined.

4.6.2.2.2 Endocrine-like nitric oxide (NO) bioavailability

Recently a feedback mechanism of NO-mediated vasoregulation has been proposed in which NO bioavailability is determined by deoxygenation-sensitive NO-containing compounds in the blood [e.g. S-nitrosothiol (SNO) in the RBC and nitrite (NO$_2^-$) in plasma] (64, 65) (Figure 4-3). With deoxygenation RBCs release SNO which, in the presence of ambient thiols, can then transfer NO to the vasculature and promote vasodilation (64). Exposure to deoxygenation also
leads to increased conversion of NO$_2^-$ to NO (66). A potential role for plasma NO$_2^-$ in exercise tolerance is supported by whole body plasma nitrite flux (change from pre- to post-exercise) being a strong predictor of peak exercise capacity across subjects ranging from cardiovascular risk-factor-but-healthy, to peripheral artery disease (PAD) to PAD + type 2 diabetes (67, 68).

Evidence for potential defects in RBC and plasma NO transport in persons with type 2 diabetes is emerging. For instance, excessive glycation of Hb results in enhanced NO binding to the RBC (69) and as a result release of NO from these RBCs at sites of low oxygen concentration may be impaired (70). Plasma nitrite levels may also be reduced (67). The current evidence supporting inadequate NO bioavailability as a potentially important contributor to impaired mO$_2$del in exercise indicates that this is a mechanism deserving closer scrutiny, especially in human in vivo conditions.

4.6.2.3 Shear-mediated support of feed-forward and feedback mechanisms: Endothelial function

Endothelial cells respond to mechanical forces (e.g. shear stress) and pharmacological agents (e.g. ATP as mentioned previously, or acetylcholine; ACh) by releasing vasodilators into the interstitium (namely NO, vasodilatory prostaglandins, and endothelium-derived hyperpolarizing factor) (71). Of these vasodilators, NO has received by far the most attention. In most studies, endothelial function is impaired in persons with type 2 diabetes versus healthy controls (34). However it is critical to point out that endothelial function has only been examined in resting muscle in persons with type 2 diabetes, and evidence to support the common contention that it is the mechanism responsible for impaired exercising mO$_2$del (7, 20, 22) is based on correlation of resting endothelial function with exercising muscle blood flow across combined datasets of healthy controls and persons with type 2 diabetes [e.g. (20)]. Such analysis must be viewed with caution as it is often the case that co-variates differing between groups are not causally linked (i.e. in this case endothelial function and MBF).
Figure 4-3. Schematic of proposed dysfunction of endocrine-like nitric oxide (NO) bioavailability.

(A) Healthy - muscle oxygen consumption in exercise leads to red blood cell (RBC) deoxygenation and S-nitrosothiol (SNO) release (1) to supplement endothelial NO synthase (eNOS) NO production (2). Excess NO converts to nitrite ($\text{NO}_2^-$) which is a biomarker of NO bioavailability (3) and eventually nitrate ($\text{NO}_3^-$). NO initiates direct and conducted vasodilation to help match oxygen delivery to demand (4).

(B) Type 2 Diabetes - muscle oxygen consumption in exercise leads to RBC deoxygenation, but SNO release is impaired (1). eNOS NO production is also impaired (2), such that plasma nitrite is now converted to NO (3) but this is not able to maintain required NO bioavailability. Therefore, direct and conducted NO-mediated vasodilation (4) is inadequate to allow matching of oxygen delivery to muscle oxygen demand in exercise. FeNO = partially nitrosylated blood, FeO$_2$ = oxygenated blood, SH = thiol functional group.
4.6.2.4 NO bioavailability: Summary

Evidence suggests that NO bioavailability is reduced at rest as a result of type 2 diabetes. However, extrapolating these findings to exercise is problematic because exercising muscle vasodilatory control demonstrates considerable redundancy (50). To determine whether impaired NO bioavailability compromises mO\textsubscript{2}del will require studies in which NO bioavailability is acutely improved during exercise in persons with type 2 diabetes. These studies are currently underway in our laboratory.

4.6.2.5 Sympathetic restraint in exercising muscle

The skeletal muscle vascular bed normally experiences “sympathetic restraint” (sympathetic adrenergic vasoconstriction) of MBF during exercise as part of the integrated cardiac and peripheral resistance regulation of arterial blood pressure. Since increasing sympathetic restraint to exercising muscle has been demonstrated to reduce MBF (72), a plausible mechanism for reduced MBF in persons with type 2 diabetes might be exaggerated sympathetic restraint in exercise.

Preliminary evidence in support of this comes from work by Hogikyan et al. (73) who observed: 1) greater vasodilation in response to alpha\textsubscript{1}-receptor blockade (phentolamine), indicative of greater underlying adrenergic vasoconstrictor tone; and 2) greater vasoconstriction in response to norepinephrine infusion, indicating greater adrenergic vasoconstrictor responsiveness, in persons with type 2 diabetes versus controls at rest. If this increased sympathetic vasoconstriction is present during exercise, then it may represent a mechanism for reduced exercising MBF in type 2 diabetes. Studies which employ alpha-adrenergic receptor blockade in exercising muscle are needed to determine if this is indeed the case. Secondary to this would be identifying the cause of heightened sympathetic restraint, with possible contenders including the CSAR, skeletal muscle metaboreflex, and/or the baroreflex. While there is
preliminary evidence for amplified CSAR function (discussed earlier), at present nothing is known about metaboreflex and/or baroreflex function in T2D.

4.6.2.6 Functional sympatholysis in exercising muscle

While sympathetic restraint of muscle does occur, it is well established that this restraint is normally blunted by local factors in the exercising muscle (74, 75). This “functional sympatholysis” effect increases with exercise intensity (75). ATP (63, 74) and NO (75, 76) have been identified as sympatholytic agents, although data in support of NO is not unequivocal and its effect may depend on an interaction with prostaglandins (77). Nevertheless, if NO bioavailability is reduced in persons with type 2 diabetes, either via reduced production or enhanced degradation as studies suggest (20, 64), then this blunting effect may also be reduced such that sympathetic vasoconstriction restrains MBF to a greater degree than in healthy individuals. However, only one study has investigated functional sympatholysis in persons with type 2 diabetes and no impairment was evident (25). These initial findings are limited in that the subjects with type 2 diabetes had intact endothelial function, and functional sympatholysis was only examined during moderate intensity exercise. Further studies are needed to replicate these findings and to examine a range of exercise intensities and patient populations to determine how these might influence functional sympatholysis, and subsequently contribute to exercising MBF.

4.7 Convective muscle O₂ delivery during exercise in type 2 diabetes: Is it really impaired?

Given the evidence for both potential central and peripheral impairments in type 2 diabetes, it is reasonable to postulate that mO₂del may be impaired in persons with type 2 diabetes. Since CaO₂ does not appear to be compromised in type 2 diabetes (8), impaired mO₂del would be dependent on MBF.
4.7.1 Is adjustment of MBF at the onset of exercise slower in type 2 diabetes?

A slower adjustment of $\text{mO}_2\text{del}$ in response to exercise can reduce the rate at which aerobic ATP production increases (termed $\dot{V}O_2$ kinetics) to meet ATP demand (78) and result in a greater accumulation of metabolites that can contribute to fatigue (e.g. ADP, Pi) (79). A slowed $\dot{V}O_2$ kinetic response has been observed in persons with type 2 diabetes at various exercise intensities (9, 80) (Figure 4-4 A), as has a faster PCr breakdown (81).

Evidence of impairment in $\text{mO}_2\text{del}$ kinetics in persons with type 2 diabetes is largely indirect, as only one study has directly measured exercising limb MBF adjustments at the onset of exercise (23). Unfortunately, the authors chose to report only the dynamics of leg vasodilation, which were slower, to infer compromised blood flow. Other studies have relied on the rate of skeletal muscle deoxygenation to infer the degree of imbalance between $\text{mO}_2\text{del}$ and utilization. In situ diabetic rat skeletal muscle has been observed to have reduced resting microvascular oxygen partial pressure (PcapO$_2$), which declines more quickly and “undershoots” the eventual steady state in response to muscle electrical stimulation compared to control (54, 55) (Figure 4-4 B). While this is consistent with slower $\text{mO}_2\text{del}$ adjustment and compromised PmyoO$_2$, PcapO$_2$ eventually stabilizes at the same steady state level in both normal and type 2 diabetes animals, suggesting steady state $\text{mO}_2\text{del}$ may not be impaired.

In humans, Bauer et al. (22) measured pulmonary $\dot{V}O_2$ and skeletal muscle hemoglobin + myoglobin deoxygenation ([HHb]; using near infrared spectroscopy (NIRS)), and estimated microvascular perfusion during exercise transitions from unloaded to moderate cycling exercise via rearrangement of the Fick principle, whereby the [HHb] is assumed to be a valid surrogate of arterio-venous oxygen difference. They observed slower $\dot{V}O_2$ and estimated microvascular blood flow kinetics in the type 2 diabetes group. However, recent work by Murias et al. (83) has exposed the failings of this approach in providing valid estimates of microvascular perfusion kinetics. Interestingly, muscle oxygenation at steady state in the Bauer et al. study was not
Figure 4-4. Schematic of proposed exercise responses in persons with type 2 diabetes (dashed lines) vs. healthy controls (solid lines).

(A) Slowed $\dot{V}O_2$ kinetics in type 2 diabetes. The magnitude of $O_2$ deficit is determined by $\dot{V}O_2$ kinetics. Dashed arrows indicate a lower $\dot{V}O_2$ at time “x” for the dashed vs. solid curve. This is significant because it indicates that type 2 diabetes results in the need for a greater reliance on substrate-level phosphorylation to meet the identical ATP demand. [Adapted from Lukin and Ralston (82)].

(B) Overshoot of microvascular deoxygenation ($PcapO_2$; partial pressure of oxygen in the capillaries) in type 2 diabetes is suggestive of a greater MBF/$\dot{V}O_2$ mismatch at the onset of exercise. Responses in panels (A) and (B) are consistent with observations in the literature.

(C) Hypothesized skeletal muscle blood flow (MBF) dynamics accounting for these responses.
different between the groups, and like the findings in animal models (54, 55) suggests that steady state mO$_2$del was not compromised.

In summary, while findings to date are consistent with slowed mO$_2$del kinetics, the evidence is indirect, limited, and comes from observations of only 20 human subjects (22, 23) and 13 rodents (54, 55). The necessary experiments to provide conclusive evidence remain to be performed.

**4.7.2 Is steady state MBF reduced in type 2 diabetes?**

Menon et al. (18) demonstrated significantly reduced MBF in persons with type 2 diabetes immediately following submaximal exercise (unloaded plantar and dorsiflexion, 80 repetitions in 150 seconds). Similarly, Young et al. (17) found that the MBF response (measured by Xe$_{133}$ clearance) to 5 minutes of cycling at 75 W was significantly lower. Kingwell et al. (20) also observed reduced steady state leg MBF responses (measured via thermodilution) to moderate intensity cycling exercise (60% $\dot{V}O_2$peak). Lalonde et al. (7) observed that steady state femoral blood flow measured using MRI during low-intensity knee extensor exercise (1.5 kg ankle weights, 60 ± 5 rpm, ~15 min), indexed to lean thigh mass, was significantly lower in persons with type 2 diabetes. Unfortunately, absolute values for MBF were not given, so it is unclear whether absolute mO$_2$del was different between groups for a given $\dot{V}O_2$. Finally, Joshi et al. (19) estimated gastrocnemius perfusion during cycling at 50 W via white light spectroscopy and laser Doppler, and found it to be blunted but only in the group whose HbA1c was ≥ 8% (64 mmol/mol). However, this measurement technique has only been validated for non-muscle perfusion (84) and may not provide valid estimates of MBF.

In contrast, Martin et al. (24) found no difference in exercising leg blood flow between type 2 diabetes and control subjects (matched for age, BMI and $\dot{V}O_2$max) during 40 minutes of bicycle exercise at 60% $\dot{V}O_2$max. This discrepancy may be due to the recruitment of a fitter cohort [peak $\dot{V}O_2$ achieved by participants was on average ~1 L/min greater than that of the
participants in the study by Kingwell et al. (20)). However, a recent investigation in diabetic rats where a relative “fitness” issue would not be manifest found no impairment to exercising MBF (85). Likewise, Thaning et al. (25) found no impairment in MBF during knee-extensor exercise at 15 W; however unlike Kingwell et al. (20) their subjects did not have impaired endothelial function. Of particular interest are the data from studies that have measured muscle deoxygenation or microvascular O₂ pressures which, while finding evidence for slower mO₂del, also observed a recovery of muscle oxygenation in steady state equal to that in controls [human study (22), rat studies (54, 55)], which seems inconsistent with a reduced steady state mO₂del. Finally, in a recent study Womack et al. (26) measured brachial artery blood flow to exercising forearm as well as contrast enhanced ultrasound measures of forearm flexor capillary recruitment and found no deficits in persons with well controlled type 2 diabetes. However, others diagnosed with microvascular complications, based on spot urine analysis for proteinuria, demonstrated considerable capillary recruitment deficits, despite no evidence of impaired brachial artery blood flow. Since measurements were made during exercise consisting of 1 s contractions separated by 20 s of rest it is possible that this reflects deficits in post-exercise hyperemia rather than steady state exercising MBF.

In summary, a definitive conclusion that mO₂del is impaired in type 2 diabetes remains premature. A problem with interpreting the cumulative evidence to date is the small number of studies conducted and the small sample sizes within these studies, and the range of potentially confounding co-morbidities (e.g. cardiovascular disease, neuropathy, dyslipidemia) and individual characteristics (e.g. age, adiposity, glycemic control) across studies that can influence outcomes of interest (see Table 4-1 for summary). Moreover, medication status during testing differs widely across studies (including only medication-free subjects, completing testing after having subjects abstain from taking their medications for a standardized period of time, or testing subjects while on their various medications) (Table 4-1). Anti-diabetic (86),
Table 4-1. A summary of key studies that examined (or allow inferences about) exercising \(\text{mO}_2\text{del}\) in humans with type 2 diabetes (T2D)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Participants: (n), age, BMI</th>
<th>Duration of Diagnosis and HbA1c in T2D</th>
<th>Medication(s) &amp; Status during Testing (SDT)</th>
<th>Study Protocol</th>
<th>Key Results</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Baldi et al., 2003 (6) | T2D: \(n=11\) (4 ♀, 7 ♂), 52 ± 6 yr 30 ± 7 kg/m\(^2\)  
C: \(n=12\) (7 ♀, 5 ♂), 49 ± 5 yr 28 ± 6 kg/m\(^2\) | 5 ± 3 yr 7.5 ± 1.1% | \(T2D:\) metformin-glipizide (73%), ACE inhibitors (18%)  
C: none  
Exclusions: >1 anti-hypertensive medication, insulin  
SDT: not reported | Incremental \(\text{VO}_2\text{max}\) test to on a cycle ergometer (2 min increments) | • ↓ \(\text{VO}_2\) at some submaximal workloads in T2D vs C  
• \(\text{VO}_2\text{max}\) and arterio-venous \(\text{O}_2\) difference at maximal exercise were lower in T2D | It is possible that \(\text{VO}_2\) did not have time to reach “steady state” levels at times of measurement in the incremental protocol |
| Bauer et al., 2007 (22) | T2D: \(n=11\) (6 ♀, 5 ♂), 47 ± 4 yr 31 ± 4 kg/m\(^2\)  
C: \(n=11\) (5 ♀, 6 ♂), 47 ± 6 yr 28 ± 3 kg/m\(^2\) | not reported 6.8 ± 1.2% | \(T2D:\) not reported  
C: none  
Exclusions: insulin, thiazolidinediones, \(\alpha\)-glucosidase inhibitors, \(\beta\)-blockers, calcium channel blockers  
SDT: not reported | Exercise transitions from unloaded to moderate cycling exercise (~85% of each subject’s lactate threshold) | • \(\text{VO}_2\) kinetics slowed in T2D vs C  
• Estimated microvascular blood flow kinetics prolonged in T2D | Methodology for estimation of capillary blood flow [i.e. calculated with the Fick Principle using measures of \(\text{VO}_2\) and [HHb] (NIRS)] may not be valid (83) |
| Brandenburg et al., 2009 (80) | T2D: \(n=8\) ♀, 37 ± 6 yr 32 ± 7 kg/m\(^2\)  
C: \(n=9\) ♀ (overweight; 37 ± 6 yr) 30 ± 4 kg/m\(^2\)  
\(n=10\) ♀ (lean; 43 ± 7 yr) 24 ± 2 kg/m\(^2\) | 3 ± 2 yr 9.5 ± 1.9% | \(T2D:\) oral agents  
C: none  
Exclusions: insulin  
SDT: not reported | -Graded \(\text{VO}_2\text{max}\) test on a cycle ergometer  
-Constant-load cycling at 20, 30 and 80 W  
Testing before and after 3 mo exercise training (3x/wk) | • \(\text{VO}_2\text{max}\) and \(\text{VO}_2\) kinetics lowest and slowest in T2D  
• T2D group had greatest improvements in \(\text{VO}_2\text{max}\) and kinetics with training | Steady state submaximal \(\text{VO}_2\) not different between groups |
| Joshi et al., 2010 (19) | T2D: Group 1: HbA1c<8, \(n=31\), 50 ± 7 yr 26 ± 3 kg/m\(^2\)  
Group 2: HbA1c≥8, \(n=38\), 50 ± 5 yr 27 ± 4 kg/m\(^2\)  
C: \(n=32\), 49 ± 5 yr 24 ± 3 kg/m\(^2\)  
(♂/♀ not indicated) | Group 1:  
~4 yr <8%  
Group 2:  
~5 yr ≥8% | \(T2D\) (Group 1, Group 2): secretagogues (71, 74%), sensitisers (74, 76%), \(\beta\)-blockers (32, 5%), antihypertensives (52, 34%), diuretics (6, 3%), statins (10, 0%)  
C: none  
Exclusions: insulin  
SDT: on medications | 10 min cycling at 50 W | • Estimated gastrocnemius perfusion blunted in T2D group with HbA1c ≥ 8  
• ↓ \(\text{O}_2\) saturation in both T2D groups vs C | Measurement technique (laser Doppler and white light spectroscopy) may not be valid for muscle-perfusion estimates (84) |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Participants: n, age, BMI</th>
<th>Duration of Diagnosis and HbA1c in T2D</th>
<th>Medication(s) &amp; Status during Testing (SDT)</th>
<th>Study Protocol</th>
<th>Key Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingwell et al., 2003 (20)</td>
<td>T2D: n=9 ♀, 48 ± 1 yr 28 ± 1 kg/m²  C: n=9 ♀, 46 ± 2 yr 26 ± 1 kg/m²</td>
<td>not reported 6.2 ± 0.4%</td>
<td>T2D: metformin (22%), gliclazide (11%) C: none SDT: held medications for 24 h before testing</td>
<td>-25 min cycling at 60% VO_{2}peak -Assessed leg blood flow (LBF) responses to intra-femoral arterial infusion of Ach and SNP</td>
<td>↓ LBF responses to Ach and exercise (but not SNP) in T2D vs C %↑ in LBF in exercise and Ach infusion were significantly correlated (r = -0.54) Subjects matched for VO_{2}peak (so workload was same absolute and relative) Measurement technique: thermodilution</td>
<td></td>
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<tr>
<td>Lalande et al., 2008 (7)</td>
<td>T2D: n=8 ♀, 53 ± 1 yr 29 ± 1 kg/m² C: n=9 ♀, 49 ± 2 yr 28 ± 1 kg/m²</td>
<td>5 ± 3 yr 6.6 ± 0.3%</td>
<td>Not reported Exclusions: insulin, any cardiovascular medications (including β-blockers, ACE inhibitors)</td>
<td>Low-intensity knee-extensor exercise (1.5 kg ankle weights, 60 ± 5 rpm, ~15 min)</td>
<td>LBF indexed to thigh lean mass was reduced in T2D vs C Absolute values of LBF not provided Measurement technique: MRI</td>
<td></td>
</tr>
<tr>
<td>MacAnaney et al., 2011 (23)</td>
<td>T2D: n=9 ♀, 49 ± 6 yr 33 kg/m² C: n=10 ♀ (heavy), 43 ± 13 yr 29 kg/m² n=8 ♀ (lean), 44 ± 8 yr 23 kg/m² BMI values are medians</td>
<td>Range: 1-4 yr 6.8 ± 1.0%</td>
<td>T2D: metformin (56%), avandien (11%), statins (33%), ACE inhibitor (11%) C: none Exclusions: insulin, β-blockers, calcium channel blockers, any other anti-hypertensive drug SDT: on medications</td>
<td>6 minutes of intermittent calf contractions (2 s static contraction: 4 s relaxation at 70% MVC)</td>
<td>Time constant (tau) of the second phase of leg vascular conductance was significantly greater in T2D vs C (resulting in a &gt; mean response time) No group differences at steady state Measurement technique: venous occlusion strain-gauge plethysmography</td>
<td></td>
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<tr>
<td>Martin et al., 1995 (24)</td>
<td>T2D: n=8 ♀, 51 ± 2 yr 24 ± 0.3 kg/m² C: n=7 ♀, 53 ± 1 yr 24 ± 1 kg/m²</td>
<td>4.7 ± 1.3 yr 7.1 ± 0.6%</td>
<td>T2D: oral hypoglycemic agents (38%) C: none SDT: held medications for 48 h before testing</td>
<td>40 min cycling exercise at 60% VO_{2}max</td>
<td>No difference in exercising LBF between groups Leg lactate output and muscle lactate were higher in T2D vs C VO_{2}peak ~1 L/min greater than that of participants in Kingwell et al., (20) Measurement technique: indocyanine green infusion in femoral artery</td>
<td></td>
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<tr>
<td>Menon et al., 1992 (18)</td>
<td>T1D: n=10 ♀, 23-40 yr T2D: n=11 ♀, 35-50 yr C: n=15 ♀, (age and BMI not reported)</td>
<td>T1D: 1-10 yr T2D: 2-10 yr HbA1c in both T1D and T2D: 9.5 ± 1.2%</td>
<td>T1D and C: not reported T2D: oral hypoglycemic agents SDT: not reported</td>
<td>Plantar and dorsiflexion exercise (80 repetitions in 150 s)</td>
<td>Median post-exercise MBF was significantly greater in C vs T1D and T2D (T1D and T2D not significantly different) Median values reported May not be appropriate to group cardiovascular responses for T1D and T2D together (90) Measurement technique: ^{133}Xe clearance</td>
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<tr>
<td>Reference</td>
<td>Study Participants: n, age, BMI</td>
<td>Duration of Diagnosis and HbA1c in T2D</td>
<td>Medication(s) &amp; Status during Testing (SDT)</td>
<td>Study Protocol</td>
<td>Key Results</td>
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| Mohler et al., 2006 (91) | T2D: n=17 (3 ♀, 14 ♂) 58 ± 6 yr 29 ± 3 kg/m²  C: n=25 (15 ♀, 10 ♂) 66 ± 10 yr 26 ± 5 kg/m² | not reported                         | not reported                                  | 30-plantar flexion over 1 min, and a progressive treadmill test | • Plantar-flexion exercise: deoxygenation and relative oxygen recovery time not different between groups  
• Treadmill test: deoxygenation and blood volume expansion not different between groups | Measurement technique: NIRS                                                                 |
| O’Connor et al., 2012 (43) | T2D: n= 32 (16 ♀, 16 ♂) 30 ± 3 kg/m²  C: n= 32 (16 ♀, 16 ♂) 29 ± 3 kg/m² 53-57 ± 10 yr | 1-11 yr ♀: 6.3 ± 0.6%  ♂: 6.9 ± 1.2% | T2D: oral hypoglycemic agents (75%); others not reported  
C: not reported  
Exclusions: β-blockers “controlled hypertensives admitted”  
SDT: not reported | 3-min unloaded cycling followed by 6-min at 80% ventilatory threshold | • Peak VO₂ was lower and VO₂ kinetics were slowed in ♀ and ♂ with T2D vs C  
• Dynamic and steady state cardiac output (CO) were not different between groups  
• No differences between ♂ and ♀ | Measurement technique for CO: inert gas rebreathing |
| Pak et al., 2010 (21) | T2D: n=4 ♂, 54 ± 4 yr 34 ± 2 kg/m²  C: n=6 ♂, 44 ± 3 yr 27 ± 1 kg/m² | 5.8 ± 2.6 yr 7.4 ± 0.9% | T2D: metformin (50%), statin (75%), anti-hypertensive (50%), aspirin (50%)  
C: none  
SDT: on medications | Two-leg knee extension/flexion exercise:  
1) incremental test (1 min at each level) to exhaustion, and  
2) step increases to low and moderate intensity work rates | • Absolute LBF was reduced at some (but not all) work rates in the incremental exercise test  
• The rapid initial increase in LBF was blunted in T2D vs C in the step exercise tests  
• No differences in VO₂ between groups in either test | Measurement technique: Doppler and Echo ultrasound  
Possible that LBF did not have time to reach “steady state” levels at times of measurement in the incremental protocol |
<table>
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<tr>
<th>Study Protocol</th>
<th>Key Results</th>
<th>Comments</th>
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<tr>
<td>Regensteiner et al., 1995 (8)</td>
<td>Graded maximal treadmill protocol (2 min increments)</td>
<td>Possible that VO&lt;sub&gt;2&lt;/sub&gt; did not have time to reach “steady state” during incremental steps of protocol</td>
</tr>
<tr>
<td>Regensteiner et al., 1998 (9)</td>
<td>7 min bouts of constant-load cycling exercise below (20 W, 30 W) and above (80 W) the lactate threshold</td>
<td>In sub-analysis published elsewhere (10), it was reported that RPE was significantly greater in T2D at 20 W (but not 30 W) even after adjusting for relative work intensity</td>
</tr>
<tr>
<td>Sanchez et al., 2011 (92)</td>
<td>MRI used to measure variations in blood volume (BV) and O&lt;sub&gt;2&lt;/sub&gt; saturation (%HbO&lt;sub&gt;2&lt;/sub&gt;) in tibialis anterior (TA; predominantly type I fibers) and extensor digitorum longus (EDL; mostly type II fibers) before, during and after 10 s isometric dorsiflexion contractions at 50% and 100% MVC</td>
<td>Measurement technique: MRI Results suggest possibly altered balance between O&lt;sub&gt;2&lt;/sub&gt; delivery and utilization in obesity that is intensity- and muscle-type-specific; weak evidence for impact of T2D specifically</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Participants: n, age, BMI</td>
<td>Duration of Diagnosis and HbA1c in T2D</td>
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<tr>
<td>Slade et al., 2011 (56)</td>
<td>T2D: n=16 (11 ♀, 5 ♂) 47 ± 2 yr 36 ± 2 kg/m²  C: n=16 (11 ♀, 5 ♂) 45 ± 2 yr 36 ± 2 kg/m²</td>
<td>median duration: 2.4 yr 7.6 ± 0.3%</td>
</tr>
<tr>
<td>Thaning et al., 2011 (25)</td>
<td>T2D: n=10 (4 ♀, 6 ♂) 55 ± 2 yr 29 ± 1 kg/m²  C: n=10 (4 ♀, 6 ♂) 55 ± 2 yr 27 ± 1 kg/m²</td>
<td>median duration: 6 yr (range 2-13) 7.6 ± 1.2%</td>
</tr>
<tr>
<td>Womack et al., 2009 (26)</td>
<td>T2D: n=22 (19 ♀, 3 ♂) 53 yr (median) 34 ± 6 kg/m²  T2D + microvascular complications (MC): n=8 (5 ♀, 3 ♂) 54 yr 35 ± 5 kg/m²  C: n=20 (11 ♀, 9 ♂) 47 yr 23 ± 3 kg/m²</td>
<td>T2D: 2.5 ± 4 yr 6.9 ± 2.2%  T2D+MC: 7.0 ± 5.0 yr 8.5 ± 2.2%</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Participants: $n$, age, BMI</td>
<td>Duration of Diagnosis and HbA1c in T2D</td>
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<tr>
<td>Young et al., 1991 (17)</td>
<td>T2D: $n=10$ ♂ 58 ± 8 yr C: $n=7$ ♂ 56 ± 5 yr (BMI not reported)</td>
<td>At least 10 years Average duration and HbA1c not reported</td>
</tr>
</tbody>
</table>

Abbreviations: ACE Inhibitor = angiotensin-converting enzyme inhibitor; Ach = acetylcholine (endothelium-dependent vasodilator); BV = blood volume; C = control; EDL = extensor digitorum longus; [HHb] = deoxygenated hemoglobin/myoglobin; %HbO$_2$ = oxygen saturation; LBF = leg blood flow; $mO_2$del = muscle oxygen delivery; MC = microvascular complications; MRI = magnetic resonance imaging; MVC = maximal voluntary contraction; NIRS = near-infrared spectroscopy; RPE = rating of perceived exertion; SDT = status during testing; SNP = sodium nitroprusside (endothelium-independent vasodilator); T1D = type 1 diabetes; T2D = type 2 diabetes; TA = tibialis anterior; ♂ = men; ♀ = women; ↓ = decrease; ↑ = increase.
anti-hypertensive (87), and anti-hyperlipidemic (88) medications have the potential to influence cardiac and vascular function directly or indirectly and could affect exercising muscle blood flow. In particular, lipophilic statins are recently receiving attention for their “pleiotropic” ability to reduce sympathetic outflow (88, 89); however, whether this effect modulates neurovascular control during exercise is unknown. Another possibility not ordinarily considered is that some persons with type 2 diabetes may have diabetes-evoked impairment while others may not and therefore the inconsistent findings may represent impaired versus unimpaired cohorts, superimposed on moderating influences of medications and co-morbidities. In other words, some persons may be more susceptible to a negative impact of the disease than others, and this propensity may depend on other moderating factors. The proposed $\dot{V}O_2$, PcapO$_2$, and MBF responses during exercise in healthy vs persons with type 2 diabetes, as based on current evidence, are depicted in Figure 4-4. Future studies are required to investigate steady state $mO_2$del in persons with type 2 diabetes to better define the specific exercise conditions and patient characteristics in which impairment does vs does not manifest.

4.8 Diffusive O$_2$ delivery: Potential mechanisms of impairment

In persons with type 2 diabetes, the diffusive flux of O$_2$ may be hindered by: 1) increased affinity of Hb for O$_2$ (affecting the pressure gradient for diffusive flux of O$_2$ into the myocyte), and 2) a reduced diffusive conductance due in part to capillary rarefaction.

4.8.1 Is Hb affinity for O$_2$ increased in type 2 diabetes?

“Glycosylated Hb”, or HbA1c, is a variant of Hb that is formed via the non-enzymatic binding of glucose to the Hb protein; the rate of formation of HbA1c is directly proportional to the ambient glucose concentration (93). The glycation of Hb increases its affinity for O$_2$, causing a leftward shift of the Hb-O$_2$ dissociation curve (94). This predicts a requirement of lower PcapO$_2$ for O$_2$ offloading from RBCs. The importance of this effect on PmyoO$_2$ and exercise tolerance in persons with type 2 diabetes has yet to be determined and will require studies which
acutely restore normal Hb-O₂ affinity and examine the effect on muscle metabolism and exercise tolerance.

**4.8.2 Is capillary morphology altered in type 2 diabetes?**

Histological analysis of skeletal muscle in type 2 diabetes reveals a structural (95) and functional (26, 96) (no compromise to anatomical capillary density, just compromised recruitment) capillary rarefaction which reduces the surface area, and increases the distance, for O₂ diffusion. Prolonged vessel non-perfusion can progress to its structural loss (97) suggesting functional rarefaction might predict structural rarefaction.

**4.9 Is disproportionate exercise intolerance in type 2 diabetes explained by impaired muscle oxygen supply?**

Direct manipulation of oxygen supply in young healthy subjects has clearly demonstrated the sensitivity of muscle metabolism, contractile function and exercise tolerance to muscle oxygenation in this group (15, 16). Current evidence suggests the possibility that both mO₂del and mDKO₂ may be impaired during exercise in persons with type 2 diabetes. Given the likely impact of these impairments on PmyoO₂, it is reasonable to hypothesize that cardiovascular dysfunction contributes to the disproportionate exercise intolerance in this population. However, current support for this hypothesis is based on the assumption that impaired O₂ delivery observed in some studies, e.g. (7, 20, 22), explains the reduced exercise tolerance observed in those and other studies. Studies in which mO₂del is acutely restored are required to identify whether and how much exercise intolerance is due to impaired mO₂del in persons with type 2 diabetes.

**4.10 Other possible contributors to exercise intolerance in type 2 diabetes**

It is important to acknowledge that, while cardiovascular dysfunction may contribute to exercise intolerance in type 2 diabetes, there are other possible factors. For instance, in type 2 diabetes there is evidence for “mitochondrial dysfunction”, manifest as a reduction in
mitochondrial content (98) with an overall reduction in oxidative phosphorylation capacity per muscle mass (99, 100). While this dysfunction is generally thought to be comprised solely of a reduced mitochondrial volume without a change in functional capacity, in one investigation the decrement in electron transport chain activity (succinate oxidase activity) was greater than could be accounted for by a reduced mitochondrial content, suggesting a functional impairment (100). In general, the mitochondrial dysfunction appears to be independent of muscle fiber type (101), present systemically in both locomotor and non-locomotor muscles (102), and related to adiposity (101). A coordinated downregulation of genes involved in oxidative phosphorylation (103, 104) and significant reduction in the expression of PGC-1α [peroxisome proliferator-activated receptor γ coactivator-1α; the “master regulator” of mitochondrial biogenesis (105)] (104) are likely responsible. Mitochondrial dysfunction could serve to limit O₂ consumption (e.g. slowed \( \dot{V}O_2 \) kinetics) or to cause a greater perturbation of the intracellular environment at a given workload, and observations of greater changes in ADP and Pi in exercising muscle in type 2 diabetes are consistent with this (81). While these alterations in mitochondrial morphology are pervasive in investigations in type 2 diabetes however, it is unclear whether they are inherent to the disease or are secondary to a sedentary lifestyle (98).

Similarly, it is possible that differences in muscle morphology contribute to decreased exercise tolerance in persons with type 2 diabetes since there is generally a trend towards reduced type I and increased type II muscle fiber content in this population (95, 106) and since type II fibers are known to have greater fatigability (107). Observations of two-fold higher leg lactate output and greater muscle lactate content during exercise (cycling at 60% \( \dot{V}O_2 \)peak) in type 2 diabetes (24) are consistent with this potential mechanism. In addition, it has been proposed that different fiber types mandate different magnitudes of contraction-induced hyperemia, such that type I fibers exhibit an augmented MBF-to- \( \dot{V}O_2 \) relationship (108). Thus it is possible that a change in muscle morphology characteristic of the population could explain the differential
microvascular oxygen response described earlier (22, 54, 55). However, these differences in fiber type composition are not always observed (101), and when they are present it is again unclear whether they result from the disease or habitual inactivity.

Lastly, psychological factors such as depression and emotional stress, which are commonly co-morbid with type 2 diabetes (109, 110), are known to increase perceived exertion and could contribute to reduced exercise tolerance in this population (111). Interestingly, there is some evidence that reduced brain derived neurotrophic factor (BDNF) may be involved in the etiology of both depression and type 2 diabetes, which could explain the clustering of these symptoms in epidemiological studies (112). BDNF also is thought to mediate the relationship between “energetic challenges” (i.e. exercise) and mood (113). Thus if persons with type 2 diabetes have less BDNF, perhaps they also experience a lesser “mood elevating effect” of exercise, which could result in reduced motivation to be active. While speculative, this possibility illustrates that there is much that remains to be determined regarding exercise intolerance in type 2 diabetes.

**4.11 Summary and future directions**

Cardiovascular support of exercising muscle can determine submaximal muscle metabolic and contractile function and thereby directly influence the capacity for, and perceived effort of, exercise, thus impacting exercise tolerance. While evidence does exist to support the hypothesis that impaired convective and diffusive exercising muscle oxygen supply occurs as a result of type 2 diabetes, a careful review of existing literature directly assessing cardiac and skeletal muscle vascular responses during exercise reveals that evidence contravening this hypothesis exists in equal measure. Therefore, it remains premature to conclude that delivery of oxygen to exercising skeletal muscle is an important contributor to exercise intolerance in persons with type 2 diabetes. The current body of scientific evidence on this issue is limited by superimposition of a range of potentially confounding co-morbidities (e.g. cardiovascular disease,
neuropathy) and individual characteristics (e.g. age, adiposity, glycemic control) that can influence outcomes of interest, as well as differences in medication status during testing across studies that can complicate interpretation.

Nevertheless, there is good evidence for the existence of specific cardiac and vascular structural and mechanistic impairments as summarized in Figure 4-5. Definitively establishing the impact of these impairments on \( \text{mO}_2\text{del} \) and exercise tolerance will require studies that are able to adequately control for the above mentioned confounds. Key research questions that need to be addressed are summarized in Table 4-2 and can be grouped into at least three categories. First, are \( \text{mO}_2\text{del} \) and/or \( \text{mDKO}_2 \) consistently compromised during exercise in type 2 diabetes? Second, what are the cardiac and peripheral vascular mechanisms that contribute to these deficiencies? Third, does compromised \( \text{mO}_2\text{del} \) and/or \( \text{mDKO}_2 \) explain some or all of the disproportionate exercise intolerance?
Table 4-2. Summary of important future directions

<table>
<thead>
<tr>
<th>Does type 2 diabetes lead to compromised convective $mO_2\text{del}$ and/or diffusive $O_2$ flux? If so: what are the cardiac and peripheral mechanisms?</th>
</tr>
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<tbody>
<tr>
<td><strong>Convective $mO_2\text{del}$</strong></td>
</tr>
<tr>
<td>o Is the initial rapid onset vasodilatory response diminished? If so:</td>
</tr>
<tr>
<td>▪ Is this due to impaired NO, prostaglandin, adenosine and/or $K^+$ mediated dilation?</td>
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<tr>
<td>o Is the rate and magnitude of increase to steady state diminished? If so:</td>
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<tr>
<td>▪ Is this due to impaired RBC ATP release or NO bioavailability?</td>
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<tr>
<td>▪ Is this due to elevated sympathetic restraint as a result of the CSAR, skeletal muscle metaboreflex or baroreflex?</td>
</tr>
<tr>
<td>• What role does impaired cardiac function play in evoking this sympathetic restraint?</td>
</tr>
<tr>
<td>▪ Is this due to diminished functional sympatholysis?</td>
</tr>
<tr>
<td>o Is there an exercise intensity dependence of impairment?</td>
</tr>
<tr>
<td>o Is there a muscle mass or exercise modality dependence of impairment?</td>
</tr>
<tr>
<td><strong>Diffusive $O_2$ Flux</strong></td>
</tr>
<tr>
<td>o Is mDKO$_2$ impaired in type 2 diabetes? If so:</td>
</tr>
<tr>
<td>▪ Is this due to increased Hb-O$_2$ affinity, and/or structural or functional capillary rarefaction?</td>
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<tr>
<th>Does dysfunctional cardiovascular support of exercising muscle in type 2 diabetes explain some or all of the disproportionate exercise tolerance?</th>
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<tbody>
<tr>
<td>• Does acute restoration of $mO_2\text{del}$ and/or mDKO$_2$ diminish or abolish the exercise intolerance in persons with type 2 diabetes?</td>
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Abbreviations: CSAR = cardiac sympathetic afferent reflex; Hb = hemoglobin; mDKO$_2$ = muscle diffusive conductance for oxygen; $mO_2\text{del}$ = muscle oxygen delivery; NO = nitric oxide; RBC = red blood cell.
Figure 4-5. Schematic of potential central and peripheral cardiovascular impairments that could compromise steady state convective and diffusive oxygen delivery to the contracting skeletal muscle myocyte, thereby contributing to exercise intolerance.

CBR = carotid baroreflex; CO = cardiac output; CSAR = cardiac sympathetic afferent reflex; MMR = muscle metaboreflex. Sympathetic restraint of exercising skeletal muscle vasodilation could be enhanced due to: a) reduced CBR inhibition of sympathetic neural outflow in order to compensate for inadequate CO and maintain arterial blood pressure, b) CSAR stimulation in a diabetic heart and/or c) MMR activation. Impaired functional sympatholysis would reduce blunting of sympathetic restraint. The vasodilatory response to increased muscle metabolic demand for oxygen in contracting skeletal muscle could be compromised due to: a) endothelial and/or b) smooth muscle dysfunction, c) impaired RBC release of ATP and/or nitric oxide (NO) with hemoglobin (Hb) deoxygenation. Diffusive flux of oxygen could be compromised by increased Hb affinity for O₂ and/or structural and functional capillary rarefaction.
4.12 References


Chapter 5

The combined influence of fat consumption and repeated mental stress on brachial artery flow-mediated dilation: a preliminary study

Published As:


5.1 Abstract

Experienced separately, both acute mental stress and high-fat meal consumption can transiently impair endothelial function, and the purpose of the present study was to investigate their combined impact. On four separate days, 10 healthy males (23 years old) underwent brachial artery flow-mediated dilation (FMD) tests, before and hourly for 4 h post-consumption of a high-fat (HFM; 54 g fat) or low-fat meal (LFM; 0 g fat; each meal ~1000 calories), with hourly mental stress (mental arithmetic, speech) or control (counting) tasks (conditions HFM+S, LFM+S, HFM and LFM). Data are presented as means ± SD. Plasma triglycerides increased and remained elevated after the high-fat meal but not the low-fat meal (P=0.004) and were not affected by mental stress (P=0.329). Indices of stress reactivity increased during mental stress tasks (mean arterial pressure, ~Δ20 mmHg; heart rate, ~Δ22 beats/min; salivary cortisol, ~Δ2.37 nmol/L; and plasma norepinephrine ~Δ0.17 ng/ml), and were not influenced by meal (P>0.05). There was no effect of meal on FMD (P=0.562), however, FMD was 4.5 ± 0.5 % in the Control conditions and 5.8 ± 0.6% in the mental stress conditions (P=0.087) and this difference was significant when normalized for the shear stress stimulus (FMD/area under the curve of shear stress, P=0.045). Overall, these preliminary data suggest that postprandial FMD was augmented with mental stress irrespective of meal type. These results are contrary to previous reports of impaired endothelial function after mental stress or fat consumption independently and highlight the need to further investigate mechanisms underlying the interactions between these factors.
5.2 Introduction

Atherosclerotic cardiovascular disease has a complex etiology that involves lifestyle factors such as diet and stress (Stone, 1990; Rozanski et al., 1999). In recent years, several investigators have found that both fat consumption (Marchesi et al., 2000; Plotnick et al., 1997; Vogel et al., 1997; Gaenzer et al., 2001) and acute mental stress (Broadley et al., 2005; Ghiadoni et al., 2000; Spieker et al., 2002; Sarabi & Lind, 2001; Jambrik et al., 2004) can result in transient (45 min to 4 h) impairments in vascular endothelial function. This is of critical importance as there is growing agreement that endothelial dysfunction precedes and plays a role in the development of atherosclerosis (Celermajer et al., 1992; Drexler & Hornig, 1999; Kawashima & Yokoyama, 2004). If brief stressful experiences and/or fat intake produce prolonged periods of endothelial dysfunction, repeated exposures could accrue to result in a clinically relevant loss of endothelium-mediated vasoprotection and thus contribute to atherosclerotic progression.

Mechanistically, mental stress induced impairments in endothelial function derive from the physiological stress response [sympathetic nervous system and hypothalamic-pituitary-adrenal axis activation and increases in norepinephrine (NE) and cortisol (Broadley et al., 2005)], while fat consumption-induced impairments result primarily from elevated blood lipid levels and an associated increase in oxidative stress (Wallace et al., 2010). However, other components of a meal may also have an impact on vascular function (e.g. phosphorus; Shuto et al., 2009). Importantly, although it has never been examined directly, there is evidence to suggest that experiencing mental stress and fat consumption simultaneously may result in an additive or synergistic impairment of endothelial function. For example, mental stress can exaggerate the rise in blood lipid levels observed after fat consumption by both inhibiting the removal of lipid from the blood (via catecholamine- and cortisol-mediated inhibition of lipoprotein lipase; Andrews & Walker, 1999; Lafontan et al., 1997) and by stimulating lipolysis (through catecholamine-mediated activation of hormone sensitive lipase; Lafontan et al., 1997). Thus,
mental stress and fat consumption may work together, via distinct and interactive pathways, to result in a greater or more universal impairment in endothelial function. This is important because mental stress is often associated with increased consumption of high-fat foods in everyday life (McCann et al., 1990).

With this as a foundation, the objective of the present study was to investigate the combined effects of mental stress and fat consumption on postprandial lipemia and endothelial function, as assessed via brachial artery flow-mediated dilation (FMD). It was hypothesized that high-fat meal consumption followed by repeated episodes of mental stress would result in a greater impairment of FMD versus either high-fat meal consumption alone or repeated episodes of mental stress following a low-fat meal. Our secondary hypothesis, related to potential mechanisms of FMD impairment with high-fat meal—mental stress interaction, was that mental stress in combination with high-fat meal consumption would evoke a larger postprandial lipemia versus consumption of a high-fat meal alone.

5.3 Methods

5.3.1 Ethical approval

The study protocol was approved by the Health Sciences Research Ethics Board at Queen’s University, which operates under the terms of the Declaration of Helsinki, and subjects gave written informed consent to participate on forms approved by this board.

5.3.2 Subjects

Ten healthy, recreationally active, non-smoking men between the ages of 18 and 30 years from the Queen’s University community participated in this study. Endothelial sensitivity to both mental stress (Spieker et al., 2002) and fat consumption (Marchesi et al., 2000) has previously been observed in this population. Based on data from previous studies examining the impact of stress and fat consumption on FMD independently (Ghiadoni et al., 2000; Vogel et al., 1997), it
was estimated that eight participants would be required to detect a 2% FMD difference between conditions with 80% power and $\alpha=0.05$ (GPower 3.1.5 Software, Universitat Kiel, Germany). As this is the first time fat and stress stimuli have been imposed in combination, we enrolled 10 participants.

Subjects visited the lab four times in addition to an initial familiarization visit, during which they completed a Seven-Day Physical Activity Recall Questionnaire (Jacobs et al., 1993). Subjects were instructed to maintain their regular physical activity levels throughout the duration of the study. Subjects abstained from taking anti-inflammatory medication within 48 h of the study, from exercising within 24 h, and from consuming food, alcohol, or caffeine for 12 h prior to each testing session (Thijssen et al., 2011). Subjects consumed a meal that was “typical” for them the night before participation and were required to replicate this meal prior to each subsequent session.

Experimental visits were separated by at least 2 days and not more than 2 weeks, and all four sessions took place within 14-29 days (mean 21.9 ± 5.1 days). Data collection commenced at 10.00 or 10.45 h (consistent within a subject) and was performed in a quiet, temperature-controlled room (~20-25°C).

### 5.3.3 Experimental procedure

This study utilized a within-subjects design, such that all subjects completed each of the following four experimental conditions on separate days: (i) consumption of a low-fat meal (LFM); (ii) consumption of a low-fat meal followed by repeated stressful tasks (LFM+S); (iii) consumption of a high-fat meal (HFM); and (iv) consumption of a high-fat meal followed by repeated stressful tasks (HFM+S). Conditions were counterbalanced across subjects.

Upon arrival at the laboratory, subjects were positioned supine and instrumented with an intravenous catheter in the right arm for repeated blood sampling. Following instrumentation, subjects rested for 30 min, after which baseline measures, including a baseline FMD test, were
conducted. Immediately following the FMD test, subjects ate either the low-fat meal or high-fat meal, after which subjects performed either Stress or Control tasks followed by FMD tests hourly for 4 h (Figure 5-1). Saliva samples (for cortisol determination), blood samples [to quantify triglycerides, total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, insulin, glucose, phosphorus, norepinephrine (NE), plasma volume (Dill & Costill, 1974) and blood viscosity] and subjective stress ratings (0-10 scale) were taken at several time points (Figure 5-1). Heart rate (HR) was monitored throughout the protocol, and mean arterial pressure (MAP) was measured continuously with finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, the Netherlands).

**Figure 5-1.** Timeline of experimental protocol.
Abbreviations: FMD, flow-mediated dilation; Hb, hemoglobin; Hct, hematocrit; HFM, high-fat meal; LFM, low-fat meal; NE, norepinephrine; S / C, Stress or Control Task.

### 5.3.3.1 Meals

The low-fat meal consisted of the following: 160 g Frosted Flakes (Kellogg Company), 500 ml skim milk and 500 ml of orange juice (990 calories, 0 g fat, 0 g saturated fat, 0 g trans-fat, 209 g carbohydrates, 23 g protein, 5 g fiber, 959 mg sodium and 687 mg phosphorus). The high-fat meal consisted of the following: an Egg McMuffin, Sausage McMuffin, two hash brown patties (McDonald’s Corporation), and water (1000 calories, 54 g fat, 16 g saturated fat, 1 g trans-fat, 94 g carbohydrates, 32 g protein, 7 g fiber, 2330 mg sodium, 559 mg phosphorus). These meals have been used previously in studies evaluating the effect of fat consumption on
endothelial function (Padilla et al., 2006; Plotnick et al., 1997; Vogel et al., 1997) and have ecological validity. Subjects were given 125-250 ml of water every hour to maintain hydration throughout the experimental visits.

5.3.3.2 Stress tasks

The mental stress tasks involved mental arithmetic (three tasks, with one repeated) or a speech (five tasks, with one repeated); all tasks were previously shown to evoke physiological stress responses (Gottdiener et al., 2003; Greeson et al., 2009; Ghiadoni et al., 2000; Biondi & Picardi, 1999; Harris et al., 2000; Sarabi & Lind, 2001; Kirschbaum et al., 1993). The tasks were administered in two “Stress Programs”, with the intention of interspersing tasks to provide a varied stimulus to minimize habituation (references refer to the sources from which the tasks were adapted). Program 1 was as follows: Shoplifting Accusation Speech Task (Ghiadoni et al., 2000); Mental Arithmetic 1 (Sarabi & Lind, 2001); Anger Interview (Greeson et al., 2009; Gottdiener et al., 2003); and Public Speaking Task (Biondi & Picardi, 1999). Program 2 was as follows: Mental Arithmetic 2 (Harris et al., 2000) and Stroop Task (Biondi & Picardi, 1999); Job Interview Speech Task (Kirschbaum et al., 1993); Public Speaking Task (Biondi & Picardi, 1999); and Mental Arithmetic 1 (Sarabi & Lind, 2001). The order of Stress Programs was counterbalanced across subjects. Speech tasks were audio recorded, and standard prompts were provided when necessary. All tasks were 10 min in duration, including instructions, preparation (speech tasks only) and speaking time.

5.3.3.3 Control task

In the non-stress conditions (LFM and HFM), a Control task was performed in which subjects listened to standardized instructions, rested for ~4 min and then counted upwards from 1 to 100 repeatedly for 5 min. This was done to control for the modest increases in heart rate and blood pressure that occur with speaking alone in the absence of emotional content (Lynch et al., 1981).
5.3.3.4 Flow-mediated dilation tests – reactive hyperemia

Brachial artery blood velocity and diameter were recorded for 2 min of baseline, the last 1 min of a 5 min cuff inflation to 250 mmHg, and for 3 min post-cuff release (Celermajer et al., 1992). The cuff was placed on the subject’s forearm, distal to the site of brachial artery ultrasound measurements. During all tests the brachial artery blood velocity was recorded during the FMD tests with Doppler ultrasound operating at 4 MHz at an insonation angle of 68 deg as previously described (Vivid i2; GE Medical Systems, Milwaukee, WI, USA; Pyke et al., 2008a). The Doppler shift frequency spectrum was analyzed via a Multigon 500P TCD spectral analyzer (Multigon Industries, Yonkers, NY, USA), from which mean blood velocity was determined as a weighted mean of the spectrum of Doppler shift frequencies. The corresponding voltage output was continuously sampled and stored (Powerlab; AD Instruments, Colorado Springs, CO, USA) for later analysis. Brachial artery images were obtained using the same ultrasound probe, operating at 12 MHz in two-dimensional B-mode. The images were transmitted to an independent computer via a VGA to USB frame grabber (Epiphan Systems Inc., Ottawa, ON, Canada), and recorded as .avi files using commercially available software (Camtasia Studio; TechSmith, Okemos, MI, USA) for later analysis. All ultrasound measurements were conducted on the left arm.

5.3.4 Data analysis

5.3.4.1 Brachial artery blood velocity

Blood velocity was analyzed offline in 3 second average time bins using the data acquisition software program LabChart (AD Instruments) as previously described (Pyke et al., 2008b).

5.3.4.2 Brachial artery diameter

Vessel diameter was quantified offline via automated wall tracking using an updated version of the software package described by Woodman et al., (2001) (Endocer FMD &
Bloodflow v3.0.3; Reed Electronics, Perth, WA, Australia) as previously described (Pyke et al., 2008b). Diameter data were compiled into 3 sec average time bins to allow for time alignment with blood velocity data and subsequent calculation of shear stress. All images were analyzed by the same researcher, who was blinded to the experimental condition and temporal sequence. Flow-mediated dilation was calculated as the percent change in artery diameter (%FMD) from baseline prior to cuff occlusion to the peak 3 s average diameter after cuff release (Pyke & Jazuli, 2011). Flow-mediated dilation was also indexed to the shear stress stimulus as the percentage FMD/area under the curve of shear stress for the first 30 s following cuff release (%FMD/AUC shear stress; Thijssen et al., 2009; Pyke & Tschakovsky, 2007).

5.3.4.3 Shear stress

The brachial artery shear stress (in dynes per square centimeter) was calculated as follows: shear stress = 4ην/D, where η is blood viscosity (Poise), ν is the mean blood velocity (in centimeters per second), and D is the mean brachial artery diameter (in centimeters; Gnasso et al., 2001). Shear stress during reactive hyperemia was expressed as the AUC of shear stress for the first 30 s following cuff release (Thijssen et al., 2009; Pyke & Tschakovsky, 2007).

5.3.4.4 Mean arterial pressure and heart rate

The MAP and HR were analyzed offline into 1 min average time bins. For the Stress and Control Tasks, the peak (1 min average) MAP and HR were obtained as indices of hemodynamic reactivity.

5.3.4.5 Cortisol analysis

Saliva samples were obtained with synthetic swabs (Sarstedt Salivette; Poll et al., 2007). Swabs were centrifuged to extract saliva (2 min at 4°C and 1070g; IEC-Centra MP4R; International Equipment Company, Needham Heights, MA, USA) for storage at -80°C. Samples were thawed, spun at 380g for 15 min and assayed in duplicate using an enzyme-linked
immunoassay (ELISA) kit according to the manufacturer’s instructions (catalogue no. 1-3002; Salimetrics, State College, PA, USA). Cortisol Reactivity for each Stress/Control Task was taken as the peak cortisol value at each task (pre-, 10 or 20 min post-task). Cortisol levels at the two pre-meal time points were not different; therefore, the mean of these two time points is reported as the baseline.

5.3.4.6 Blood sample analysis

Blood samples were centrifuged at 4°C for 10 min at 1070g, and the plasma was separated and stored at -80°C. Plasma lipids, glucose and phosphorus were analyzed via Beckman Coulter UniCel® DxC 600/800 SYNCHRON® Clinical System(s) and SYNCHRON® Systems Multi Calibrator (Beckman Coulter, Fullerton, CA, USA) at Kingston General Hospital. All triglyceride levels were corrected for changes in plasma volume according to the equations of Dill and Costill (1974). Plasma NE and insulin concentrations were assayed in duplicate by ELISA according to the manufacturer’s instructions (2-CAT Fast Track BA E 6500; Labor Diagnostika Nord GmbH & Co. KG; and 80-INSHU-E01.1, ALPCO Diagnostics, Salem, NH, USA, respectively). Hematocrit and hemoglobin were determined either in house (StatProfile M Blood Gas Analyzer; Nova Biomedical, Mississauga, ON, Canada), or at Kingston General Hospital (Beckman Coulter LH 785 Hematology System). Whole blood viscosity was analyzed at a shear rate of 225 s⁻¹ (Brookfield Viscometer DV-II+ Pro, Middleboro, MA, USA) at 37 ± 2°C.

5.3.5 Statistical analysis

In order to assess whether subject characteristics changed over the course of the four experimental visits, one-way repeated-measures (RM) ANOVA tests were performed. Three-way RM ANOVAs were used to compare the effects of Meal (high-fat or low-fat), Stress condition (Stress or Control) and Time (baseline, 1, 2, 3 and 4 h postprandial) on shear stress, FMD, plasma volume, metabolic variables (triglycerides, glucose, insulin and phosphorus) and stress reactivity.
parameters (MAP, HR, cortisol, NE, subjective stress ratings). Significant interactions were explored with pairwise comparisons (Tukey’s test).

The relationships between the change from baseline %FMD ($\Delta$%FMD) and factors reflecting stress reactivity [change from baseline in MAP ($\Delta$MAP$_{\text{peak}}$), HR ($\Delta$HR$_{\text{peak}}$), cortisol ($\Delta$cortisol) and NE ($\Delta$NE)], and between $\Delta$%FMD and factors related to metabolism (plasma $\Delta$triglyceride, $\Delta$glucose and $\Delta$insulin concentration) were assessed with linear regression. Linear regression was also used to assess the relationships between NE and postprandial triglycerides (average of NE at tasks 1 and 4 versus triglycerides at 4 h within HFM+S) and between cortisol and triglycerides (average of peak cortisol at tasks 1-4 versus triglyceride at 4 h within HFM+S).

Statistical significance was set at P<0.05. Three-way ANOVAs were conducted using IBM® SPSS® Statistics (version 19; SPSS Inc., Armonk, NY, USA); all other statistics were calculated using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA). All data are expressed as means ± SD.
5.4 Results

5.4.1 Subject characteristics

There were no systematic changes in subject weight or blood lipid parameters over the duration of the study (P>0.05); these values were averaged across experimental visits to provide single descriptive values (Table 5-1). All blood parameters were within the normal range for healthy subjects of this age (Fletcher et al., 2005).

Table 5-1. Subject characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.2 ± 3.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.5 ± 6.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.3 ± 6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4 ± 2.4</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Fasting plasma triglycerides (mmol/L)</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>7-day PAR Score (kcal/kg/week)</td>
<td>260.1 ± 30.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PAR, physical activity recall.

5.4.2 Shear stress and FMD parameters

Diameter and shear stress parameters during the FMD tests are shown in Table 5-2. Baseline artery diameter did not differ by Meal (P=0.460), Stress condition (P=0.416) or Time (P=0.226). Baseline shear stress varied modestly over time and between conditions; although there were significant Meal x Time (P=0.007) and Stress x Time (P=0.029) interactions, post hoc testing within each time point revealed that the only significant difference was an effect of Meal at 2 h postprandial (P=0.036). Shear stress in the last minute of occlusion was modestly higher in the stress conditions (P=0.021). However, the shear stress stimulus following cuff release (AUC of shear stress for the first 30 s) was stable over time and between conditions (Meal, P=0.200; Stress, P=0.787; Time, P=0.100).
Table 5-2. Diameter and shear stress parameters during the flow-mediated dilation tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline artery diameter (cm)</td>
<td>LFM</td>
<td>0.387 ± 0.05</td>
<td>0.387 ± 0.05</td>
<td>0.393 ± 0.04</td>
<td>0.389 ± 0.004</td>
<td>0.385 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>HFM</td>
<td>0.383 ± 0.05</td>
<td>0.379 ± 0.05</td>
<td>0.386 ± 0.05</td>
<td>0.388 ± 0.05</td>
<td>0.388 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>LFM+S</td>
<td>0.383 ± 0.05</td>
<td>0.382 ± 0.05</td>
<td>0.388 ± 0.05</td>
<td>0.385 ± 0.05</td>
<td>0.386 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>HFM+S</td>
<td>0.379 ± 0.06</td>
<td>0.382 ± 0.05</td>
<td>0.385 ± 0.05</td>
<td>0.373 ± 0.04</td>
<td>0.389 ± 0.04</td>
</tr>
<tr>
<td>Baseline SS (dyn/cm$^2$)*†</td>
<td>LFM</td>
<td>3.7 ± 1.5</td>
<td>2.9 ± 0.9</td>
<td>4.6 ± 2.9$^&lt;$</td>
<td>4.4 ± 2.8</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>HFM</td>
<td>5.3 ± 2.8</td>
<td>3.4 ± 1.8</td>
<td>3.4 ± 2.1</td>
<td>3.7 ± 1.6</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>LFM+S</td>
<td>3.7 ± 1.7</td>
<td>3.1 ± 1.3</td>
<td>4.7 ± 2.5$^&lt;$</td>
<td>5.0 ± 3.2</td>
<td>4.3 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>HFM+S</td>
<td>3.9 ± 2.4</td>
<td>3.4 ± 1.3</td>
<td>3.5 ± 1.5</td>
<td>3.9 ± 1.6</td>
<td>4.0 ± 1.6</td>
</tr>
<tr>
<td>Mean SS during last minute of cuff occlusion (dyn/cm$^2$)$^\parallel$</td>
<td>LFM</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>HFM</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>LFM+S</td>
<td>1.4 ± 0.7</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.6</td>
<td>1.4 ± 0.5</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>HFM+S</td>
<td>1.2 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.7</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>AUC SS for the first 30 s following cuff-release</td>
<td>LFM</td>
<td>543.1 ± 110.9</td>
<td>542.9 ± 136.5</td>
<td>604.9 ± 163.0</td>
<td>603.9 ± 163.1</td>
<td>638.1 ± 179.8</td>
</tr>
<tr>
<td></td>
<td>HFM</td>
<td>577.4 ± 131.9</td>
<td>541.2 ± 178.9</td>
<td>508.1 ± 150.6</td>
<td>580.8 ± 183.7</td>
<td>616.4 ± 196.0</td>
</tr>
<tr>
<td></td>
<td>LFM+S</td>
<td>588.4 ± 278.4</td>
<td>555.8 ± 171.2</td>
<td>653.3 ± 242.6</td>
<td>641.0 ± 240.5</td>
<td>614.1 ± 167.2</td>
</tr>
<tr>
<td></td>
<td>HFM+S</td>
<td>564.0 ± 105.0</td>
<td>513.8 ± 100.5</td>
<td>549.6 ± 136.4</td>
<td>557.6 ± 105.9</td>
<td>581.5 ± 137.0</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve; HFM, high-fat meal; LFM, low-fat meal; S, Stress condition; and SS, shear stress. *Significant Stress x Time interaction (P<0.05). †Significant Meal x Time interaction (P<0.05). $^\parallel$Main effect of Stress (P<0.05). $^<$Significantly different from HFM condition (P<0.05).
Meal and Time did not affect the %FMD [P=0.562 and P=0.157, respectively; 95% confidence interval (CI) for the mean difference between low-fat and high-fat meal conditions, -2.77 to 0.42]. However, %FMD was non-significantly higher in the stress conditions relative to control (5.8 ± 0.6 versus 4.5 ± 0.5%; P=0.087; 95% CI for the mean difference between Stress and Control conditions, -0.23 to 2.82%) (Figure 5-2 A). There was a significant main effect of stress when FMD was normalized for the shear stress stimulus (%FMD/AUC shear stress, P=0.045; 95% CI for mean difference between Stress and Control conditions, 0.065 to 4.68; Figure 5-2 B).

5.4.3 Hemodynamic, hormonal and subjective stress indices

The P-values for all main effects and significant interactions are provided in Figure 5-3.

The MAP and HR were not different between stress and control conditions at baseline (P>0.05). The MAP and HR increased from baseline during both stress and control tasks (P<0.05), but were significantly greater during the stress tasks (P<0.05; Figure 5-3 A and B). At the fourth task only, HR was greater in LFM and LFM+S versus HFM and HFM+S (P=0.010; Figure 5-3 B). There was no evidence of habituation of MAP and HR (levels at tasks 1-4 not different from one another, P>0.05).

At baseline, cortisol, NE and subjective stress ratings were not different between conditions (P=0.508, P=0.769 and P=0.748, respectively).

Cortisol levels were greater in response to stress tasks than control tasks (P=0.012), with evidence of habituation over time (2-4 h task peaks were significantly less than the 1 h task peak, P<0.001; Figure 5-3 C).

Norepinephrine levels were greater following the stress tasks than the control tasks at both the first (P<0.001) and fourth tasks (P=0.003; Figure 5-3 D). Norepinephrine was greater than baseline following both the first (P<0.001) and fourth (P<0.001) stress tasks, and NE was
Figure 5-2. Flow mediated dilation.

(A) %FMD. 95% Confidence intervals for mean difference between: Stress and Control conditions, -0.23 to 2.82%; and low-fat meal and high-fat meal conditions, -1.35 to 0.79%.

(B) %FMD normalized for the area under the curve (AUC) of the shear stress stimulus for the first 30 s following cuff release. The normalized data are multiplied by 1000 to give whole numbers for ease of visual comparison. 95% confidence intervals for mean difference between: Stress and Control conditions, 0.065 to 4.68; and low-fat meal and high-fat meal conditions, -2.77 to 0.42. Data are means ± SD. Abbreviations: NS, not significant (indicates no difference between conditions at baseline); M, Meal; S, Stress condition; and T, Time. *Main effect of Stress condition; P<0.05.
Figure 5-3. Stress indices.
Mean arterial pressure (MAP; A) and heart rate (HR; B) at baseline and peak (1 min average) during Stress/Control tasks 1-4. (C) Cortisol levels at baseline (average of the two pre-meal time points) and the peak levels at each hourly Stress/Control task. (D) Norepinephrine (NE) concentration at baseline and immediately after the 1 and 4 h Stress/Control tasks. (E) Subjective stress ratings (0-10 scale) at baseline (average of the two pre-meal time points) and after the Stress/Control tasks. Data are means ± SD. Inset boxes identify P-values for all main effects and interactions (M, Meal; S, Stress condition; and T, Time). NS, not significant (indicates no difference between conditions at baseline). *Significantly different from Control conditions (P<0.05). †Significantly different from all other time points (P<0.05). ‡Significantly different from Task 1 (P<0.05). ¥Significantly different from Baseline (P<0.05). §Effect of Meal (P<0.05).
non-significantly lower at the fourth task versus the first (P=0.073). Norepinephrine was significantly elevated from baseline following the first control task (P=0.021), but not following the fourth control task (P=0.403).

Subjective stress ratings (scale of 0-10) were significantly greater following stress tasks than control tasks (all P<0.001; Figure 5-3 E).

5.4.4 Metabolic variables

The P-values for all main effects and interactions are provided in Figure 5-4.

5.4.4.1 Triglycerides (Figure 5-4 A)

Plasma volume was not significantly affected by Meal (P=0.094), Stress condition (P=0.161) or Time (P=0.216; data not shown). Correcting the triglyceride concentrations for plasma volume shifts did not alter the results, so only uncorrected concentrations are shown to reflect the circulating concentrations. There was no impact of Stress on triglycerides (P=0.329); however there was an interaction between Meal and Time (P<0.001). Following consumption of the low-fat meal (LFM and LFM+S conditions), plasma triglyceride levels were not affected by Time (P=0.271). Following consumption of the high-fat meal (HFM and HFM+S conditions), plasma triglyceride levels were significantly increased from baseline by 2 h postprandial (P=0.005) and remained elevated at 3 and 4 h (P<0.001). Plasma triglyceride levels at 3 and 4 h postprandial were not significantly different (P=0.987).

5.4.4.2 Glucose and insulin (Figure 5-4 B and C)

For glucose there was a significant Meal x Time interaction (P<0.001). Glucose concentrations were increased from baseline at 2 h following meal consumption in the low-fat conditions (LFM and LFM+S; P=0.012), but were unchanged from baseline in the high-fat meal conditions (HFM and HFM+S; all P>0.05). Glucose was greater in the low-fat versus high-fat
Figure 5-4. Metabolic variables at baseline and for 4 h following consumption of the low-fat meal or high-fat meal in stress and control conditions. Inset boxes identify P-values for all main effects and interactions (M, Meal; S, Stress condition; and T, Time). (A) Triglyceride. †Significantly different from baseline within HFM conditions (P<0.05). ‡Main effect of Meal (P<0.05). (B) Glucose. *Significantly different from baseline in low-fat meal conditions (P<0.05). ‡Low-fat meal conditions significantly different from high-fat meal conditions (P<0.05). §Significantly different from baseline in Stress conditions (P<0.05). ‡Stress conditions significantly different from Control conditions (P<0.05). (C) Insulin. †Main effect of Meal (P<0.05). *Main effect of stress within LFM conditions (P<0.05). ‡Significantly different from baseline within LFM conditions (P<0.05). §Significantly different from 1 and 2 h time points in LFM conditions (P<0.05). †Significantly different from baseline within HFM conditions (P<0.05).
meal conditions at 2 and 3 h post-meal (P<0.010). There was also a significant Stress x Time interaction (P<0.005). Plasma glucose was increased from baseline at 1-3 h postprandial in the Stress conditions (LFM+S and HFM+S; all P<0.05), but was unchanged from baseline within Control conditions (LFM and HFM; all P>0.05), and was greater in Stress versus Control conditions at 1-4 h postprandial (all P<0.05).

For insulin there were significant Meal x Stress and Meal x Time interactions (P<0.010). Insulin concentrations were greater in LFM+S than LFM (P=0.025), but were not different between HFM+S and HFM (P=0.852). Insulin levels were increased from baseline at 1-3 h post-meal in the low-fat meal conditions (P<0.05), and at 1 h post-meal only in the high-fat meal conditions (P<0.001).

5.4.4.3 Phosphorus (Table 5-3)

There was a main effect of Time (P=0.001) but no main effect of Meal (P=0.782) or Stress (P=0.085) on phosphorus levels. There were significant Meal x Stress (P=0.001), Stress x Time (P=0.017) and Meal x Time (P=0.001) interactions. We explored the significant Stress x Time interaction to provide insight regarding the effect of stress on normalized FMD and found that phosphorus levels were not different between Stress (LFM+S and HFM+S) and Control (LFM and HFM) conditions at baseline (P=0.703) but were significantly lower in the Stress conditions at both 2 h (P=0.040) and 4 h postprandial (P=0.012). In the Stress conditions, phosphorus was significantly reduced from baseline at 2 h (P=0.009) but not different from baseline at 4 h (P=0.993), while in the Control conditions, phosphorus was not different from baseline at 2 h (P=0.862) and was significantly increased at 4 h (P=0.010).
### Table 5.3. Plasma phosphorus (mmol/l)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time point</th>
<th>Baseline</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>LFM</td>
<td>1.04 ± 0.15</td>
<td>0.99 ± 0.20 *</td>
<td>1.25 ± 0.19 **</td>
</tr>
<tr>
<td></td>
<td>HFM</td>
<td>1.04 ± 0.15</td>
<td>1.04 ± 0.16</td>
<td>1.11 ± 0.12</td>
</tr>
<tr>
<td>Stress</td>
<td>LFM+S</td>
<td>1.05 ± 0.20</td>
<td>0.85 ±0.24 †</td>
<td>1.06 ± 0.22 ‡</td>
</tr>
<tr>
<td></td>
<td>HFM+S</td>
<td>1.06 ± 0.15</td>
<td>0.99 ± 0.16</td>
<td>1.04 ± 0.16</td>
</tr>
</tbody>
</table>

Significant Stress x Time interaction (P=0.017). *Significantly different from Stress conditions (P<0.05). †Significantly different from Baseline (P<0.05). ‡Significantly different from 2 h time point (P<0.05).

#### 5.4.4.4 Linear regressions

Only $\Delta HR_{\text{peak}}$ was significantly related to $\Delta\%\text{FMD}$, and this was a very weak association ($r^2 = 0.0503$, P=0.004). Neither NE nor cortisol was significantly related to plasma triglycerides ($r^2 = 0.320$, P=0.088 and $r^2 = 0.270$, P=0.124, respectively, within HFM+S).

#### 5.5 Discussion

The objective of this study was to investigate the combined impact of mental stress and fat consumption on postprandial lipemia and endothelial function. This is the first investigation to examine the impact of repeated bouts of acute mental stress, as well as a mental stress plus high-fat meal combination, on endothelial function in humans. It was hypothesized that mental stress would enhance the magnitude of lipemia following consumption of a high-fat meal and that this would result in an exacerbation of a mental stress- and fat intake-induced reduction in FMD. Contrary to our hypotheses, the main findings of this preliminary study are the following: (i) repeated mental stress tasks had no impact on postprandial lipemia following consumption of either a high- or low-fat meal; and (ii) meal fat content did not affect FMD, but normalized FMD was depressed in the “stress-free” postprandial period (independent of meal type) relative to when this period was accompanied by mental stress. These outcomes occurred in the presence of the following responses: (i) robust physiological stress responses in the mental stress conditions [Figure 5-3, involving both sympathetic nervous system activation (Figure 5-3 A, B and D) and hypothalamic-pituitary-adrenal axis activation (Figure 5-3 C)] that are in good agreement with
previous research examining the impact of acute mental stress on FMD in healthy subjects (Ghiadoni et al., 2000; Speker et al., 2002); and (ii) the expected plasma triglyceride response following consumption of low- and high-fat meals (Figure 5-4). These provocative preliminary results demonstrate the absence of a simple relationship between stress reactivity, fat consumption, and endothelial function, and indicate that in young, healthy individuals acute mental stress may have a modest beneficial effect on endothelial function during the postprandial period.

5.5.1 The impact of acute mental stress on postprandial lipemia

It was hypothesized that mental stress would enhance postprandial lipemia, because previous studies have demonstrated that short-term mental stress increases triglyceride and cholesterol levels in humans (Dimsdale & Herd, 1982; Niaura et al., 1992). In the present study, while the degree of postprandial lipemia following consumption of the high-fat meal was similar to that attained in other studies in which the same test meal was provided (Vogel et al., 1997; Plotnick et al., 1997), there was no impact of mental stress on blood lipid levels. This lack of effect was observed despite significant increases in NE and cortisol. This is in contrast to one previous study with a similar test meal, in which it was reported that repeated stressor application enhanced postprandial lipemia (Le et al., 1999). More frequent application of the stress tasks over a longer duration in this previous study (10 min task every 30 min for 5 h) may have contributed to the disparate results.

5.5.2 The impact of meal and mental stress on FMD

In the present study, the meal type (high- or low-fat) did not affect FMD, but normalized FMD was modestly enhanced in the stress conditions (LFM+S and HFM+S) relative to control conditions (LFM and HFM, P=0.045; without normalization: %FMD was 5.8 ± 0.6% versus 4.5 ± 0.5%; P=0.087). This effect persisted when the baseline time point was excluded from the comparison (main effect of Stress for %FMD/AUC shear stress, P=0.032), which, together with
the lack of significant difference in baseline normalized FMD (P=0.746), suggests that the main effect of Stress was driven by the post-intervention time points. There is no universally accepted method to account for variability in the reactive hyperemia shear stress stimulus and, while ratio normalization may have limitations (Thijssen et al., 2011), it has been shown to be useful in isolating differences in endothelial function (Padilla et al., 2009; Wray et al., 2006). The shear stress stimulus upon cuff release did not differ between conditions, and while there were statistically significant differences in baseline and cuff-occlusion shear stress levels, the magnitude of differences was modest (equivalent to ~1 cm/s differences in blood velocity during occlusion) and unlikely to influence the FMD results (Wray et al., 2011). The effect of stress (Figure 5-2 B) was driven by an overall pattern of a non-significant decrease in normalized FMD from baseline in the postprandial period in the LFM and HFM conditions and a non-significant increase from baseline with the addition of repeated bouts of acute mental stress (LFM+S and HFM+S conditions).

Researchers in our laboratory have demonstrated highly repeatable within- and between-observer analysis of FMD (Szijgyarto et al., 2013). In the present preliminary study, the magnitude of the significant difference in normalized FMD between the pooled Stress and Control conditions (2.4%; Stress greater than Control) was similar to the range of the group average pre-intervention baseline values (2.2%; Figure 5-2 baseline). While the size of the significant relative enhancement of the normalized FMD in the Stress versus Control conditions may be interpreted with caution, these results are clearly in contrast with previous studies in which consistent and large decreases in FMD (magnitude halved) were observed following mental stress tasks in fasted conditions (Broadley et al., 2005; Ghiadoni et al., 2000; Spieker et al., 2002; see ‘The impact of mental stress on FMD’ below).
5.5.3 The impact of meal consumption on FMD

The collective postprandial “impairment” in normalized FMD in the Control conditions (LFM and HFM) relative to the Stress conditions (LFM+S and HFM+S) occurred in the presence of dissimilar changes in metabolic factors between meals (i.e. greater increases in insulin and glucose following the low-fat meal, and greater increases in triglycerides after the high-fat meal). Hyperglycemia and hypertriglyceridemia are both thought to impair endothelial function primarily via an increase in oxidative stress (Wallace et al., 2010; Williams et al., 1998). However, blood glucose and triglyceride levels following the LFM and HFM were similar to those in previous studies, in which FMD was found to decline from a fasting baseline following the high-fat meal but to remain unchanged following the low-fat meal (Plotnick et al., 1997; Vogel et al., 1997), suggesting that these metabolic measures do not consistently translate to an impact on FMD. Indeed, glucose was higher in Stress versus Control conditions and yet FMD was not impaired. As with previous reports examining the impact of meal fat content on FMD (Padilla et al., 2006; Plotnick et al., 1997; Vogel et al., 1997), the low-fat meal but not the high-fat meal included orange juice, which is high in vitamin C. However, this did not appear to result in a beneficial antioxidant effect because we did not observe a higher FMD in the low-fat meal conditions.

The impact of high-fat meal consumption on FMD has some demonstrated variability (Johnson et al., 2011; Plotnick et al., 2003), and a number of factors that may moderate the acute interaction between meal consumption and endothelial function have been identified [e.g. antioxidant therapy (Anderson et al., 2006; Plotnick et al., 2003) or regular physical activity (Johnson et al., 2011)]. It is possible that these factors may have influenced our observed postprandial FMD responses. If anything however, they would be expected to attenuate any postprandial reduction in FMD, and while they might explain the absence of a greater relative dysfunction following the high-fat meal they do not account for the overall presence of depressed postprandial normalized FMD in the Control relative to the Stress Conditions. While the
mechanism driving the effect of stress is unclear, it may involve an effect of stress on plasma phosphorus, as described in the following section.

5.5.4 The impact of mental stress on FMD

As identified above, most previous studies have demonstrated an adverse effect of mental stress on conduit artery endothelial function when measured shortly following the stress experience (Broadley et al., 2005; Ghiadoni et al., 2000; Spiker et al., 2002), although this finding is not universal (Jambrik et al., 2004; Harris et al., 2000) and may depend on individual characteristics [e.g. hypnotizability (Jambrik et al., 2004) and hostile affect (Gottdiener et al., 2003)]. Such impairment has been observed immediately (Jambrik et al., 2004) and from ~10 (Jambrik et al., 2004; Broadley et al., 2005; Ghiadoni et al., 2000; Spiker et al., 2002) to 90 min (Ghiadoni et al., 2000) following only a brief (2-10 min) bout of stress. In contrast, in the present study FMD was not depressed versus the “stress free” conditions (LFM and HFM; normalized FMD significantly greater in Stress versus Control conditions). This occurred despite robust stress responses, similar to those in the above-mentioned studies in which post-stress FMD was impaired.

Only one study has demonstrated an improvement in FMD with a mental stress task (7-8 min of mental arithmetic; Harris et al., 2000), however, the FMD was measured during the mental stress task (rather than post-stress), and it is likely that different mechanisms influence FMD during versus after a stressful experience (Poitras & Pyke, 2013). Notably, the preservation of FMD with mental stress in the present study occurred superimposed on the postprandial state, whereas in previous investigations where a stress-mediated reduction in FMD was observed it occurred in a fasting condition (Jambrik et al., 2004; Broadley et al., 2005; Ghiadoni et al., 2000; Spiker et al., 2002). Thus, contrary to our hypothesis, perhaps experiencing stress with food consumption protects against a negative influence of both stimuli.
One factor that may have contributed to our finding of preserved endothelial function with acute mental stress concerns the interaction of stress with dietary phosphorus. Dietary phosphorus, in amounts similar to that in the test meals in the present study (559 and 687 mg for the high- and low-fat meals, respectively), has been shown to acutely impair FMD (Shuto et al., 2009). We observed that phosphorus levels were lower in the Stress conditions, in agreement with previous findings that circulating concentrations of phosphorus are significantly reduced by an acute experience of mental stress (Stroop Test; Joborn et al., 1990) and by infusions of the stress hormone epinephrine (Joborn et al., 1990; Kjeldsen et al., 1996). Therefore, if phosphorus exerted a negative impact on endothelial function, the stress-induced attenuation of the postprandial rise in phosphorus may have contributed to the tendency for a higher FMD in the Stress conditions. Future work is required to dissect the effects of all single meal components and their interactions with stress.

A second possible mechanism relates to perception of the stress tasks. Positive emotions and mental stress may have divergent effects on endothelial function despite similar physiological responses (Sugawara et al., 2010; Miller et al., 2006; Miller et al., 2010). In the present study, the length of the protocol posed a potential for boredom or negative emotions in control conditions, and if the subjects viewed the stress tasks as a welcome distraction rather than an unpleasant experience, this may have impacted their FMD responses.

5.5.5 Limitations

This study had a small sample size and used a homogeneous population (results cannot be generalized to women, older or disease populations). As described in the Methods section, the sample size was selected via calculations based on previous work that employed a similar stress task protocol and identified a large and consistent deleterious effect of stress on FMD (FMD magnitude cut in half post stress; Ghiadoni et al., 2000). We were also influenced by other work that identified a large deleterious impact of high-fat meal intake on FMD (FMD values halved
post meal; Vogel et al., 1997). However, the FMD responses in the present study were directionally opposite from what was anticipated in our a priori power calculations. With respect to Meal, when pooled across Time and Stress the mean high-fat meal FMD was slightly higher than the low-fat meal FMD (high-fat meal 5.2%, low-fat meal 4.9%, P=0.562). With respect to Stress, when pooled across Time and Meal the FMD was higher in the Stress condition (Stress 5.8%, Control 4.5%), and this difference was significant when normalized to the stimulus (P=0.045). Therefore, while variability and power remain of concern when interpreting results with a small sample size, these findings are provocative and warrant repetition in a larger group of subjects.

We cannot exclude the possibility that the subjects recruited here represent a subset of the population who inherently have reduced vulnerability to stress- and/or fat-induced decreases in endothelial function, because in previous studies susceptibility to these impairments has been shown to depend on individual characteristics [e.g. hypnotizability (Jambrik et al., 2004), hostility (Gottdiener et al., 2003), and fitness (Johnson et al., 2011)]. Thus, perhaps our sample was biased in that the impacts of stress and/or fat intake on FMD were systematically underestimated relative to their impacts at the population level. Indeed, the probability of type II error (failing to find an effect in the sample that truly exists in the population) is increased with small sample sizes as in the present study. Additionally, we did not utilize a fasting control group; thus, it is unknown to what extent repeated bouts of mental stress in the absence of the postprandial state may have impacted endothelial function in this subject population. Larger studies that follow this preliminary work can attempt to tease out “phenotypes” with respect to vulnerability to stress and dietary fat intake.

The laboratory mental stress tasks employed in this study may be less potent stimulators of the physiological stress responses than real life events (Rozanski et al., 1999), and as a result may fail to emulate the true impact of acute stress on postprandial lipemia and endothelial
function. However, the present study used similar stress tasks and elicited similar stress responses as previous work in which directionally opposite changes in FMD were observed post-stress (Ghiadoni et al., 2000; Spieker et al., 2002; Broadley et al., 2005). Thus, the results of the present study demonstrate that at a given level of stress reactivity, experiences of mental stress are not universally detrimental to endothelial function.

5.5.6 Conclusions

In the present study we observed depressed normalized FMD after consumption of a single meal (high or low in fat) followed by non-stressful stimuli (control tasks) relative to when the postprandial state was accompanied by intermittent stressful tasks over a 4 h period. These results suggest that in young, healthy men, the postprandial state may have a modest negative impact on vascular endothelial function, whereas acute experiences of mental stress in this state may confer protection, possibly due in part to a reduction in plasma phosphorus levels. This is in contrast to previous studies, in which mental stress and fat consumption have been independently shown to transiently impair endothelial function in a similar subject population. While the present findings may be considered preliminary, and should be interpreted in the context of the small sample size, the unanticipated impacts of meal consumption and stress are provocative and suggest that we have more to learn regarding the integration of these complex signals and their impact on the cardiovascular system in vivo. The lack of demonstrated consistency in the impact of mental stress or fat consumption on endothelial function highlights the need to investigate the following: (i) the mechanisms underlying the interactions between these factors; (ii) the moderating influences of individual subject characteristics; and (iii) the potential differences with respect to acute versus chronic exposures to mental stress and fat consumption.
5.6 References


Chapter 6

Evidence that meal fat content does not impact hemodynamic reactivity to or recovery from repeated mental stress tasks

Published As:

6.1 Abstract

The magnitude (“reactivity”) and duration (“recovery”) of hemodynamic stress responses are predictive of cardiovascular risk, and fat intake has been shown to enhance hemodynamic reactivity to psychological stress tasks. The objective of this study was to determine the impact of a high fat meal (HFM) on the magnitude and stability of hemodynamic stress reactivity and recovery. This was assessed by: 1) the peak changes from baseline to during stress for: heart rate (HR); mean, systolic and diastolic blood pressure; cardiac output; and total peripheral resistance; and 2) the residual arousal in hemodynamic parameters at two points post-stress (“early” and “late” recovery). On different days, 10 healthy males (23.2 ± 3.3 yr) consumed either a HFM (54 g fat) or low-fat meal (LFM; 0 g fat) (~1000 calories each), followed by 4 hourly 10 min Stress tasks (mental arithmetic and speech tasks). Pre-Stress (baseline) parameters did not differ between HFM and LFM conditions (all P>0.05). Plasma triglycerides were greater following the HFM vs the LFM (P=0.023). No reactivity or recovery parameters differed between meals (all P>0.05). Stress reactivity and recovery parameters were stable over the 4 stress tasks (main effects of Time all P>0.05), with the exception of HR (P<0.05). Contrary to previous reports, meal fat content did not impact hemodynamic reactivity to laboratory stressors. These data also provide the first evidence that meal fat content does not impact hemodynamic recovery from repeated mental stress tasks.
6.2 Introduction

Experiences of psychosocial stress result in activation of the physiological stress response systems [the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis], and subsequent hemodynamic and hormonal responses (including increases in heart rate and blood pressure). While in the short-term these responses may be adaptive (i.e. the “fight or flight” response that allows an organism to react to an immediate threat), hyperactivity or prolonged duration of responses is maladaptive and can promote the pathogenesis of disease (Rozanski et al. 1999; Black and Garbutt 2002).

Stress “reactivity” is defined as the magnitude of elevation in parameters (e.g. heart rate and blood pressure) in response to an acute stressful stimulus (Moseley and Linden 2006), while “recovery” refers to the duration of the stress response as quantified by the time required to return to pre-stress levels or the extent of elevation that remains during the post-stress period (Moseley and Linden 2006; Stewart et al. 2006). Greater cardiovascular reactivity to and poor recovery from laboratory mental stress tasks has been shown to predict higher blood pressure and incident hypertension, atherosclerosis, coronary artery disease, and stroke up to 36 years later (Chida and Steptoe 2010; Kamarck et al. 1997; Krantz et al. 1999; Everson et al. 2001), independent of traditional risk factors such as age, lipid levels, and obesity (reviewed in Chida and Steptoe 2010). Thus responsiveness to laboratory stress tasks is informative in terms of long-term cardiovascular health status.

Dietary fat intake is also an independent risk factor for cardiovascular disease (Stone 1990), and there is some evidence that both a single high fat meal (Faulk and Bartholomew 2012; Jakulj et al. 2007) and short-term elevations in dietary fat intake (Straznicky et al. 1993) may enhance hemodynamic reactivity to mental stress. The mechanisms of this effect are unclear but have been proposed to involve fat intake induced endothelial dysfunction (Faulk and Bartholomew 2012; Jakulj et al. 2007) and nutrient effects on neural (Barnes et al. 2003; Prior et
al. 2010) and hormonal (Choi et al. 1996) stress responses. Since stress reactivity is associated with cardiovascular risk, such interactions with diet are important. There are substantial gaps in the literature however, as first, no studies have investigated the impact of consumption of a fat-rich meal on an index of hemodynamic stress recovery with adequate resolution. This is important because this aspect of stress responsiveness affords additional predictive value beyond that of hemodynamic reactivity alone in terms of long-term cardiovascular risk (Chida and Steptoe 2010; Rutledge et al. 2000; Stewart and France 2001). Second, all studies investigating an interaction between fat consumption and acute stress responses to date have assessed hemodynamic reactivity at a single time point (~2 h postprandial), but metabolic parameters are known to change over several hours post-meal and thus the time course of the interaction is unknown.

With this as a foundation, the objective of the present study was to determine the impact of a high-fat meal on the magnitude and stability of hemodynamic reactivity to, and recovery from, varied mental stressors over a 4-hour period. The data for the present investigation were derived from a larger protocol examining the impact of stress and fat consumption on endothelial function (Poitras et al. 2014). It was hypothesized that: a) stress reactivity would be augmented, and b) stress recovery would be impaired following consumption of a high- versus low-fat meal. It was predicted that these differences would be greatest at 3-4 hours post-meal consumption when postprandial lipemia is expected to peak (Parks 2001).

6.3 Methods

6.3.1 Subjects

Ten healthy, non-smoking, normolipidemic, recreationally active males (23.2 ± 3.3 yr) from the Queen’s University community participated in this study (for detailed subject characteristics see Poitras et al. 2014). Health status was established with a medical screening questionnaire. The study protocol was approved by the Health Sciences Human Research Ethics
Board at Queen’s University, and subjects gave written consent to participate on forms approved by this board. A Seven-Day Physical Activity Recall Questionnaire was administered to estimate subjects’ habitual physical activity levels (1997) and did not influence eligibility for inclusion in the study.

6.3.2 Measurements

Heart rate (HR; 3 lead electrocardiography) and mean arterial pressure (MAP; photoplethysmography) were acquired continuously throughout the protocol with detailed methods reported previously (Poitras et al. 2014). Systolic and diastolic blood pressure (SBP, DBP) were measured continuously with finger photoplethysmography (Finometer PRO, Finapres Medical Systems). Stroke volume (SV) was estimated continuously via the Finometer and BeatScope Easy software (BeatScope Easy v.01, Finapres Medical Systems) using the Modelflow method (i.e. computation and integration of aortic flow waveform from finger pressure; Bogert and Van Lieshout 2005). SV was used in conjunction with HR to estimate cardiac output (CO; where estimated CO = SV x HR). Total peripheral resistance (TPR) was calculated as: TPR = MAP/CO. Methods for blood sampling (to quantify viscosity, hematocrit, hemoglobin, lipids, glucose and insulin concentrations) and for plasma volume correction were described previously (Poitras et al. 2014).

6.3.3 Experimental procedure

Subjects visited the lab on two different days, separated by 14 ± 5.4 days. Subject preparation, blood sampling and stress task timing were described in detail previously (Poitras et al. 2014). Briefly, subjects were instructed to abstain from taking anti-inflammatory drugs within 48 hours of the study, from exercising within 24 hours, and from consuming food, alcohol, or caffeine for 12 hours prior to each testing session (Thijssen et al. 2011). Subjects participated in the study after an overnight fast and after having been instructed to consume a meal that was “typical” for them the night before, which they then replicated prior to their second testing
session. Data collection commenced at 10:00 or 10:45 am (consistent within a subject), and was performed in a quiet, temperature-controlled room (~20-25°C).

Upon arrival at the laboratory, subjects were positioned supine and were instrumented with an intravenous catheter for repeated blood sampling. Following instrumentation, subjects rested quietly for 30 minutes, after which baseline measures were taken. Subjects then ate the low-fat or high-fat meal (order counterbalanced across subjects), and underwent 4 hourly stress tasks that were 10 minutes in duration. Blood samples were taken to quantify metabolic variables, viscosity and plasma volume (at baseline and hourly postprandial).

6.3.3.1 Meals

As previously described (Poitras et al. 2014), meals were the following:

_Low-fat meal (LFM):_ 160 g Frosted Flakes (Kellogg Company), 500 ml skim milk, and 500 ml of orange juice (990 calories, 0 g fat, 0 g saturated fat, 0 g trans-fat, 209 g carbohydrates, 23 g protein, 5 g fiber, 959 mg sodium).

_High-fat meal (HFM):_ an Egg McMuffin, Sausage McMuffin, two hash brown patties (McDonald’s Corporation), and water (1000 calories, 54 g fat, 16 g saturated fat, 1 g trans-fat, 94 g carbohydrates, 32 g protein, 7 g fiber, 2330 mg sodium).

Subjects were given 125-250 ml of water every hour to maintain hydration throughout the experimental visits.

6.3.3.2 Stress tasks

The stress tasks have been described in detail previously (Poitras et al. 2014). Briefly, the stress tasks included mental arithmetic (3 tasks – 1 repeated) or a speech (5 tasks – 1 repeated) and the Stroop color-word conflict task; all tasks were previously shown to evoke physiological stress responses (Gottdiener et al. 2003; Greeson et al. 2009; Ghiadoni et al. 2000; Biondi and Picardi 1999; Harris et al. 2000; Sarabi and Lind 2001; Kirschbaum et al. 1993). The tasks were administered in two counterbalanced “Programs” with the intention of interspersing tasks to
provide a varied stimulus to minimize adaptation. Speech tasks were audio recorded, and researchers provided standard prompts to continue if needed. All tasks were 10 minutes in duration, including instructions, preparation (speech tasks only) and speaking time.

6.3.4 Data analysis

6.3.4.1 Cardiovascular parameters

MAP, SBP, DBP, HR, CO and TPR were analyzed offline into 1-minute average time bins. Stress reactivity was assessed as the peak (1-minute average) change from baseline (2-minute average) during the Stress Tasks (△MAP_peak, △SBP_peak, △DBP_peak, △HR_peak; Szijgyarto et al. 2014; Greeson et al. 2009). The change from baseline in CO and TPR (i.e. △CO_peak, △TPR_peak) was assessed at the time of △MAP_peak, because these variables are the central and peripheral determinants of MAP. In addition, △MAP_peak during the task with the highest postprandial lipemia (individually determined for each subject) in the HFM condition was determined for comparison to △MAP_peak at the corresponding task number in the LFM condition. Stress recovery was assessed as “Early Recovery” [change from baseline to the 1 minute average taken from minutes 2-3 post-stress (Moseley and Linden 2006); △MAP_R1, △SBP_R1, △DBP_R1, △HR_R1, △CO_R1, △TPR_R1] and “Late Recovery” [change from baseline to the 1 minute average taken from minutes 7-8 post-stress (Heponiemi et al. 2007); △MAP_R2, △SBP_R2, △DBP_R2, △HR_R2, △CO_R2, △TPR_R2].

6.3.4.2 Blood sample analysis

Blood sample analysis has been described previously (Poitras et al. 2014). Briefly, blood viscosity analysis was performed with a cone-plate viscometer operating at a shear rate of 225 s⁻¹ (Brookfield Viscometer DV-II+ Pro, Brookfield Engineering Lab Inc.) at 37 ± 2°C. Hematocrit and hemoglobin were determined either in-house (StatProfile M Blood Gas Analyzer, Nova Biomedical), or at Kingston General Hospital (KGH; Beckman Coulter LH 785 Hematology System). Plasma lipids and glucose were analyzed via Beckman Coulter UniCel® DxC 600/800
SYNCHRON® Clinical System(s) and SYNCHRON® Systems Multi Calibrator at KGH. Plasma insulin concentrations were analyzed by ELISA according to the manufacturer’s instructions (80-INSHU-E01.1, ALPCO Diagnostics).

6.3.5 Statistical analysis

Paired t-tests were used to test the day-to-day stability of baseline parameters (MAP, SBP, DBP, HR, CO, TPR, viscosity, and plasma volume). Two-way repeated measures analysis of variance tests (ANOVAs) were conducted to evaluate the impact of Meal (LFM, HFM) and Time on stress reactivity and recovery parameters (at Tasks 1-4), and on blood viscosity, and plasma volume, triglycerides, glucose and insulin (at baseline and hours 1-4 postprandial). Significant differences were further investigated using Tukey’s post hoc tests. ∆MAP_peak during the task with the greatest postprandial lipemia (HFM condition) was compared to the ∆MAP_peak during the corresponding task number in the LFM condition with a paired t-test. The relationship between postprandial lipemia and MAP reactivity at all postprandial time points was assessed with linear regression. A paired t-test was used to determine whether there was habituation in ∆MAP_peak (i.e. visit 1 vs visit 2). Statistical significance was set at P<0.05. Statistics were calculated using SigmaPlot 11.0 (Systat Software Inc.; San Jose, CA, USA). Data are expressed as means ± SD.

6.4 Results

The baseline (Table 6-1) and peak MAP and HR values used to derive MAP and HR reactivity change scores, and the metabolic values (Table 6-2) were previously reported with distinct statistical analysis involving a greater number of conditions than the present investigation (Poitras et al. 2014). No hemodynamic parameters differed at baseline from day-to-day (baseline MAP, SBP, DBP, HR, CO, and TPR, all P>0.05; Table 6-1).
Table 6-1. Baseline parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>P-value (visit 1 vs 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>88.9 ± 9.0</td>
<td>0.151</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>127.5 ± 11.6</td>
<td>0.291</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>71.3 ± 8.1</td>
<td>0.250</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>53.6 ± 7.0</td>
<td>0.179</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>5.5 ± 1.3</td>
<td>0.684</td>
</tr>
<tr>
<td>TPR, mmHg/L/min</td>
<td>17.1 ± 4.1</td>
<td>0.453</td>
</tr>
</tbody>
</table>

Values are means ± SD. CO = cardiac output; DBP = diastolic blood pressure; HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure; TPR = total peripheral resistance.

6.4.1 Metabolic parameters

The triglyceride, glucose and insulin concentrations are reported in Table 6-2. Triglycerides, glucose and insulin did not differ between meal conditions at baseline (P>0.05). For triglycerides, there was a Meal x Time interaction (P<0.001). Following consumption of the HFM plasma triglycerides increased significantly (P<0.05 for 2 h, 3 h and 4 h postprandial vs baseline and vs LFM), but were unchanged from baseline in the LFM condition (all P>0.05). For glucose there was a Meal x Time interaction (P=0.006), such that glucose was unchanged from baseline in the HFM condition (P>0.05), but was greater than baseline at 1-3 hours postprandial in the LFM condition (P<0.05) and significantly greater than in the HFM condition at 2 and 3 hours postprandial (P<0.05). There was a Meal x Time interaction for insulin (P<0.001). Insulin concentrations were increased from baseline at 1 h postprandial only in the HFM condition (P=0.015) and at 1-3 hours following consumption of the LFM (all P<0.05). Insulin concentrations were greater in LFM than HFM at 1-3 hours postprandial (all P<0.05).

6.4.2 Viscosity and plasma volume

Viscosity and plasma volume did not differ between meal conditions at baseline (P>0.05). Whole blood viscosity increased modestly from ~4.0 cP at baseline to ~4.3 cP postprandially (P<0.001 for 1 h, 2 h, 3 h and 4 h vs baseline), but was not different between meals
<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Time Point</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>1 h</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>HFM</td>
<td>0.76 ± 0.61</td>
<td>0.97 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>0.71 ± 0.36</td>
<td>0.78 ± 0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>HFM</td>
<td>4.83 ± 0.35</td>
<td>5.42 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>4.85 ± 0.40</td>
<td>5.68 ± 1.23*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>HFM</td>
<td>3.16 ± 1.92</td>
<td>24.58 ± 16.84*†</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>3.35 ± 2.30</td>
<td>56.21 ± 24.61*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concentrations at baseline and for 4 hours following consumption of the low fat meal (LFM) or high fat meal (HFM). These values were reported previously as part of a larger study that involved additional conditions (Poitras et al. 2014). Data are means ± SD. *Significantly different from baseline, †Significantly different from LFM, P<0.05. M = Meal, T = Time, TG = triglyceride.
(P=0.351). For plasma volume there was a main effect of Time (P=0.015) and a main effect of Meal (P=0.017; Meal x Time: P=0.856), with plasma volume being significantly greater in the HFM versus LFM condition (57.2 ± 4.11 vs 55.9 ± 3.42 % respectively) and significantly lower than baseline at 1 h (P=0.015) and 4 h (P=0.041) post-meal.

6.4.3 Hemodynamic stress reactivity and recovery (Figure 6-1, Table 6-3, Table 6-4)

No stress reactivity or recovery parameters were affected by Meal (all P>0.05). Only HR reactivity, and early and late HR recovery were affected by time (main effects of Time, P=0.045, P=0.045, and P=0.032 respectively; Figure 6-1 G, H and I), with no specific differences revealed by post hoc testing (all P>0.05). SBP, DBP and HR reactivity and recovery are shown in Figure 6-1 A-I. As seen in Table 6-3, MAP increased with stress, however there were no differences between meal conditions in how the pressor response was achieved (i.e. ΔCO\text{peak} and ΔTPR\text{peak}) or sustained (i.e. ΔCO\text{R1}, ΔCO\text{R2}, ΔTPR\text{R1}, ΔTPR\text{R2}; Table 6-4) either between meals or across Time (effect of Meal and Time all P>0.05). ΔMAP\text{peak} during the task with the highest postprandial lipemia in the HFM condition (individually determined for each subject) did not differ from the ΔMAP\text{peak} at the corresponding task number in the LFM condition (27.9 ± 14.0 mmHg vs 29.2 ± 12.2 mmHg; P=0.626). In addition, there was no relationship between postprandial lipemia and ΔMAP\text{peak} (r² =0.08; P=0.095; data not shown). When divided by visit (visit 1 vs visit 2) rather than meal (meal order was counterbalanced) there was no difference in ΔMAP\text{peak} (P=0.301), supporting a lack of habituation to the stress tasks over the two testing days.
Figure 6-1. Hemodynamic stress reactivity and recovery parameters.
Grey bars = HFM, White bars = LFM. (A-C) Systolic blood pressure (SBP) reactivity and early and late SBP recovery. (D-F) Diastolic blood pressure (DBP) reactivity and early and late DBP recovery. (G-I) Heart rate (HR) reactivity and early and late HR recovery. Data are means ± SD. M = Meal, T = Time.
### Table 6-3. Hemodynamic stress reactivity

<table>
<thead>
<tr>
<th>Variable: Reactivity</th>
<th>Condition</th>
<th>Time Point</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Task 1</td>
<td>Task 2</td>
</tr>
<tr>
<td>ΔMAP, mmHg</td>
<td>HFM</td>
<td>21.0 ± 10.8</td>
<td>30.1 ± 19.8</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>33.8 ± 10.0</td>
<td>31.1 ± 17.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCO, L/min</td>
<td>HFM</td>
<td>2.3 ± 1.2</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>1.7 ± 2.4</td>
<td>2.1 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔTPR, mmHg/L/min</td>
<td>HFM</td>
<td>-2.5 ± 2.1</td>
<td>1.8 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>-0.9 ± 4.8</td>
<td>-0.6 ± 6.4</td>
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<td></td>
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</tr>
</tbody>
</table>

Values are means ± SD. MAP, CO and TPR reactivity for each task in the high fat and low fat meal conditions. P-values refer to the 2-way ANOVA analysis (M = Meal, T = Time, M x T = Meal by Time interaction). CO = cardiac output; HFM = high-fat meal; LFM = low-fat meal; MAP = mean arterial pressure; TPR = total peripheral resistance.
Table 6-4. Hemodynamic stress recovery

<table>
<thead>
<tr>
<th>Variable: Recovery</th>
<th>Condition</th>
<th>Task 1</th>
<th>Task 2</th>
<th>Task 3</th>
<th>Task 4</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>∆MAP, mmHg</td>
<td>HFM</td>
<td>7.9 ±  6.3</td>
<td>5.4 ±  6.2</td>
<td>12.7 ± 14.8</td>
<td>9.8 ± 12.7</td>
<td>11.9 ± 11.2</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>11.8 ± 7.6</td>
<td>12.8 ± 10.7</td>
<td>15.2 ± 15.3</td>
<td>11.8 ± 14.6</td>
<td>12.9 ± 16.7</td>
</tr>
<tr>
<td>∆CO, L/min</td>
<td>HFM</td>
<td>1.3 ±  0.7</td>
<td>1.3 ±  0.8</td>
<td>0.6 ±  0.7</td>
<td>0.8 ±  0.8</td>
<td>0.8 ±  0.8</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>1.3 ±  1.2</td>
<td>0.9 ±  1.2</td>
<td>1.1 ±  1.7</td>
<td>0.6 ±  1.6</td>
<td>0.8 ±  1.4</td>
</tr>
<tr>
<td>∆TPR, mmHg/L/ min</td>
<td>HFM</td>
<td>-2.3 ± 2.2</td>
<td>-2.7 ± 2.6</td>
<td>0.8 ± 4.0</td>
<td>-0.7 ± 2.1</td>
<td>-0.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>LFM</td>
<td>-1.8 ± 3.8</td>
<td>3.0 ± 12.0</td>
<td>-0.5 ± 6.9</td>
<td>0.4 ± 5.9</td>
<td>-0.4 ± 6.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. MAP, CO and TPR early and late recovery in each task for both the high fat and low fat meal conditions. P-values refer to the 2-way ANOVA analysis at early (R1) and late (R2) recovery (M = Meal, T = Time, M x T = Meal by Time interaction). CO = cardiac output; HFM = high-fat meal; LFM = low-fat meal; MAP = mean arterial pressure; R1 = early recovery; R2 = late recovery; TPR = total peripheral resistance.
6.5 Discussion

This is the first study to examine the impact of acute fat consumption on both hemodynamic stress reactivity and hemodynamic stress recovery over a 4 h postprandial period in humans. This study also provided the first examination of the relationship between postprandial lipemia and blood pressure reactivity. Contrary to our hypothesis, meal fat content did not significantly impact hemodynamic reactivity to, or recovery from, varied mental stress tasks over a 4 hour postprandial period, and we found no association between postprandial lipemia and blood pressure reactivity. These data also provide evidence of minimal habituation in hemodynamic responses to varied mental stress tasks performed 8 times over 2 laboratory visits.

6.5.1 The link between acute stress responsiveness and cardiovascular disease

Cardiovascular diseases develop gradually over decades with clinical symptoms typically manifesting later in life. Interestingly, traditional risk factors (including family history, obesity, smoking, diabetes mellitus and hypercholesterolemia) only predict ~50% of future cardiovascular disease (Treiber et al. 2003), signifying that other risk factors remain to be identified. Stress reactivity and recovery parameters have been found to be additional independent predictors of cardiovascular disease, including hypertension, atherosclerosis, coronary artery disease and stroke (Everson et al. 1996; Everson et al. 1997; Everson et al. 2001; Kamarck et al. 1997; Barnett et al. 1997; Markovitz et al. 1998; Matthews et al. 1998; Kaplan et al. 1991). For instance, hemodynamic stress reactivity typically explains 4-13% of the variation in future blood pressure after adjusting for standard risk factors (Light et al. 1992; Matthews et al. 1993; Flaa et al. 2008). Thus stress response parameters can provide important information regarding cardiovascular disease risk.

Although associations between responses to acute stress and future negative cardiovascular outcomes do not establish a causal connection, it has been hypothesized that heightened stress responses play a mechanistic role in disease development. Proposed pathways
are abundant and include: 1) injury and dysfunction of the vascular endothelium and subsequent atherosclerosis (Barnett et al. 1997; Beevers et al. 2001; Kaplan et al. 1991), and 2) SNS-mediated increases in TPR leading to myocardial ischemia and/or development of hypertension over time (Rozanski et al. 1988; Folkow 1990). Delayed post-stress recovery is thought to represent prolonged activation of these deleterious processes (Steptoe and Marmot 2006).

6.5.2 The acute interaction between meal fat content and stress responsiveness

In the present study, no stress reactivity or recovery parameters were significantly affected by meal fat content, nor, with the exception of HR, did they change across the series of stress tasks presented over a 4 hour period. These findings are in agreement with one previous study (Sauder et al. 2012) in which SBP, DBP and TPR reactivity to a stress task were not influenced by meals that were either low in fat or high in saturated or polyunsaturated fat (speech and cold pressor task, ~2.5 h post-meal), but are in contrast with other previous findings that blood pressure (Jakulj et al. 2007; Faulk and Bartholomew 2012) and TPR (Jakulj et al. 2007) reactivity were greater in response to laboratory stressors presented 2 hours following consumption of a HFM versus a LFM. The reason for these disparate findings is unclear as all studies used similar subject populations (young, healthy adults), stress tasks (variations of speech tasks, mental arithmetic, and cold-pressor/ischemia) and quantities of fat (~40-54 g).

Mechanisms responsible for previously observed fat intake dependent differences in postprandial hemodynamic stress reactivity are uncertain, but it has been suggested that transient impairments in endothelial function and reduced flow-mediated vasoactivity with fat consumption (e.g. Vogel et al. 1997; Tsai et al. 2004) may be responsible (Faulk and Bartholomew 2012; Jakulj et al. 2007) (i.e. endothelial dysfunction resulting in a relatively more vasoconstricted state may augment blood pressure and TPR responses to stress). However, impaired endothelial function is not a generalized response to fat consumption (e.g. Sejda et al. 2002; Muntwyler et al. 2001), and indeed endothelial function was assessed via reactive
hyperemia flow mediated dilation (FMD) 10 min following each stress task in the larger protocol involving these participants (Poitras et al. 2014) and was found to be similar over the 4 hour time period following both meals. This may have contributed to the lack of difference in blood pressure recovery parameters between conditions. However, endothelial function was not measured during stress, which is the period relevant to hemodynamic reactivity. Unfortunately endothelial function was not quantified by previous authors (Jakulj et al. 2007; Sauder et al. 2012; Faulk and Bartholomew 2012), so it is not possible to determine whether this factor played a role in their results.

6.5.3 Time course of the postprandial response

Notably, this is the first study to examine the interaction of meal and stress responses at several time points in the postprandial period; this is important as metabolic parameters continue to change over several hours post-meal (Table 6-2). We predicted that any differences between meals would be greatest at 3-4 hours postprandial when blood lipids are expected to peak (Parks 2001). While the anticipated postprandial changes in triglycerides, glucose and insulin did occur (Table 6-2), they did not appear to moderate postprandial stress responses. The magnitude of postprandial lipemia in this study was similar to that in the previous study in which no impact of fat consumption on stress reactivity was observed (Sauder et al. 2012).

To further probe a potential impact of postprandial lipemia we examined the correlation between postprandial lipemia and MAP reactivity. We found no relationship between these variables, and also no significant difference between MAP reactivity during the task that coincided with the greatest lipemia in the HFM and the MAP reactivity in the corresponding task number in the LFM condition. This suggests that the range of postprandial lipemia values observed did not have an impact on reactivity. The studies in which reactivity was found to be enhanced post-fat consumption did not measure blood lipids (Jakulj et al. 2007; Faulk and Bartholomew 2012), and since there can be wide variability in post-meal lipid levels (Bansal et
it is possible that those subjects had a greater postprandial lipemia which had an impact on reactivity. Endothelial dysfunction, one of the mechanisms thought to connect high fat meal intake and reactivity, has been shown to be exacerbated with greater postprandial lipemia (Gaenzer et al. 2001), and on average the highest lipemia levels in that report were substantially greater than those in the present study. Future work will be required to determine whether there is a threshold level of lipemia that is obligatory for an impact on reactivity to be manifest, and/or whether there is a dose-response relationship between lipemia and heightened stress responsiveness if higher levels of lipemia are reached.

6.5.4 Meal selection

With respect to the meals utilized in the present study: 1) similar meals have previously been found to enhance hemodynamic reactivity (Jakulj et al. 2007), 2) these same meals have been used in studies investigating other acute cardiovascular responses [i.e. investigation of postprandial endothelial function, (Plotnick et al. 1997; Vogel et al. 1997)], and 3) they are ecologically valid. These meals were previously criticized for their differences in protein content, with the rationale that the greater protein quantity in the HFM could promote enhanced reactivity by increasing blood viscosity (and therefore potentially increasing hemodynamic responses secondary to an impact on vascular resistance) and/or by stimulating the renin-angiotensin pathway and increasing angiotensin (promotes vasoconstriction) and aldosterone (increases sodium and water retention) with subsequent increases in blood pressure (Dickson et al. 2007). In the present study however, blood viscosity did not differ between the meal conditions and thus did not influence the results. In addition, while plasma volume was marginally higher in the HFM versus LFM condition (~57.2 vs 55.9%), suggesting greater activity of the renin-angiotensin pathway, this was not accompanied by enhanced stress responsiveness, and plasma volume was actually either unchanged or reduced from fasting levels in the postprandial period.
6.5.5 Habituation of stress responses

In order to investigate stress responses over time, the stress tasks employed must evoke measurable and sustained responses in the study participants. In the present study there was evidence of minimal habituation in hemodynamic responses to varied mental stress tasks performed 8 times over 2 laboratory visits. Only HR reactivity and recovery were affected by Time, although there were no specific differences identified in post hoc testing. An explanation for this non-systematic effect of Time is not obvious, although it may have been generated by variability in responses secondary to the variety of stress tasks presented. Overall, these data demonstrate sustained stress responses over repeated stress tasks, and suggest that the psychological stress tasks utilized in this study can be used to activate physiological stress responses reliably on two separate days in future studies.

6.5.6 Limitations

This study had a small sample size that consisted of young, healthy men. The variability in responses was greater than some previous observations (Faulk and Bartholomew 2012) and this lowers the power to detect small differences between meals. However, opposite to our hypothesis, we observed a larger MAP reactivity in the LFM condition that almost reached significance (P=0.064). This suggests that the small sample size does not account for the discrepancy with previous work, because it is unlikely that enrolling more subjects would have reversed our observed trend to result in detecting significantly exaggerated reactivity in the HFM condition. These data in combination with the findings of Sauder et al. 2012 clearly indicate a lack of a consistent or robust amplifying impact of acute fat consumption on stress responses. Future studies examining the time course of meal and reactivity/recovery interactions in this population would benefit from a larger sample size in order to more conclusively detect the presence or absence subtle effects.
Importantly our results cannot be generalized to women or older/disease populations. For instance, there is evidence that diet modification has a greater impact on blood pressure in persons with hypertension versus normotension (Appel et al. 1997), and that post-stress recovery tends to be slowed with age (following years of repeated stress exposure; Steptoe and Marmot 2005), and therefore it is possible that older/clinical populations may be more vulnerable to an impact of fat consumption on stress responses.

Although we classified the meals in this study based on their fat content (HFM vs LFM), the meals differed in other components as well (e.g. sodium and protein). However, this does not alter the primary conclusion of the study, that meal composition did not significantly impact stress responsiveness. The aim of this study was to assess the acute effects of fat consumption on stress responses, and long-term effects of habitual diet cannot be inferred from the present data.

The stress tasks presented in this study may evoke less potent physiological responses than real-life stressors (Rozanski et al. 1999), and may limit the generalizability of these data to real-world cardiovascular function. However, the present study used similar stress tasks and elicited similar responses to previous work in which an impact of fat consumption on stress reactivity was identified (Jakulj et al. 2007; Faulk and Bartholomew 2012). Thus in the event that the stress tasks evoked smaller than real-life responses, the results of the present study in combination with the previous contrasting findings still suggest that consumption of a high-fat meal does not universally impact stress responses.

We did not include stress hormone reactivity or recovery analysis. In our previous investigation in these participants (Poitras et al. 2014) we reported baseline and peak cortisol (tasks 1-4) and norepinephrine (tasks 2 and 4) levels. That statistical analysis involved more conditions than reported here, however, the results did not support an impact of meal fat content on hormonal reactivity [calculation of hormone reactivity (peak – baseline) and statistical analysis with the conditions included in the present study (data not shown) confirmed no significant
impact of meal fat content]. These data provided the first insight regarding the interaction between fat consumption and hormonal reactivity to stress (Poitras et al. 2014) however future research is required to examine the impact of meal fat content on stress hormone recovery.

6.5.7 Conclusion

Contrary to some previous reports (Jakulj et al. 2007; Faulk and Bartholomew 2012), meal fat content did not impact hemodynamic reactivity to varied mental stressors. These data also provide the first evidence that meal fat content does not impact hemodynamic recovery from repeated mental stress tasks (examined over 4 postprandial hours). In addition, we did not detect a relationship between postprandial lipemia and blood pressure reactivity. This suggests that an impact of fat intake on stress responsiveness may not necessarily play a role in increasing cardiovascular risk in this population. Further research is required to investigate: 1) the impact of fat intake on stress responsiveness in older individuals and/or persons with underlying cardiovascular risk factors, 2) the potential differences with respect to acute versus chronic exposures to fat intake and mental stress.
6.6 References


Chapter 7

Lack of independent effect of type 2 diabetes beyond characteristic co-morbidities and medications on small muscle mass exercising muscle blood flow and exercise tolerance

Under review in the *Journal of Applied Physiology* with the following authors:

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7.1 Abstract

Persons with type 2 diabetes (T2D) are believed to have reduced exercise tolerance; this may be partly due to impaired exercising muscle blood flow (MBF). Whether there is an impact of T2D on exercising MBF within the typical constellation of co-morbidities (hypertension, dyslipidemia, obesity) and their associated medications has not been investigated. We tested the hypothesis that small muscle mass exercise tolerance is reduced in persons with T2D versus Controls (matched for age, BMI, fitness, co-morbidities, non-T2D medications) and that this is related to blunted MBF. Eight persons with T2D and 8 Controls completed a forearm critical force (fCF\textsubscript{impulse}) test as a measure of exercise tolerance (10 min intermittent maximal-effort forearm contractions; the average contraction impulse in the last 30 s quantified fCF\textsubscript{impulse}). Forearm blood flow (FBF; ultrasound) and mean arterial pressure (MAP; finger photoplethysmography) were measured; forearm vascular conductance (FVK) was calculated. Data are means ± SD, T2D vs Control. fCF\textsubscript{impulse} was not different between groups (136.9 ± 47.3 vs 163.1 ± 49.7 N·s, P=0.371), nor was the ∆FBF from rest to during exercise at fCF\textsubscript{impulse} (502.9 ± 144.6 vs 709.1 ± 289.2 ml/min, P=0.092), or its determinants ∆FVK and ∆MAP (both P>0.05), although there was considerable inter-individual variability. ∆FBF was strongly related to fCF\textsubscript{impulse} (r=0.727, P=0.002), providing support for the relationship between oxygen delivery and exercise tolerance. We conclude that small muscle mass exercising MBF and exercise tolerance are not impaired in representative persons with T2D versus appropriately-matched Controls. This suggests that peripheral vascular control impairment does not contribute to reduced exercise tolerance in this population.
7.2 Introduction

It is often stated that persons with type 2 diabetes (T2D) have exaggerated intolerance to exercise beyond what might be explained by sedentary behavior [for review see (46)] and that this contributes to poor adherence to physical activity, which is an important treatment modality (55, 59). Advocates for this view cite observations of slowed oxygen uptake kinetics and reduced peak oxygen consumption capacity ($\dot{V}O_2\text{peak}$) relative to (self-reported) activity-matched Controls (3, 25, 44, 45). Importantly however, evidence of reduced exercise tolerance when matched for $\dot{V}O_2\text{peak}$ is absent from the literature, and since $\dot{V}O_2$ (kinetics and peak) responds normally to exercise training in this population (46) it is unclear how much of the “impairment” in tolerance is due to relative inactivity versus deficiency that is inherent to the disease. While a single study reported that ratings of perceived exertion were greater after adjusting for relative work intensity in persons with T2D versus weight-matched Controls ($n=13$ per group), this was only true at a low-level work rate (~3 metabolic equivalents of task; METs), these ratings are subjective, and the underlying physiological mechanism was not elucidated (14).

If in fact exaggerated exercise intolerance is present in persons with T2D, it has been proposed that reduced exercising muscle blood flow and concomitant impaired oxygen delivery, factors which are known to increase fatigue progression (2, 20), are at least partly responsible (23, 25). Type 2 diabetes is characterized by vascular pathologies (13, 16, 34, 58) which could impact vascular responsiveness in exercising muscle, blunting oxygen delivery and exercise tolerance. Previous studies have investigated exercising muscle oxygen delivery either in persons with T2D who are co-morbidity- and medication-free compared to weight- and age-matched controls (4, 25) or who had medications discontinued during the study (23, 31, 65), or did not have control groups matched for co-morbidities and their related medications (18, 29, 60, 65). However, persons with T2D typically also present with obesity (53), hypertension (51), and/or dyslipidemia (6) and are
taking one or more associated medications, as well as medications for T2D, and as such findings from previous studies do not have ecological validity for this population.

Whether there is an impact of T2D on exercising muscle blood flow within the characteristic constellation of co-morbidities and medications has not been investigated. In addition, if impaired muscle blood flow is present in this population, the extent to which it impacts exercise tolerance is unknown. Reduced exercising muscle blood flow could be due to impaired peripheral vascular control (i.e. reduced vasodilation), but it could also be due to impaired cardiac function which might evoke an increase in sympathetic vasoconstriction in exercising muscle (49, 68), and thus investigation of peripheral vascular dysfunction specifically requires the use of a small muscle mass modality that would be unlikely to be limited by impaired cardiac function. Therefore the aim of this study was to assess small muscle mass exercising muscle blood flow and exercise tolerance in persons with T2D versus Control participants matched for age, body mass index (BMI), aerobic fitness, co-morbidities and non-T2D medications. We hypothesized that the T2D group would have reduced exercising muscle blood flow and that this would be associated with reduced exercise tolerance. If present, reduced muscle blood flow and associated impaired small muscle mass exercise tolerance would be one factor contributing to whole-body exercise intolerance in this population.

We addressed the limitations of the current literature by: 1) matching T2D and Control participants for peak aerobic capacity (and other potentially confounding factors including BMI and medication use), 2) utilizing a small-muscle mass exercise modality that enables isolation of peripheral vascular contributions to exercising muscle blood flow (49, 52), and 3) using a novel test of small muscle mass exercise tolerance that has been shown to be sensitive to differences in oxygen delivery: the forearm critical force (fCF_impulse) test (22).
7.3 Methods

7.3.1 Participants

Ten men with T2D and 10 matched Controls participated in this study. Participants were recruited via the Cardiac Rehabilitation Centre at Hotel Dieu Hospital (HDH; Kingston, Ontario, Canada), and all testing was completed in the interim between their screening for, and commencement of, this program. The important rationale for this approach to recruitment was to enable investigation of the impact of T2D within the constellation of prevalent co-morbidities and medications and to match Control participants for these characteristics in order to isolate an effect of T2D.

Inclusion criteria were the following: 1) BMI of 24-35 kg/m², 2) achievement of 4-10.0 METs on a symptom-limited graded treadmill exercise test, 3) clear presence or absence of T2D [T2D: fasting plasma glucose ≥ 7.0 mmol/L and/or oral glucose tolerance test (OGGT) 2 h plasma glucose ≥ 11.1 mmol/L; Control: fasting plasma glucose < 6.1 mmol/L and OGGT 2 h plasma glucose <7.8 mmol/L], and 4) successful completion of the forearm critical force test (see “Experimental Protocol” below). Participants were excluded from the study if they demonstrated: 1) Stage 3 (or more advanced) renal disease, 2) current smoking or smoking within the past 12 months, or 3) if they were taking exclusionary medications (β-blockers, nitroglycerine or other nitric oxide donors). Participants taking β-blockers were not excluded if they could safely withdraw these medications for 48 hours prior to testing, under medical supervision (Dr. Stephen LaHaye, Cardiologist at HDH) (n=1 in each group). Participants completed testing while continuing any other medications (Table 7-1). See Figure 7-1 for a flow-chart of participant recruitment.

Prior to the day of testing, participants visited the lab to be screened for clear blood velocity signals (Doppler ultrasound) and images (Echo ultrasound) of the brachial artery, and to
Figure 7-1. Flow-chart of participant recruitment.

Note that potential participants may have been deemed ineligible due to one or more criteria (therefore the n values for the reasons participants were “not eligible” do not sum to the total number of participants who were not eligible). *Other reasons for exclusion were: myocardial ischemia during exercise (n=1), Churg-Strauss syndrome (autoimmune vasculitis; n=1), heart transplant patient (n=1), age (n=2; 17 and 85 y), unable to do baseline assessment of aerobic fitness (n=3; due to: wheelchair, high blood pressure, musculoskeletal issues), participation vetoed by cardiologist without provided reason (n=2), impaired glucose tolerance (n=6). BMI = body mass index; CR = cardiac rehabilitation; fCF = forearm critical force; METs = metabolic equivalents.
perform a forearm critical force test (fCF\textsubscript{impulse}; described below) for purposes of familiarization (22). The study protocol was approved by the Health Sciences Human Research Ethics Board at Queen’s University, and participants gave written consent to participate on forms approved by this board.

7.3.2 Experimental protocol

Participants were instructed to abstain from taking anti-inflammatories within 48 hours of the study (excluding daily low-dose Aspirin; \(n=7\) in T2D Group, \(n=6\) in Control Group), from exercising within 24 hours, and from consuming alcohol or caffeine for 12 hours and food for 3 hours prior to the testing session (61). Data collection was performed in a quiet, temperature-controlled room (19-23.5°C).

Upon arrival at the laboratory, participants were positioned supine with the exercising (left) arm extended 90° at heart level. Participants performed 3 maximal effort isometric forearm handgrip contractions using a handgrip dynamometer, each separated by 1 minute of rest. The largest force was taken to represent the maximum voluntary contraction (MVC). Participants then rested for \(~10-20\) minutes to allow their hemodynamic parameters to return to resting levels (confirmed by brachial artery blood velocity measurement of \(\leq5\) cm/s). Following instrumentation, participants performed the fCF\textsubscript{impulse} test.

7.3.2.1 Forearm critical force (fCF\textsubscript{impulse}) test

The fCF\textsubscript{impulse} test was conducted as previously described (22). Briefly, after 1 minute of resting baseline measures, participants performed MVCs in time with a metronome (1 s contraction: 2 s relaxation) for 10 minutes. A Powerlab oscilloscope display provided continuous force output feedback (Powerlab, AD Instruments; Figure 7-2 A), and the MVC target force was identified on the screen. Participants were not given feedback on elapsed time, but were given verbal encouragement and coaching (i.e. reminded to use the muscles of the forearm only, to relax between contractions, and to maintain the appropriate cadence).
Figure 7-2. Force output during the fCF\textsubscript{impulse} test for a representative participant. (A) Raw handgrip force tracing. Inset shows 3 contractions (shading indicates computation of the force impulse as the area under the curve). (B) Force impulse. Each dot represents the area under the curve of a single contraction. Dashed box indicates that the curve fit was used to calculate the average force impulse during the last 30 s of the test; this value is the fCF\textsubscript{impulse}. The area between the fCF\textsubscript{impulse} and the curve fit is the W'.
Achievement of a valid test result is contingent on the participant having a high degree of motivation and a willingness to give maximum effort for each handgrip contraction. Even with prior test familiarization and persistent encouragement, 2 participants in each group performed poorly on the test (i.e. did not deliver maximal effort with each contraction, resulting in repeated cycles of declines in force production followed by recovery) and failed to reach the requisite plateau in force production. These participants were excluded from the study (data not shown; final $n=8$ per group).

7.3.3 Measurements

7.3.3.1 Anthropometric measurements, blood sampling and analysis

Anthropometric measures (height, weight, BMI, waist circumference) and blood samples for determination of fasting plasma glucose, HbA1c, triglycerides, and total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were taken during the participants’ screening process for the Cardiac Rehabilitation Program at HDH. A symptom-limited graded maximal effort exercise tolerance test was performed on a treadmill (ramp protocol) and the maximum metabolic equivalents of task (METs) achieved were calculated based on the final treadmill speed and grade. Forearm volume (ml) was measured via water displacement in a custom volumeter, and forearm girth (cm) was measured with a tape measure at the point of greatest forearm circumference.

7.3.3.2 Mean arterial blood pressure (MAP)

MAP was measured continuously with finger photoplethysmography on the non-exercising arm positioned at heart level (Finometer MIDI, Finapres Medical Systems).
7.3.3.3 **Brachial artery diameter**

Images of the brachial artery were obtained using a 10 MHz linear Echo ultrasound probe (Vivid i2, GE Medical Systems), operating in two-dimensional B-mode, and recorded continuously in Digital Imaging and Communications in Medicine (DICOM) format.

7.3.3.4 **Brachial artery mean blood velocity**

Mean blood velocity (MBV) was measured distal to the Echo probe placement with a 4 MHz Doppler ultrasound probe (Multigon 500B, Transcranial Doppler, Multigon Industries). The corresponding voltage output was recorded continuously at 200 Hz in the data acquisition software program LabChart (ADInstruments) for later analysis. To quantify absolute mean blood velocity the ultrasound probe was calibrated as follows. Briefly, the ultrasound probe was immersed in a water bath and positioned to insonate tubing of known internal diameter (angle of insonation when probe is parallel to the tube is 70°). Water with ultrasound-reflecting particles (corn starch) was pumped through the tubing at a series of known flow rates (measured volumetrically as the rate of change of fluid volume in a collecting container) representing actual mean velocities of ~2-120 cm/s. The voltage output was then plotted against the known mean flow velocity, giving a linear calibration slope ($r^2=0.98$). This procedure was repeated with the ultrasound probe positioned at different insonation angles, manipulated to achieve 1° increments with a range of 70 +/- 15°, representing the observed range in brachial artery blood vessel orientation relative to the skin. For each participant, the brachial artery was imaged at the site of Doppler probe placement to quantify the actual angle of insonation (using an on-screen protractor), and the appropriate voltage-to-velocity calibration was applied prior to data analysis. This enabled accurate absolute blood velocity measurements allowing for between-participant comparisons.
7.3.3.5 Handgrip force

Handgrip force was obtained using an electronic handgrip dynamometer connected to a data acquisition system (Powerlab, ADInstruments) and recorded on a personal computer (LabChart, ADInstruments).

7.3.4 Data analysis

7.3.4.1 Forearm critical force \( (fCF_{\text{impulse}}) \)

The area under the curve (AUC) of the handgrip force tracing for each forearm muscle contraction was computed (in kg·s) and multiplied by gravitational acceleration (9.8067 m/s\(^2\)) to give the time-tension integral (i.e. the “force impulse”) in Newton-seconds (N·s). The force impulse data were then fit with nonlinear regression (exponential decay; SigmaPlot 11.0, Systat Software Inc.). The regression equation was used to calculate the average force impulse in the last 30 s (~10 contractions) of the exercise test, and this value was taken as the \( fCF_{\text{impulse}} \) (Figure 7-2 B). Our lab has demonstrated excellent trial-to-trial repeatability of this test [test-retest coefficient of variation (CV) 6.7%] (22).

7.3.4.2 Curvature constant of the force-time relationship \( (W') \)

The \( W' \) constitutes the maximum amount of work that can be performed above the critical force and primarily represents a fixed anaerobic energy reserve (17). The \( W' \) was calculated as the excess impulse above \( fCF_{\text{impulse}} \) for all contractions (Figure 7-2 B). This was derived by subtracting the calculated \( fCF_{\text{impulse}} \) from the force impulse predicted by the line of best fit for each contraction during the test, and summing these values. This is conceptually equivalent to subtracting the AUC of a horizontal line plotted at \( y = fCF_{\text{impulse}} \) from the AUC of the regression equation fit to the force impulse data (from 0 to 600 s).
7.3.4.3 MBV and MAP

After applying the appropriate voltage-to-velocity calibration, the data collected at 200 Hz were filtered using a low-pass filter with a cut-off frequency of 0.2 Hz (lowpass.xfm, SigmaPlot 11.0, Systat Software Inc.) to eliminate higher frequency noise (i.e. higher frequencies associated with muscle contraction and cardiac cycle) (9). The data were then resampled at 10 Hz (i.e. from the 200 Hz data set, every 20<sup>th</sup> datum was retrieved). Resampling was necessary to reduce the overall number of data points (from ~132,000 to 6,600) in order to fit the data within the constraints of the software fitting algorithm capacity. Parameter estimates have been shown to be unaffected by resampling at 10 Hz (9). MAP data were similarly sampled at 200 Hz, filtered and resampled.

7.3.4.4 Brachial artery diameter

Vessel diameter was quantified at 30 second intervals using automated wall tracking as previously described (42). Diameter data were then plotted over time and an exponential or sigmoidal line of best fit was determined, depending on the type of diameter response profile observed (SigmaPlot 11.0, Systat Software Inc.). Using the curve fit equation, diameter values were calculated for each time point at which blood velocity values were obtained. This approach minimizes the effect of random diameter measurement error on calculated blood flow.

7.3.4.5 Forearm blood flow (FBF), forearm vascular conductance (FVK) and MAP

FBF was used as a surrogate for oxygen delivery and was calculated according to the formula:

(Eq. 1) \( FBF = MBV \times 60 \, s/min \times \pi \times (diameter/2)^2 \)

where the FBF is in ml/min, the MBV is in cm/s, and brachial artery diameter is in cm. Intravenous catheterization and measurements of blood oxygen content (CaO<sub>2</sub>) were not employed in the present study to calculate oxygen delivery directly, since our lab previously demonstrated that changes in FBF accounted for almost all of the between-participant differences
in oxygen delivery in fCF_{impulse} tests (forward stepwise multiple regression was used to evaluate the independent contributions of FBF and CaO_2 to oxygen delivery, and the change in r^2 by their addition to the model was 0.944 and 0.0515 respectively; total r^2=0.996, P<0.001) (21). FVK was calculated as:

(Eq. 2) \[ FVK = \left( \frac{FBF}{MAP} \right) \times 100 \]

where FVK is in ml/min/100mmHg such that the values for FVK are quantitatively similar to those for FBF.

Where measurement error occurred (due to compromised signal), data was removed. FBF, FVK and MAP data were curve-fit with two-component exponential non-linear regression (Figure 7-3; SigmaPlot 11.0, Systat Software Inc.). For one participant in the T2D group, a one-component exponential curve was fit to the MAP data, and since the FBF and FVK showed a visible decline after the initial two-component increase, this part of the data was fit separately with linear regression. The curve fit equations were used to calculate the average FBF, FVK and MAP at rest (“baseline”; 1 min average) and in the last 30 s of the test (i.e. “steady state” hemodynamic values at the time of fCF_{impulse}), and the change from baseline to steady state was calculated (i.e. ΔFBF, ΔFVK, ΔMAP). The AUC of the FBF curve fit was computed as a measure of the total oxygen delivered during the fCF_{impulse} test (including the resting baseline).

7.3.4.6 Contributions of ΔFVK and ΔMAP to ΔFBF

To determine how the increases in FBF (ΔFBF) during the fCF_{impulse} tests were achieved (i.e. from baseline to steady state), the proportional contributions of changes in FVK and MAP were calculated as follows:

(Eq. 3) \[ ΔBF_{due to ΔFVK} = \left( \frac{ΔFVK \times MAP_{baseline}}{ΔFBF} \right) \times 100 \]

(Eq. 4) \[ ΔBF_{due to ΔMAP} = 100 - ΔBF_{due to ΔFVK} \]
Figure 7-3. Hemodynamic response at rest and during the fCF_idle test in a representative participant. (A) FBF, (B) FVK, (C) MAP. Dashed lines indicate the start of exercise. Curve fits were used to determine parameter values at baseline and during the last 30 s of the test. AUC of the FBF curve fit was used as a measure of “total” oxygen delivery during the test (including the resting baseline).
In other words, we calculated how much FBF would have changed if only FVK increased (assuming MAP was constant) as a proportion of the actual increase in FBF, with the remainder of the change in FBF being attributed to the increase in MAP.

### 7.3.4.7 Vasodilatory capacity

In addition, to quantify the vasodilatory “capacity” (i.e. independent of the impedance effect of muscle contraction and/or the potential immediate muscle relaxation-induced negative venous pressure), we examined the equivalent of a cardiac cycle in the relaxation phase between contractions that was unaffected by either muscle contraction or relaxation (FBF\textsubscript{relax}, FVK\textsubscript{relax}; Figure 7-4) (64). To quantify the impact of muscle contraction, we also assessed the FBF and FVK during the contraction phase of the duty cycle (FBF\textsubscript{contract}, FVK\textsubscript{contract}). These assessments were done during the final 30 s of the test (i.e. at the time of fCF\textsubscript{impulse}).

### 7.3.5 Statistical analysis

Unpaired t-tests were used to compare the effect of Group (T2D, Control) on participant characteristics and on baseline hemodynamic parameters (FBF, FVK, MAP). Data for ∆FBF, FBF\textsubscript{relax}, ∆FVK and FVK\textsubscript{relax} were log-transformed (natural log); this was: a) since the data were bound by zero, b) since the data were positively skewed, and c) to allow a multiplicative interpretation. Unpaired t-tests were performed to compare the effect of Group on the primary [fCF\textsubscript{impulse}; ∆FBF and FBF\textsubscript{relax} (raw and log-transformed)] and secondary [total FBF; ∆FVK and FVK\textsubscript{relax} (raw and log-transformed); FBF\textsubscript{contract}, FVK\textsubscript{contract}; ∆MAP; ∆FBF\textsubscript{due to ∆FVK} and ∆FBF\textsubscript{due to ∆MAP}; and W’] outcome variables. Transforming the data did not change the statistical interpretation; raw (non-transformed) data are shown in figures for ease of interpretation with associated P-values from the transformed data statistical analysis. The reverse of the log-transformation was applied (exponentiation) to show the mean fold-difference between groups. Bootstrapping (10,000 bootstrap samples) was used to obtain valid 95% confidence intervals (CI; bias corrected accelerated) without relying on the assumption of normality. Linear regression
Figure 7-4. Mean blood velocity across the duty cycle of handgrip contraction and relaxation for a representative participant.

Note that during contraction the mean blood velocity is transiently negative, indicating retrograde flow. Boxes identify the cardiac cycles used to assess the relaxation-phase FBF and FVK (FBF_{relax} and FVK_{relax}). In this participant, FBF at the time of fCF_{impulse} from the curve fit (i.e. FBF across the duty cycle) was 910 ml/min, while FBF_{relax} (i.e. single cardiac cycles of unimpeded flow) was 1,058 ml/min and FBF_{contract} was 71.5 ml/min.
Data for one participant in the T2D group was removed from comparisons involving fCF\textsubscript{impulse} (between groups and in regression analysis) since: a) the fCF\textsubscript{impulse} had a z-score >2, b) in fCF\textsubscript{impulse} vs FBF regressions the standardized residual was >2, and c) most importantly, an fCF\textsubscript{impulse} of this magnitude would be highly unlikely given the participant’s small FBF magnitude and the known dependency of fCF\textsubscript{impulse} on oxygen delivery (21). This participant’s quantified fCF\textsubscript{impulse} therefore likely represents the result of a handgrip position that altered the mechanical advantage (such that the measured contraction force was achieved with lower actual muscle contraction force) (22), and thus this participant’s data was excluded from statistical comparisons and is not included in the group mean values (but is still plotted with distinct data points). Statistical significance was set at P<0.05, and all statistics were calculated using IBM\textsuperscript{®} SPSS\textsuperscript{®} Statistics (version 22; SPSS Inc.). All data are expressed as means ± SD.

### 7.4 Results

#### 7.4.1 Participant characteristics

Participant characteristics are shown in Table 7-1. The T2D and Control groups did not differ with respect to age, height, weight, BMI, waist circumference, fasting blood lipids, METs achieved on a graded treadmill exercise test, MVC, forearm volume and circumference, and number of non-T2D medications (all P>0.05). As expected, the T2D group had greater fasting plasma glucose (P=0.001) and HbA1c levels (P=0.009) versus Control.

#### 7.4.2 fCF\textsubscript{impulse} and W’

Force output for a representative participant is shown in Figure 7-2. fCF\textsubscript{impulse} was not significantly different between groups (P=0.317; mean difference 26.2 N·s, 95% CI: -16.8 to 69.9
N·s) (Figure 7-5). W’ was not different between T2D and Control groups (5,583.4 ± 3,588.3 vs 4,848.8 ± 2,420.0 N·s respectively; P=0.639).

7.4.3 Hemodynamic parameters – FBF, FVK, MAP

Hemodynamic parameters at rest and during the fCF\textsubscript{impulse} test for a representative participant are shown in Figure 7-3. No hemodynamic parameters differed between groups at baseline (for FBF, FVK and MAP: P=0.359, P=0.460, P=0.542; Figure 7-6 A, Figure 7-7 A, Figure 7-8 A).

7.4.3.1 FBF (Figure 7-6)

The increase in FBF from baseline to during fCF\textsubscript{impulse} (ΔFBF) was not significantly different between groups (P=0.092) although there was considerable variability in responses. On average, the ΔFBF in the T2D group was 0.727 times that in the Control group (95% CI: 0.511 to 1.02 times). The ΔFBF was achieved primarily via an increase in FVK; overall the proportional contributions of ΔFVK and ΔMAP to ΔFBF were 73.7 ± 6.9% and 26.3 ± 1.7% respectively, with no differences between groups (both P=0.256; Figure 7-6 B).

Similarly, the absolute FBF during the relaxation phase of the duty cycle (FBF\textsubscript{relax}) was not significantly different between groups (P=0.368; mean fold-difference 0.831; 95% CI for fold-difference between groups 0.553 to 1.212), nor was FBF during the contraction phase (FBF\textsubscript{contract}; P=0.647; Figure 7-4, Figure 7-6 C, D).

Total FBF during the test was not different between groups (5,629.4 ± 2,148.0 vs 6,873.77 ± 3,068.1 ml for T2D vs Control; P=0.363).

7.4.3.2 FVK (Figure 7-7)

The ΔFVK was not significantly different between groups (P=0.095; mean fold-difference between groups 0.735; 95% CI: 0.513 to 0.994; Figure 7-7 B). FVK\textsubscript{relax} was not different between groups (P=0.399), nor was FVK\textsubscript{contract} (P=0.762; Figure 7-7 C, D).
7.4.3.3 MAP (Figure 7-8)

On average, MAP was 89.0 ± 10.6 mmHg at baseline and increased to 116.9 ± 18.6 mmHg at the time of fCF\textsubscript{impulse}; the increase was not different between groups (P=0.259).

7.4.4 Linear regressions

Absolute FBF at fCF\textsubscript{impulse}, ∆FBF, FBF\textsubscript{relax}, and total FBF were significantly related to fCF\textsubscript{impulse} (all P<0.05; Figure 7-9, Table 7-2).
### Table 7-1. Participant characteristics

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<th>Control Group</th>
<th>T2D Group</th>
<th>P value</th>
</tr>
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<tr>
<td>Age, y</td>
<td>62.6 ± 10.7</td>
<td>61.8 ± 8.9</td>
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<tr>
<td>Diabetes Duration, y</td>
<td>--</td>
<td>6.9 ± 6.2</td>
<td>--</td>
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<td>Height, cm</td>
<td>180.0 ± 6.6</td>
<td>175.1 ± 5.3</td>
<td>0.122</td>
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<tr>
<td>Weight, kg</td>
<td>96.0 ± 19.6</td>
<td>97.7 ± 16.4</td>
<td>0.861</td>
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<td>BMI, kg/m²</td>
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<td>31.8 ± 4.4</td>
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<td>WC, cm</td>
<td>104.6 ± 9.8</td>
<td>109.8 ± 12.6</td>
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<td>Fasting plasma glucose, mmol/L</td>
<td>5.1 ± 0.4</td>
<td>8.1 ± 2.0</td>
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<td>HbA1c, %</td>
<td>5.6 ± 0.4</td>
<td>7.3 ± 1.6</td>
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<td>Fasting triglycerides, mmol/L</td>
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<td>1.6 ± 1.1</td>
<td>0.767</td>
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<td>Total Cholesterol, mmol/L</td>
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<td>HDL Cholesterol, mmol/L</td>
<td>1.1 ± 0.3</td>
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<td>LDL Cholesterol, mmol/L</td>
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<td>Forearm Volume, ml</td>
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<td>Number of non-T2D medications</td>
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### Number (n) using medications:

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<th>T2D Group</th>
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<td>COX Inhibitor (Aspirin)</td>
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<tr>
<td>ACE Inhibitor</td>
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<tr>
<td>Angiotensin II receptor blocker</td>
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<tr>
<td>HMG-CoA Reductase Inhibitor (“statin”)</td>
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<td>8 (5 hydrophilic; 3 lipophilic)</td>
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<td>Thienopyridine (or derivative; “anti-platelet”)</td>
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<tr>
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### Cardiovascular Health History (n)

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<td>2</td>
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</tr>
<tr>
<td>History of cardiomyopathy</td>
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<td>1</td>
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</tr>
<tr>
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<tr>
<td>Transient ischemic stroke</td>
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Values are means ± SD. ACE = angiotensin-converting-enzyme; BMI = body mass index; COX = cyclooxygenase; GXT = graded exercise test (treadmill); HDL = high density lipoprotein; HMG-CoA Reductase = 3-hydroxy-3-methyl-glutaryl-coA reductase; LDL = low density lipoprotein; METs = metabolic equivalents; MVC = maximum voluntary contraction; WC = waist circumference. *P<0.05 compared with Control Group.
Figure 7-5. Forearm critical force.

Data are means (excluding outlier in T2D group) ± SD, with individual participant points (grey point = outlier excluded from analysis).
Figure 7-6. Forearm blood flow.

(A) Absolute FBF at rest and during the last 30 s of the fCF_impulse test (“steady state”) in the Control (grey bars) and T2D (white bars) groups. Lines represent individual participant data (solid = Control participants; dashed = T2D participants). (B) Change in FBF from resting baseline to the last 30 s of the fCF_impulse test. White shading represents the predicted ∆FBF if only FVK changed (MAP constant), with the remaining grey shading due to the addition of a change in MAP. Inset percent values indicate the proportional contribution of ∆FVK to ∆FBF. (C) Absolute FBF_relax and (D) FBF_contract during the last 30 s of the fCF_impulse test. Data are means ± SD; closed symbols = Control group, open symbols = T2D group.
Figure 7-7. Forearm vascular conductance.

(A) Absolute FVK at rest and during the last 30 s of the fCF_impulse test (“steady state”) in the Control (grey bars) and T2D (white bars) groups. Lines represent individual participant data (solid = Control participants; dashed = T2D participants). (B) Change in FVK from resting baseline to the last 30 s of the fCF_impulse test. (C) Absolute FVK_{relax} and (D) FVK_{contract} during the last 30 s of the fCF_impulse test. Data are means ± SD; closed symbols = Control group, open symbols = T2D group.
Figure 7-8. Mean arterial pressure.
(A) Absolute MAP at rest and during the last 30 s of the fCF\textsubscript{impulse} test ("steady state") in the Control (grey bars) and T2D (white bars) groups. Lines represent individual participant data (solid = Control participants; dashed = T2D participants). (B) Change in MAP from resting baseline to the last 30 s of the fCF\textsubscript{impulse} test. Data are means ± SD; closed symbols = Control group, open symbols = T2D group.
Figure 7-9. Relationship between the increase in FBF during the fCF impulse test and fCF impulse. \( \Delta \text{FBF} = \) change in FBF from baseline to steady state. Squares are group means ± SD; circles are individual participant points (closed symbols = Control; open symbols = T2D; grey data point = outlier excluded from analysis).

Table 7-2. Linear regressions: Relationship between the following variables and fCF impulse

<table>
<thead>
<tr>
<th>Variable</th>
<th>( r )</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute FBF at steady state (ml/min)</td>
<td>0.690</td>
<td>0.476</td>
<td>0.004</td>
</tr>
<tr>
<td>( \Delta \text{FBF} ) (ml/min)</td>
<td>0.727</td>
<td>0.528</td>
<td>0.002</td>
</tr>
<tr>
<td>( \text{FBF}_{\text{relax}} ) (ml/min)</td>
<td>0.664</td>
<td>0.441</td>
<td>0.007</td>
</tr>
<tr>
<td>Total FBF (ml/min)</td>
<td>0.663</td>
<td>0.440</td>
<td>0.007</td>
</tr>
</tbody>
</table>

\( n=15 \) (outlier excluded from regression analysis)
7.5 Discussion

The objective of this study was to test the hypothesis that T2D, when present in an ecologically valid constellation of co-morbidities and medications, compromises small muscle mass exercise tolerance and that this is related to blunted exercising muscle blood flow. Contrary to our hypothesis, the major findings of this study are: 1) small muscle mass exercise tolerance ($fCF_{impulse}$) was not impaired in T2D relative to matched Controls, and 2) there was no difference in forearm muscle blood flow (or its determinants, FVK and MAP) during exercise at $fCF_{impulse}$ between T2D and Control groups, with considerable variability between individuals. These data also provide evidence for a strong relationship between oxygen delivery and exercise tolerance ($fCF_{impulse}$).

7.5.1 Use of the forearm critical force ($fCF_{impulse}$) test to assess exercise tolerance

Traditionally, small muscle mass exercise tolerance is assessed with one of two models: “time to exhaustion” at a given intensity, or a progressive exercise test to the limit of tolerance. The former is subject to considerable variability however (33), and the latter only identifies “capacity” without an indication of submaximal tolerance per se. In contrast, the $fCF_{impulse}$ test is a small muscle mass analogue of the whole body critical power test [reviewed in (17)] which can expose differences in submaximal exercise tolerance that are not revealed by a test of peak aerobic capacity (36). The $fCF_{impulse}$ test was recently validated in our laboratory (22). As previously described (Methods), the $fCF_{impulse}$ test comprises 10 minutes of intermittent maximal effort forearm contractions, during which time the amount of force the participant can produce declines exponentially to a reproducible plateau [test-retest CV 6.7% (22)], referred to as the “forearm critical force”.

This level of force production is physiologically relevant as it represents the maximal force production that can be sustained while maintaining a stable metabolic environment (i.e. a plateau in PCr, [lactate], pH and $\dot{V}O_2$) (17). In other words, the $fCF_{impulse}$ is the maximal force
production at which aerobic metabolism completely matches metabolic demand without requiring subsidization by substrate level phosphorylation, and as such represents the boundary between the “high-intensity” and “severe-intensity” exercise domains (intensities below the critical force are sustainable, while intensities above the critical force result in a short time to exhaustion) (17). Since the fCF
impulse represents the boundary between tolerable and intolerable exercise, it can be taken as a measure of exercise tolerance; the higher the fCF
impulse, the lower the relative intensity of any given exercise intensity below fCF
impulse.

Since oxygen delivery is a crucial determinant of an individual’s fCF
impulse (21), quantifying FBF during this test allows for between-group comparisons of “exercise tolerance” (fCF
impulse) in relation to FBF.

7.5.2 Small muscle mass exercise tolerance (fCF
impulse) in T2D

This is the first study to our knowledge to examine the impact of T2D on small muscle mass exercise tolerance when accompanied by the typical constellation of co-morbidities and associated medications in this population. Contrary to our hypothesis, fCF
impulse was not different between persons with T2D and matched Controls. In addition, W’ (the curvature constant of the force-time relationship) was not different between groups, indicating that the finite amount of work that could be performed above fCF
impulse was also not affected by T2D. These findings have functional significance since small muscle mass exercise tolerance is central to conducting activities of daily living.

Evidence for impaired exercise tolerance in T2D comes primarily from observations of reduced peak oxygen consumption capacity (\(\tilde{\text{VO}_2}\)peak; ~20% reduction versus controls matched for age, BMI, and self-reported physical activity level) (3, 25, 44, 45). However, this evidence is potentially problematic since self-reported activity has limited reliability and validity (54), and since overweight/obese populations are known to systematically overestimate levels of physical activity (41). Indeed, objective measures of physical activity (accelerometry) confirmed that
among sedentary adults (<1h/wk of moderate intensity physical activity), those with T2D spent significantly more time being inactive and less time at all levels of intensity (light, moderate, vigorous) than those without T2D (matched for age and BMI) (39). Thus it is possible that the findings of impaired peak aerobic function in T2D are due to a more sedentary lifestyle (46, 62) versus disease-related dysfunction. In the present study, the T2D and Control groups were matched for VO$_{2}$peak (maximal METs achieved on a graded treadmill exercise test) in order to remove fitness as a potential confounder. The current findings do not support the hypothesis that T2D, on top of the typical constellation of co-morbidities present in this population, impairs small muscle mass exercise tolerance.

7.5.3 Exercising muscle oxygen delivery in T2D – muscle blood flow

FBF during exercise at fCF$_{impulse}$ did not differ between T2D and Control groups in the present study, and the similar FBF was achieved via similar FVK and MAP responses (Figure 7-6, Figure 7-7, Figure 7-8). This was confirmed by examining FBF and FVK in terms of the mean response, as well as specific to relaxation and contraction phases of the duty cycle (the relaxation phase being an indicator of the vasodilatory response, and the contraction phase being a gauge of the mechanical impedance to flow during the maximal effort test), and the total FBF across the test (AUC).

These results were unexpected given the evidence for impaired vasodilatory mechanisms in T2D (13, 16, 34, 58) and the findings of two recent studies which formed the basis for our hypothesis (23, 25). First, Kingwell et al. (23) observed reduced steady state leg blood flow (LBF) in persons with T2D versus Controls (matched for age, VO$_{2}$peak and body weight) during moderate-intensity exercise (25 min supine cycling at 60% VO$_{2}$peak). Similarly, Lalande et al. (25) demonstrated that steady state LBF, albeit indexed to lean thigh mass, was significantly lower in T2D participants versus healthy Controls (matched for age, BMI and fat-free mass) during low-intensity leg-kicking exercise. In contrast to these previous investigations however,
the present study is the first to evaluate the impact of T2D on exercising muscle blood flow and exercise tolerance within the typical clustering of co-morbidities and medications in this population, and it is within this important context that we suggest possible explanations for the current findings.

1) One or a combination of the other co-morbidities may have equally impaired exercising muscle blood flow in both groups.

First, it is possible that one or more co-morbidities contributed to impaired FBF such that both groups were impacted to the same extent independent of T2D. For instance, chronic hypertension is associated with vascular remodeling (and increased media/lumen ratio), increased artery stiffness (15) and damaged capillaries (27), while dyslipidemia is known to result in heightened inflammation, impaired endothelial function (6) and atherosclerosis (30), all of which might be expected to impact exercising muscle blood flow. Similarly, obesity could compromise exercising muscle blood flow via myogenic, endothelium-dependent, and metabolic control mechanisms (12), although impairment is not always demonstrated (28).

Therefore, it may be that T2D has no independent effect, or that it has no additional effect when superimposed on the co-morbidities that usually accompany the disease (i.e. due to a “floor effect”). This hypothesis is supported by previous studies in which an impairment in persons with T2D was observed only when compared with lean (but not weight-matched) Controls (45, 50). In previous investigations in which control participants were not matched for these characteristics (18, 65) the conclusion of an impact of T2D per se may have been confounded by the presence of clinical sequelae (i.e. spurious associations due to co-morbid conditions but falsely attributed to T2D). While T2D may have an independent effect when unaccompanied by co-morbidities (4, 25), it should be recognized that these otherwise healthy individuals represent a minority of those with T2D. These possibilities do not alter the interpretation of the present study
however, which is that in the “average” person with T2D, exercising FBF is not reduced relative to an otherwise similar individual in the absence of T2D.

2) *Regular medications may moderate the impact of T2D on exercising muscle blood flow.*

We also elected to test participants without disruption of their regular medications. As previously mentioned, the important rationale for this was that the majority of persons with T2D take anti-diabetic, anti-hypertensive, and anti-hyperlipidemic agents (24, 51), making such participants representative of the general T2D population and improving the ecological and external validity of our findings.

For instance, up to 80% of persons with T2D also have hypertension (51), and of those more than 65% require ≥2 medications to achieve the target blood pressure of <130/85mmHg, with angiotensin-converting-enzyme (ACE) inhibitors being the first prescribed (57). Even in persons with T2D and “normal” blood pressure, ACE inhibitors are often prescribed since they are known to reduce the progression of renal (43) and cardiovascular (67) disease in this population (57). ACE inhibitors act by blocking the conversion of Angiotensin I to Angiotensin II (ANG II) in the plasma, which results in reduced sympathetic nervous system stimulation of the heart and blood vessels (i.e. reducing cardiac output and increasing vascular conductance), reduced blood volume (due to a reduced sodium absorption at the kidney), and improved endothelial function (5, 40). Angiotensin receptor-blockers have the same effects as ACE inhibitors but exert their effects by acting directly on the ANG II receptors (5). Similarly, statins are prescribed for treatment of dyslipidemia; they act to reduce LDL cholesterol, oxidative stress, and vascular inflammation, as well as increase the stability of atherosclerotic lesions (30) and improve endothelial function (63). Based on their mechanisms of action, both of these classes of medications might be anticipated to influence exercising muscle blood flow, and indeed this has been demonstrated for anti-hypertensives (7, 8, 66). Importantly, these medications were well-
matched between groups in the present study (Table 7-1) and thus any potential impacts would be exerted equally on both groups.

In contrast, in the T2D group there was the additional potential impact of T2D-specific medications. Metformin, a biguanide which acts primarily by suppressing hepatic glucose output, is the most common anti-diabetic agent and is prescribed either alone or in combination with a sulphonylurea (stimulates insulin secretion) or thiazolidinedione (improves insulin action via gene regulation) and eventually insulin as hyperglycemia progress (24, 38). Anti-diabetic agents are known to improve endothelial function (32) and there is some (limited) evidence that they may increase exercising muscle blood flow (10). The absence of anti-diabetic medications in the studies mentioned earlier could potentially explain the findings of impaired muscle blood flow in T2D, since Lalande et al. enrolled only medication-free participants (25), and Kingwell et al. included only two T2D participants who took anti-diabetic agents but completed testing after a 24 h drug-free period (23).

In the present study, 7 T2D participants were taking Metformin, with one also taking insulin. While these medications may therefore have contributed to the conserved FBF responses, we cannot estimate the magnitude of their effect (if any) (3). In addition, others have observed no impact of T2D on steady state muscle blood flow when medications were held for 48 h before testing (31), or impaired muscle blood flow when participants were tested while on their regular medications [only when HbA1c ≥ 8% (18)]. Thus, differences in medication status cannot fully account for differences in exercising muscle blood flow responses in T2D across studies.

3) The impact of T2D may depend on the active muscle mass and/or the specific vascular bed.

It is possible that there is an impairment in muscle blood flow during leg but not arm exercise in persons with T2D, as a result of differences in active muscle mass and/or possible differences in muscle blood flow regulatory mechanisms across vascular beds (37, 48).
Importantly however, we (unpublished observations) and others (29, 31, 56, 60) have found no impairment to steady state leg blood flow during low- to moderate-intensity leg exercise (comparable to that in Kingwell et al. and Lalande et al.) in persons with T2D versus Controls. This suggests that differences in muscle mass and/or vascular beds do not present a straightforward explanation for disparate findings across studies.

4) There may be different cohorts within persons with T2D [such that (dys)function exists along a continuum].

Lastly, we propose that some persons may be more susceptible to a negative impact of T2D than others, such that some exhibit diabetes-evoked impairment and some do not. This hypothesis is supported by the substantial inter-individual variability in FBF responses in the present study. In the T2D group, the range in ∆FBF responses from baseline to during exercise at fCF$_{\text{impulse}}$ was ~270 to 700 ml/min (i.e. the participant with the largest ∆FBF had an increase in FBF more than double that of the individual with the lowest ∆FBF, for a difference of 430 ml/min; see Figure 7-6 B). This between-participant range in responses is considerable and would be expected to translate to functional impairment. (Indeed, the difference in fCF$_{\text{impulse}}$ between these two participants was ~100 N·s). Thus while at the group level impairment was not observed, the large demonstrated variability in responses suggests that impaired and unimpaired cohorts may exist within this population, and this could explain some of the inconsistency in the literature. Note however that there was also large variability within the Control group, and thus the inter-individual differences may be moderated by factors other than T2D specifically. Future work will be required to identify whether different phenotypes exist and the specific conditions in which impairment does versus does not manifest.

7.5.4 Implications of preserved exercising FBF

Type 2 diabetes is characterized by both macro- and micro-vessel endothelial and smooth muscle dysfunction (34), impaired release of ATP from red blood cells (58), reduced
bioavailability of nitric oxide (16), and potentially heightened sympathetic nervous system activity (13), all of which would be anticipated to reduce exercising muscle blood flow. Given this evidence for vascular dysfunction in persons with T2D, the present finding of preserved FBF during exercise at fCF\textsubscript{impulse} was unexpected. It is known that there is considerable redundancy in mechanisms controlling exercise hyperemia (19, 26), and the present results suggest that compensatory responses may function to defend exercising muscle oxygen delivery in T2D.

7.5.5 The relationship between oxygen delivery and exercise tolerance

In the present study, a strong positive relationship was observed between FBF (whether expressed as absolute FBF, ∆FBF, FBF\textsubscript{relax}, or Total FBF) and exercise tolerance (fCF\textsubscript{impulse}) (Figure 7-9, Table 7-2). This is in agreement with previous observations in young, healthy participants (21) and suggests that differences in FBF (as determined by vasodilatory and pressor responses) may be an underlying mechanism explaining the inter-individual differences in exercise tolerance.

7.5.6 Limitations

In this study, statistical analysis of the increase in blood flow and vasodilation from rest for the T2D group versus the Control group resulted in P-values of P=0.092 and P=0.095 respectively. This raises the possibility of a type II error (i.e. failing to detect an effect in the sample that truly exists in the population), due to the limitation of a small sample size. However, additional analyses support a conclusion of no difference between groups, or at most, a clinically insignificant difference. First, when measuring the FBF and FVK during relaxation phases between contractions (i.e. un-confounded by the potential impact of contraction-induced movement artifact on the brachial artery blood velocity measures), the P-values for between-group comparisons were large (FBF\textsubscript{relax}, P=0.368; FVK\textsubscript{relax}, P=0.399), signifying a highly improbable difference between groups. This was also the case for total FBF (P=0.363). Furthermore, bootstrapping analysis achieved valid confidence intervals of the fold-difference in
responses between T2D and Control groups, and the confidence interval for ∆FBF crossed 1 (i.e. no difference between groups). Finally, individual variation in the increase in FBF was a strong predictor of individual variation in fCF_{impulse} \left( r^2 = 0.528, P=0.002 \right) for ∆FBF vs fCF_{impulse}, yet there was no difference between groups in fCF_{impulse} \left( P=0.317 \right), meaning that if there was an effect of T2D on the increase in FBF that we were unable to detect, it was so small as to have no effect on fCF_{impulse}. Moreover, post hoc power analysis indicates that in order to detect an effect of the magnitude reported in this study with the present variability (i.e. between groups difference in FBF_{relax}) with 80% power and α=0.05, we would need to enroll 69 participants per group. This is much larger than the sample size typically employed in studies of this nature [i.e. a sample size of 8-10 per group is standard, e.g. (23, 25, 31, 35)]. Thus the current sample size does not alter the important conclusion allowed by the present data: that considerable inter-individual variability exists but there are no consistent or robust differences at the group level in FBF or fCF_{impulse} responses. Findings cannot be generalized to the minority of T2D patients who do not have co-morbidities or who are not taking anti-hypertensive, anti-hyperlipidemic or anti-diabetic medications, or to women since there is some evidence that their exercise tolerance may be disproportionately reduced (1, 47).

We cannot exclude the possibility that some T2D patients may have declined to participate in the study on the basis of reduced exercise capacity (50), thereby biasing our study sample in that it would systematically underestimate the true level of exercise intolerance experienced in the general T2D population. This seems unlikely however since the number of participants who voluntarily excluded themselves was greater in the Control group \left( n=13 \right) than the T2D group \left( n=7 \right) (Figure 7-1). Similarly, the 4 patients who had invalid fCF_{impulse} tests and were excluded from analysis may have had reduced exercise capacity (i.e. leading to their poor performance on the test). However, since there were two participants with invalid tests per group, this is unlikely to represent any systematic effect of T2D. The fCF_{impulse} test requires participants
to have a high level of motivation and to give maximal effort. Measuring the force impulse allowed us to quantify the actual amount of work done, but there was variability in how well the test was executed.

The use of ultrasound quantifies brachial artery blood flow, however it may fail to detect microvascular pathology. For instance, Womack et al. observed an impairment in microvascular perfusion that was unaccompanied by a reduction in bulk flow during low- and high-intensity handgrip exercise (25% and 80% MVC) in persons with T2D and microvascular complications versus healthy Controls (65). While we cannot reject the possibility that an impairment in blood flow distribution occurred at the microvascular level in the present study, this seems improbable since no impact on exercise tolerance was observed.

Lastly, we collected our data in the fasted state and others have shown that exercise hyperemia is attenuated in obese, insulin-resistant participants when exercise is performed in hyperinsulinemic conditions (11). Thus it is possible that FBF and fCF\textsubscript{impulse} may have been differentially affected in the postprandial state.

### 7.5.7 Conclusions

The present study is the first to investigate the impact of T2D on small muscle mass exercising muscle blood flow and exercise tolerance within the typical constellation of comorbidities and medications that are present in this population. Contrary to our hypothesis, small muscle mass exercise tolerance and exercising muscle oxygen delivery were not impaired in representative persons with T2D (relative to Controls matched for age, aerobic fitness, BMI, comorbidities and non-T2D medications). This suggests that localized peripheral vascular control impairment does not contribute to reduced whole body exercise tolerance in this population. There was a strong positive relationship between FBF and fCF\textsubscript{impulse} suggesting that interindividual differences in exercising muscle oxygen delivery (as determined by the magnitude of the vasodilatory and pressor responses) are responsible for differences in exercise tolerance.
Given the large inter-individual variability in exercise hyperemia and exercise tolerance responses, future studies are warranted to more definitively establish the presence or absence of a T2D-specific impairment, and to determine whether these results can be generalized to women.
7.6 References


Chapter 8

Independent effect of type 2 diabetes beyond characteristic co-morbidities and medications on immediate but not continued knee extensor exercise hyperemia

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8.1 Abstract

We tested the hypothesis that type 2 diabetes (T2D), when present in the characteristic constellation of co-morbidities (obesity, hypertension, dyslipidemia) and medications, slows the dynamic adjustment of exercising muscle perfusion and blunts the steady state relative to Controls (matched for age, BMI, fitness, co-morbidities, non-T2D medications). Thirteen persons with T2D and 11 Controls performed rhythmic single-leg isometric quadriceps exercise (rest-to-6kg-to-12kg; 5 min at each intensity). Measurements: Leg blood flow (LBF, femoral artery; ultrasound), mean arterial pressure (MAP; finger photoplethysmography), leg vascular conductance (LVK; calculated). Dynamics were quantified using mean response time (MRT). Measures of amplitude were also used to compare response adjustment: the change from baseline to 1) the “peak initial response” (greatest 1 s average in first 10 s; ∆LBF_{PIR}, ∆LVK_{PIR}) and 2) the “on-transient” (average from curve fit at 15, 45, 75 s; ∆LBF_{ON}, ∆LVK_{ON}). ∆LBF_{PIR} was significantly blunted in T2D vs Control (P=0.037); this was due to a tendency for reduced ∆LVK_{PIR} (P=0.063). In contrast, the overall response speed was not different between groups (MRT, P=0.856; ∆LBF_{ON}, P=0.150) nor was the change from baseline to steady state (P=0.204). ∆LBF_{PIR}, ∆LBF_{ON} and LBF MRT did not differ between rest-to-6 kg and 6-to-12 kg workload transitions (all P>0.05). Despite a transient amplitude impairment at the onset of exercise, there is no robust or consistent impact of T2D, on top of the co-morbidities and medications typical of this population, on the overall dynamic adjustment of LBF or the steady state levels achieved during low- or moderate-intensity exercise.
8.2 Introduction

Type 2 diabetes (T2D) is one of the most common chronic diseases, and the growth in prevalence and incidence continues at unprecedented rates (5). Exercise is widely recognized as a critical component of the management of T2D (38, 40), with positive effects on blood glucose and lipid levels, blood pressure, cardiovascular events, mortality, and quality of life (5). However, exercise intolerance is considered a major complication of T2D (1, 13, 32), which may contribute to poor exercise adherence. Indeed, 60-80% of adults with T2D do not exercise sufficiently (31). This limits the use of exercise as a treatment modality in the long-term management of the disease.

Recent evidence indicates that this exercise intolerance may be due, at least in part, to impaired muscle blood flow (MBF) and compromised oxygen delivery (15, 17), factors which are known to hasten the development of fatigue (43). However, key knowledge gaps currently exist. First, in terms of the dynamics of the oxygen delivery response (i.e. adjustment of MBF to steady state), an impairment has been inferred from observations of slowed \( \dot{V}O_2 \) kinetics (2, 32) and a transient undershoot in muscle oxygenation at the onset of exercise in T2D (1, 27). However, these observations could also indicate impaired oxygen consumption, which could potentially occur in T2D (e.g. due to mitochondrial dysfunction (29)). Understanding the mechanistic basis of impairment is critical for the development of effective intervention strategies. In addition, the dynamics of the oxygen delivery response per se have only been examined in one study, and this was done in terms of vascular conductance (versus blood flow specifically) and only in the transition from rest-to-exercise at a single intensity (19). While an important initial investigation, our understanding remains limited because: 1) there is some evidence that \( \dot{V}O_2 \) kinetic impairment is intensity-dependent in T2D (32), 2) in young healthy persons some kinetic response characteristics are sensitive to basal exercise intensity (34, 35) and thus propensity for impairment may be different in rest-to-exercise versus exercise-to-exercise transitions in T2D.
and 3) much of daily living involves transitioning between different intensities of physical activity, rendering exercise-to-exercise transitions particularly relevant. Impairment at the onset of exercise (or in the transition from one intensity to another) would be important because the dynamics of oxygen delivery establish intra-myocellular oxygenation such that a slower adjustment of oxygen delivery can result in a greater oxygen deficit which contributes to increased fatigue (6).

Second, whether there is an impact of T2D on exercising muscle oxygen delivery within the constellation of co-morbidities and medications characteristic of this population has not been investigated. T2D is typically co-morbid with obesity (37), hypertension (36) and dyslipidemia (3), and patients routinely require associated medications (16, 39). However, previous studies investigated exercising muscle oxygen delivery in persons with T2D who were free from co-morbidities and medications versus age- and weight-matched controls (1, 17) or who withdrew medications prior to testing (15, 21, 48), or did not have control groups matched for co-morbidities and their associated medications (14, 19, 41, 48). Therefore, previous findings lack ecological validity for this population. Mechanistically, impaired steady state MBF could result from peripheral vascular dysfunction, but it could also be due to vasoconstriction secondary to impaired cardiac function (24, 51); therefore, assessment of peripheral vascular control specifically requires the use of a small muscle mass modality that would not be limited by central hemodynamic responses. Impaired MBF at steady state would result in a lower myocellular oxygenation at a given submaximal \( \dot{V}O_2 \) and thereby increase the rate of fatigue progression (45).

With this as a foundation, the objective of the present study was to characterize the MBF response during exercise in persons with T2D and to determine whether and/or how this is impaired relative to Controls matched for age, body mass index (BMI), aerobic fitness, co-morbidities and non-T2D medication use. We aimed to bridge the current gaps in the literature by: 1) enrolling representative T2D participants (i.e. with common co-morbidities and
medications) and matching Control participants for these and other important characteristics; 2) characterizing the MBF response directly in terms of (a) the dynamics of the rest-to-exercise response, (b) the dynamics of a low-to-moderate intensity exercise transition, and (c) the steady state response at each intensity; and 3) utilizing a single-leg isometric exercise model to better isolate local peripheral contributions during exercise. It was hypothesized that the T2D group would have reduced MBF (rate and amount) per workload compared to the Control group.

8.3 Methods

8.3.1 Participants

Thirteen men with T2D and 11 matched Control participants took part in this study. Participants were recruited via the Cardiac Rehabilitation Centre at Hotel Dieu Hospital (HDH; Kingston, Ontario, Canada), and all testing was completed between their screening for and commencement of this program. Inclusion criteria were: 1) BMI of 24-35 kg/m$^2$, 2) achievement of 4-10.0 metabolic equivalents of task (METs) on a graded treadmill exercise test, 3) baseline femoral artery diameter of $\geq 0.79$ cm (9) and 4) clear presence or absence of T2D [T2D: fasting plasma glucose $\geq 7.0$ mmol/L and/or oral glucose tolerance test (OGGT) 2 h plasma glucose $\geq 11.1$ mmol/L; Control: fasting plasma glucose $< 6.1$ mmol/L and OGGT 2 h plasma glucose $< 7.8$ mmol/L]. Exclusion criteria were: 1) presence of Stage 3 (or more advanced) renal disease, 2) current smoking or smoking within the past 12 months, or 3) usage of exclusionary medications (β-blockers, nitroglycerine or other nitric oxide donors). Participants taking β-blockers were not excluded if they withdrew these medications for 48 hours prior to testing under medical supervision (Dr. Stephen LaHaye, Cardiologist at HDH) ($n = 1$ in each group). Participants completed testing while taking all other medications (Table 8-1). See Figure 8-1 for a flowchart of participant recruitment.

Prior to the day of testing, participants visited the lab to be screened for clear blood velocity signals (Doppler ultrasound) and images (Echo ultrasound) of the femoral artery, and to
Figure 8-1. Flow-chart of participant recruitment.
Note that potential participants may have been excluded for more than one reason (therefore the n values for the reasons participants were “not eligible” do not sum to the total number of participants who were not eligible). *Other reasons for ineligibility were: myocardial ischemia during exercise (n=1), Churg-Strauss syndrome (autoimmune vasculitis; n=1), heart transplant patient (n=1), age (n=2; 17 and 85 y), unable to do baseline assessment of aerobic fitness (n=3; due to: wheelchair, high blood pressure, musculoskeletal issues), participation vetoed by cardiologist without provided reason (n=2), impaired glucose tolerance (n=6). BMI = body mass index; CR = cardiac rehabilitation; METs = metabolic equivalents.
practice the exercise for purposes of familiarization. The study protocol was approved by the Health Sciences Human Research Ethics Board at Queen’s University, and individuals gave written consent to participate on forms approved by this board.

8.3.2 Experimental protocol

Participants were instructed to abstain from taking anti-inflammatory medications within 48 hours of the study (excluding daily low-dose Aspirin, \( n=8 \) in T2D Group, \( n=9 \) in Control Group), from exercising within 24 hours, from consuming alcohol or caffeine for 12 hours and food for 3 hours prior to the testing session (42). Data collection was performed in a quiet, temperature-controlled room (19-23.5°C).

Participants were positioned in a semi-reclined position (~30-35° from horizontal) with their knees bent at 90° on a custom leg-ergometer (Figure 8-2). The left ankle was secured to a custom-built force system using a nylon strap. The system consisted of a force transducer (Interface model SM-2000N Load Cell, Durham Instruments) that was mounted on a steel plate running horizontally behind the participant’s leg and connected to a data acquisition system (Powerlab, ADInstruments). Participants performed isometric knee-extension exercise, and the force produced during the exercise was digitized, sampled at 200 Hz and recorded on a personal computer with live visual feedback provided to the participant. Isometric (versus dynamic) exercise was chosen to reduce the potential for movement artifact which could increase femoral artery Doppler ultrasound measurement error.

After 1 minute of resting baseline measurements, participants performed single-leg isometric knee-extension exercise at an absolute intensity equivalent to 6 kg, with live computer feedback displaying force output, in time with a metronome having a 1:2-s work-rest duty cycle. After 5 minutes at this intensity, the workload was increased to 12 kg for an additional 5 minutes. This series of rest followed by step increases in workload was repeated 2 additional times, separated by at least 10 minutes of rest to allow hemodynamic parameters to return to
baseline (Figure 8-3). These workloads were shown in pilot work to: 1) achieve an adequate response magnitude such that the kinetics of the muscle blood flow response could be quantified, and 2) be moderate enough to avoid cumulative fatigue over repeated trials.

Figure 8-2. Experimental set-up. Arrow indicates that participants performed isometric exercise by kicking against the strap to produce force ($\vec{F}$).

Figure 8-3. Timeline of experimental protocol. This series of rest followed by step increases in workload to 6 and 12 kg (1:2 s work:rest) was completed 3 times (separated by at least 10 min of rest). Data for all 3 trials were time-aligned to the start of exercise and were averaged together prior to analysis.
8.3.3 Measurements

8.3.3.1 Participant characteristics

Anthropometric measures (height, weight, BMI, waist circumference) and blood samples for determination of fasting plasma glucose, HbA1c, triglycerides, and total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were taken during the participants’ screening process for the Cardiac Rehabilitation Program at HDH. Participants also completed a graded treadmill exercise test and their maximal METs achieved were estimated based on the treadmill speed and grade. The maximum voluntary contraction (MVC) in the exercising (left) leg was measured during the screening visit to the lab by having participants perform 3 maximal effort single-leg isometric knee extensions, each separated by 1 minute of rest. The largest value was taken to represent the MVC.

8.3.3.2 Mean arterial blood pressure (MAP)

MAP was measured continuously with finger photoplethysmography on the participants’ right hand positioned at heart level via an arm sling (Finometer MIDI, Finapres Medical Systems).

8.3.3.3 Femoral artery diameter and mean blood velocity (MBV)

Images of the common femoral artery on the exercising (left) leg were obtained at rest using a 10 MHz linear Echo ultrasound probe (Vivid i2, GE Medical Systems), operating in two-dimensional B-mode. Images were recorded in Digital Imaging and Communications in Medicine (DICOM) format for later analysis. MBV was measured ~2-3 cm proximal to the femoral artery bifurcation at the site imaged with the Echo probe with a 4 MHz Doppler ultrasound probe (Multigon 500B, Transcranial Doppler, Multigon Industries). The corresponding voltage output was recorded continuously at 200 Hz in the data acquisition software program LabChart (ADInstruments) for later analysis. To quantify absolute MBV, voltage-to-velocity calibrations were performed with known flow velocities at a range of
insonation angles (in 1° increments to achieve a range of ± 15° from the 70° angle of insonation inherent to the probe). For each participant, the femoral artery was imaged at the site of Doppler probe placement to quantify the actual angle of insonation (using an on-screen protractor), and the appropriate voltage-to-velocity calibration was applied prior to data analysis. This enabled accurate absolute blood velocity measurements allowing for between-participant comparisons.

8.3.3.4 Leg force

Leg force was obtained using a force transducer (Interface model SM-2000N Load Cell, Durham Instruments) connected to a data acquisition system (Powerlab, ADInstruments) and recorded on a personal computer (LabChart, ADInstruments).

8.3.4 Data analysis

8.3.4.1 MBV and MAP

After applying the appropriate voltage-to-velocity calibration to the 200 Hz MBV data, a frequency-domain filtering procedure was used to eliminate higher-frequency noise due to heart rate and muscle contraction (i.e. a low-pass filter with a cut-off frequency of 0.2 Hz; lowpass.xfm, SigmaPlot 11.0, Systat Software Inc.) (8). The data were then resampled to retrieve every 20th datum to reduce the total number of data points (from ~132,000 to 6,600) to enable curve-fitting on a personal computer. Blood flow kinetic parameter estimates have been found to be unaffected by this resampling procedure and are superior to parameter estimates via beat-by-beat or contraction-relaxation cycle averaging (8). MAP data were similarly sampled at 200 Hz, filtered and resampled.

8.3.4.2 Femoral artery diameter

Vessel diameter was quantified at baseline using automated wall tracking via an updated version of the software package described by Woodman et al. 2001 (49) as previously described (28) and manual caliper measurements. Several values were averaged to obtain a single femoral
artery diameter value to minimize the effect of random diameter measurement error on calculated blood flow. Resting femoral artery diameter values were used to calculate leg blood flow because previous work has shown that femoral arteries of a baseline diameter of 0.79 cm or greater do not dilate at any time point of an exercise transient (9).

8.3.4.3 Leg blood flow (LBF), leg vascular conductance (LVK) and MAP

LBF was used as a surrogate for oxygen delivery and was calculated according to the formula:

\[ LBF = MBV \times 60 \text{ s/min} \times \pi \times (\text{diameter}/2)^2 \]

where LBF is in ml/min, MBV is in cm/s, and femoral artery diameter is in cm. LVK was calculated as:

\[ LVK = (LBF/MAP) \times 100 \]

where LVK is in ml/min/100mmHg such that the values for LVK are quantitatively similar to those for LBF.

Data for all 3 trials were time-aligned to the onset of exercise and averaged together. In one T2D participant there was evidence of measurement error (i.e. compromised signal) in one of the three trials; this trial was excluded and the remaining two were averaged together. LBF and LVK for the transitions from rest-to-6 kg and 6 kg-to-12 kg were fit with one-, two- or three-component exponential models (SigmaPlot 11.0, Systat Software Inc.; details in “Kinetics Analysis”), while MAP data were fit with linear or one- or two-component exponential models as appropriate. The regression equations were used to calculate the average values for LBF, LVK and MAP at rest (“baseline”; 1 min average) and in the last 30 s at each workload (i.e. “steady state” at 6 kg and 12 kg). The change from baseline to steady state was calculated (i.e. \( \Delta LBF_{ss} \), \( \Delta LVK_{ss} \), \( \Delta MAP_{ss} \)), where the “baseline” for the 12 kg workload was the steady state at 6 kg. For the 12 kg workload, the change from the overall baseline was also calculated (\( \Delta LBF_{ss-12} \), \( \Delta LVK_{ss-12} \), \( \Delta MAP_{ss-12} \)).
8.3.4.4 Kinetics analysis

As mentioned, the time course plots for LBF and LVK from rest-to-6 kg and 6 kg-to-12 kg were fit with one-, two- or three-component exponential models for analysis of dynamic response characteristics. The models have a baseline component ($G_0$) and one or more amplitude terms ($G_1$, $G_2$ and/or $G_3$), time constants ($\tau_1$, $\tau_2$, and/or $\tau_3$), and time delays ($TD_1$, $TD_2$ and/or $TD_3$), consistent with the number of phases of the response (34). $G$ represents the magnitude of the response, $\tau$ (“tau”) the time it takes to achieve 63% of the response magnitude (describing the rate at which LBF or LVK increases), and TD gives a measure of the delay in onset of response (i.e. how quickly the vascular control systems can begin to respond following the onset of muscle contraction or increase in intensity). The model is described by the following equation:

(Eq. 3) $Y(t) = G_0 + G_1[1 - e^{-(t-TD_1)/\tau_1}] \cdot \mu_1 + G_2[1 - e^{-(t-TD_2)/\tau_2}] \cdot \mu_2 + G_3[1 - e^{-(t-TD_3)/\tau_3}] \cdot \mu_3,$

where

$\mu_1 = 0$ for $t < TD_1$ and $\mu_1 = 1$ for $t \geq TD_1$

$\mu_2 = 0$ for $t < TD_2$ and $\mu_2 = 1$ for $t \geq TD_2$

$\mu_3 = 0$ for $t < TD_3$ and $\mu_3 = 1$ for $t \geq TD_3$

where $t$ is time in seconds and $Y(t)$ is the time-dependent variation in LBF or LVK.

The mean response time (MRT) quantifies the time to reach 63% of the overall amplitude of the response from baseline, and was calculated as a weighted sum of the time delay and time constant of each response phase (20):

(Eq. 4) $MRT = \left[ \frac{G_1}{G_1+G_2+G_3} \right] \cdot (TD_1 + \tau_1) + \left[ \frac{G_2}{G_1+G_2+G_3} \right] \cdot (TD_2 + \tau_2) + \left[ \frac{G_3}{G_1+G_2+G_3} \right] \cdot (TD_3 + \tau_3)$

8.3.4.5 Adjustment amplitude

Measures of amplitude at different time points during the dynamic increase were also used in order to compare the adjustment of the response. This was done via: 1) the change from baseline to the “peak initial response” (PIR) of LBF and LVK [defined as the greatest 1 s average (11 data points) within the first 10 s of exercise at each intensity; $\Delta LBF_{PIR}$, $\Delta LVK_{PIR}$], and 2) the
change from baseline to the “on-transient” (ON) of LBF, LVK and MAP (mean of 1 s averages from curve fit at 15, 45, and 75 s of exercise; ∆LBF<sub>ON</sub>, ∆LVK<sub>ON</sub>, ∆MAP<sub>ON</sub>; see Figure 8-6 A). In other words, the initial amplitude (“peak initial response”) and amplitude of the response at discrete time points in the exercise transient (obtained from the best “fit” of the data) are indicative of the dynamics of the response in that a greater magnitude equates to a greater speed (either within the first 10 s or averaged at 15, 45, 75 s). For MAP, the “peak initial response” (∆MAP<sub>PRI</sub>) was calculated at the time that corresponded with the ∆LBF<sub>PRI</sub>, since pressure is a determinant of blood flow.

The proportion of the total increase (to steady state) in LBF and LVK achieved with the PRI was calculated as:

\[
\text{(Eq. 5) } \% \Delta \text{LBF}_{PRI-SS} = \frac{\Delta \text{LBF}_{PRI}}{\Delta \text{LBF}_{SS}} \times 100
\]

\[
\text{(Eq. 6) } \% \Delta \text{LVK}_{PRI-SS} = \frac{\Delta \text{LVK}_{PRI}}{\Delta \text{LVK}_{SS}} \times 100
\]

8.3.4.6 Force impulse

The area under the curve (AUC) of the leg force tracing for each isometric contraction was computed to give the time-tension integral (i.e. the “force impulse”, in kg·s). The average force impulse for the duration of each of the 6 and 12 kg workloads was calculated. In addition, the change in force impulse (∆F<sub>i</sub>) from baseline (resting baseline or 6 kg steady state “baseline” as appropriate) was calculated during each phase of the response (i.e. the first 10 s for the PRI, the first 90 s for the ON, and the final 30 s at each workload for SS).

8.3.5 Statistical analysis

Unpaired t-tests were used to compare the effect of Group (T2D, Control) on participant characteristics, resting baseline hemodynamic parameters (LBF, LVK, MAP), and the change in hemodynamic variables from resting baseline to 12 kg steady state (∆LBF<sub>SS-12</sub>, ∆LVK<sub>SS-12</sub>, ∆MAP<sub>SS-12</sub>). Two-way repeated measures analysis of variance (RM ANOVA) were used to
assess the impact of Group (T2D, Control) and Workload Transition (rest-to-6 kg, 6-to-12 kg) on: force impulse (absolute and delta); LBF and LVK MRT; the change in LBF, LVK and MAP from baseline to each phase of the response (PIR, ON, SS); and the proportion of the steady state LBF and LVK achieved with the PIR (%ΔLBF_{PIR,SS}, %ΔLVK_{PIR,SS}). Two-way RM ANOVAs were also used to assess the change in LBF, LVK and MAP across Time (i.e. the different time phases of the response: PIR, ON, SS) and Workload Transition (rest-to-6 kg, 6-to-12 kg) within each group. Significant differences were further investigated with Tukey’s post hoc tests. Linear regression was used to evaluate the relationship between ΔLBF at each phase of the response (PIR, ON, SS) and glycemic control (HbA1c) within the T2D group. Statistical significance was set at P<0.05, and all statistics were calculated using IBM® SPSS® Statistics (version 22; SPSS Inc.). All data are expressed as means ± SD.

8.4 Results

8.4.1 Participant characteristics

Participant characteristics are shown in Table 8-1. The T2D and Control groups did not differ with respect to age, weight, BMI, waist circumference, fasting blood lipids, METs achieved on a graded treadmill test, or leg MVC. As anticipated, the T2D group had greater fasting plasma glucose (P<0.001) and HbA1c (P=0.001). The 6 kg and 12 kg workloads represented intensities of approximately 19% MVC and 38% MVC (not different between Groups; both P=0.693).

8.4.2 Force impulse

The average force impulse at each of the target workloads was not significantly different between groups (for 6 and 12 kg targets in T2D vs Control groups respectively: 5.7 ± 0.8 vs 5.9 ± 0.8 kg·s, and 11.4 ± 2.1 vs 12.2 ± 1.7 kg·s; P=0.343; Figure 8-4). ΔF_{i} was not different between
Table 8-1. Participant characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group</th>
<th>T2D Group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td>N/A</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.7 ± 11.2</td>
<td>63.2 ± 9.5</td>
<td>0.921</td>
</tr>
<tr>
<td>Diabetes Duration, y</td>
<td>--</td>
<td>5.9 ± 5.4</td>
<td>--</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.6 ± 6.2</td>
<td>172.7 ± 6.8</td>
<td>0.039*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>97.4 ± 19.6</td>
<td>95.8 ± 15.1</td>
<td>0.823</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.4 ± 5.0</td>
<td>32.1 ± 4.9</td>
<td>0.404</td>
</tr>
<tr>
<td>WC, cm</td>
<td>105.2 ± 12.5</td>
<td>110.2 ± 12.8</td>
<td>0.349</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.1 ± 0.3</td>
<td>8.2 ± 2.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.6 ± 0.3</td>
<td>7.3 ± 1.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>Fasting triglycerides, mmol/L</td>
<td>1.8 ± 2.2</td>
<td>1.5 ± 0.9</td>
<td>0.700</td>
</tr>
<tr>
<td>Total Cholesterol, mmol/L</td>
<td>3.5 ± 1.2</td>
<td>3.5 ± 1.2</td>
<td>0.986</td>
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<tr>
<td>HDL Cholesterol, mmol/L</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>0.633</td>
</tr>
<tr>
<td>LDL Cholesterol, mmol/L</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 1.0</td>
<td>0.614</td>
</tr>
<tr>
<td>Baseline GXT, METs</td>
<td>8.3 ± 0.8</td>
<td>8.2 ± 1.8</td>
<td>0.817</td>
</tr>
<tr>
<td>Leg MVC, kg</td>
<td>33.4 ± 8.1</td>
<td>36.4 ± 13.6</td>
<td>0.525</td>
</tr>
<tr>
<td>% MVC at 6 kg workload</td>
<td>19.1 ± 5.6</td>
<td>18.2 ± 5.5</td>
<td>0.693</td>
</tr>
<tr>
<td>% MVC at 12 kg workload</td>
<td>38.3 ± 11.2</td>
<td>36.5 ± 10.9</td>
<td>0.693</td>
</tr>
<tr>
<td>Number of non-T2D medications</td>
<td>4.2 ± 1.1</td>
<td>4.6 ± 1.8</td>
<td>0.484</td>
</tr>
<tr>
<td>Number (%) using medications:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX Inhibitor</td>
<td>9 (82%)</td>
<td>8 (62%)</td>
<td>--</td>
</tr>
<tr>
<td>ACE Inhibitor</td>
<td>7 (64%)</td>
<td>13 (100%)</td>
<td>--</td>
</tr>
<tr>
<td>HMG-CoA Reductase Inhibitor (“statin”)</td>
<td>10 (91%) (8, 73% HP; 2, 18% LP)</td>
<td>13 (100%) (9, 69% HP; 4, 31% LP)</td>
<td>--</td>
</tr>
<tr>
<td>Thienopyridine (or derivative; “anti-platelet”)</td>
<td>4 (36%)</td>
<td>4 (31%)</td>
<td>--</td>
</tr>
<tr>
<td>Cholesterol Absorption Inhibitor</td>
<td>0 (0%)</td>
<td>3 (23%)</td>
<td>--</td>
</tr>
<tr>
<td>Biguanide</td>
<td>0 (0%)</td>
<td>9 (69%)</td>
<td>--</td>
</tr>
<tr>
<td>Insulin / Analogue</td>
<td>0 (0%)</td>
<td>1 (8%)</td>
<td>--</td>
</tr>
<tr>
<td>Other</td>
<td>9 (82%)</td>
<td>7 (54%)</td>
<td>--</td>
</tr>
<tr>
<td>Cardiovascular Health History: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>3 (27%)</td>
<td>3 (23%)</td>
<td>--</td>
</tr>
<tr>
<td>Angioplasty</td>
<td>5 (45%)</td>
<td>6 (46%)</td>
<td>--</td>
</tr>
<tr>
<td>Coronary artery bypass surgery</td>
<td>5 (45%)</td>
<td>3 (23%)</td>
<td>--</td>
</tr>
<tr>
<td>History of cardiomyopathy</td>
<td>0</td>
<td>1 (8%)</td>
<td>--</td>
</tr>
<tr>
<td>Heart valve repair / replacement</td>
<td>1 (9%)</td>
<td>4 (31%)</td>
<td>--</td>
</tr>
<tr>
<td>Transient ischemic stroke</td>
<td>1 (9%)</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

Values are means ± SD. ACE = angiotensin converting enzyme; BMI = body mass index; COX = cyclooxygenase; GXT = graded exercise test (treadmill); HDL = high density lipoprotein; HMG-CoA Reductase = 3-hydroxy-3-methyl-glutaryl-coA reductase; HP = hydrophilic; LDL = low density lipoprotein; LP = lipophilic; METs = metabolic equivalents; MVC = maximum voluntary contraction; WC = waist circumference. *P<0.05 compared with Control Group.
Figure 8-4. Force impulse at each of the target workloads.

Grey bars and closed symbols = Control; White bars and open symbols = T2D; Dashed lines indicate target workloads at 6 and 12 kg.
T2D and Control groups at any time point (for change from baseline to PIR, ON and SS respectively: P=0.316, P=0.476, P=0.436), nor was it different between 6 and 12 kg workloads at any time point (at PIR, ON and SS respectively: P=0.213, P=0.070, P=0.934), confirming that the exercise was done as prescribed.

8.4.3 Hemodynamic parameters

Hemodynamic parameters at rest and during exercise for a representative participant are shown in Figure 8-5. LBF, LVK and MAP were not different between groups at the overall resting baseline (P=0.148, P=0.361 and P=0.095 respectively). Baseline values for LBF, LVK and MAP in T2D vs Control groups were the following: 319.7 ± 83.2 vs 404.2 ± 182.5 ml/min, 419.2 ± 128.2 vs 489.3 ± 233.2 ml/min/100mmHg, and 78.5 ± 7.5 vs 84.9 ±10.6 mmHg.

8.4.3.1 Dynamic adjustment of hemodynamic parameters

8.4.3.1.1 Mean response time (MRT)

For LBF, the MRT was not different between groups (P=0.856) or between rest-to-6 kg and 6-to-12 kg workload transitions (P=0.881) (values for rest-to-6 kg and 6-to-12 kg workload transitions in T2D vs Control groups respectively: 39.8 ± 41.1 vs 43.4 ± 47.7 s; 49.3 ± 17.8 vs 49.7 ± 35.0 s). Similarly for LVK, the MRT was not different between groups (P=0.442) or between workloads (P=0.616; for 6 and 12 kg workloads in T2D vs Control groups: 40.6 ± 40.6 vs 44.8 ± 49.0 s; 61.3 ± 53.2 vs 37.2 ± 29.4 s). Note that while the difference between groups at 12 kg may appear to be large (~24 s), this can be attributed to an “outlier” participant in the T2D group whose LVK response was tri-phasic and had a calculated MRT of 214 s (and a z-score of 2.9). Removal of this participant’s datum from analysis did not alter the result, and therefore it is included in the overall group mean.
Figure 8-5. Hemodynamic responses in a representative participant.  
(A) LBF, (B) LVK, (C) MAP. Curve fits were used to determine values at: 1) baseline, 2) 15, 45 and 75 s of exercise (i.e. during the on transient; ON), and 3) steady state (SS; change indicated by delta symbols and double-headed arrows). The largest 1 s average within the first 10 s was identified (arrows) as the amplitude of the “peak initial response” (PIR) and the change from baseline was quantified. Dashed vertical lines indicate the transitions from rest-to-6 kg and 6 kg-to-12 kg; dotted horizontal lines indicate the parameter values at baseline, 6 kg SS and 12 kg SS.
8.4.3.1.2 Peak initial response (PIR) (Figure 8-6 – Bi, Ci, Di)

ΔLBF_{PIR} was significantly lower in the T2D group versus Control (P=0.037), but was not different between rest-to-6 kg and 6-to-12 kg workload transitions (P=0.305). Similarly, ΔLVK_{PIR} tended to be lower in the T2D group versus Control (P=0.063), but was not different between workload transitions (P=0.421). ΔMAP_{PIR} was not different between groups (P=0.731) and was significantly smaller at the 6-to-12 kg transition than the rest-to-6 kg transition (P<0.001). The proportion of steady state LBF achieved with the PIR (%ΔLBF_{PIR,SS}) was not different between groups (P=0.287) but was significantly lower at the 6-to-12 kg vs rest-to-6 kg workload transition (P=0.009; for rest-to-6 kg and 6-to-12 kg in T2D vs Control groups respectively: 71.6 ± 32.5 vs 75.5 ± 31.0%, and 47.6 ± 19.6 vs 63.1 ± 21.2%). Similarly, %ΔLVK_{PIR,SS} was not different between groups (P=0.392) but was significantly lower at 6-to-12 kg vs rest-to-6 kg (P=0.027; for rest-to-6 kg and 6-to-12 kg in T2D vs Control groups respectively: 73.7 ± 36.8 vs 79.6 ± 37.1%, and 52.2 ± 29.5 vs 66.3 ± 28.2%).

8.4.3.1.3 On-Transient (ON) (Figure 8-6 – Bii, Cii, Dii)

ΔLBF_{ON} and ΔLVK_{ON} were not different between groups (P=0.150, P=0.237 respectively) or across workload transitions (P=0.167, P=0.346). ΔMAP_{ON} was not different between groups (P=0.638) but was significantly lower at 6-to-12 kg vs rest-to-6 kg (P<0.001).

8.4.3.2 Steady state hemodynamic responses

8.4.3.2.1 Change from baseline to SS, where “baseline” for 12 kg workload was SS for 6 kg (Figure 8-6 – Biii, Ciii, Diii)

ΔLBF_{SS} and ΔLVK_{SS} were not different between groups (P=0.204, P=0.347) but were significantly greater at the 6-to-12 kg vs rest-to-6 kg workload transition (P=0.006, P=0.034). ΔMAP_{SS} was not different between groups (P=0.821) but was significantly smaller at the 6-to-12 kg vs rest-to-6 kg workload transition (P<0.001).
Figure 8-6. Hemodynamic responses during isometric leg exercise (1 s contraction: 2 s relaxation) at 6 kg and 12 kg.

(A) Schematic representation of the absolute LBF response to illustrate the different phases of the response. (i) identifies the “peak initial response” (PIR; the largest 1 s average in the first 10 s of exercise; indicated by circle), (ii) identifies the amplitude of the “on-transient” (ON; average of 1 s averages from curve fit at 15, 45 and 75 s at each work rate; indicated by “x” symbols), (iii) steady state (SS; average of final 30 s from curve fit at each work rate). Note that the x-axis is not to scale. (B-D) ∆LBF, ∆LVK and ∆MAP from baseline to (i) PIR, (ii) ON, (iii) SS. For the 12 kg workload, the “baseline” is the 6 kg steady state for each variable. Data are means ± SD; symbols are individual participant data (grey bars and closed symbols = Control; white bars and open symbols = T2D). *Main effect of Group; †Main effect of Workload (P<0.05).
8.4.3.2.2 Change from overall resting baseline to 12 kg SS (Figure 8-7)

\( \Delta \text{LBF}_{\text{SS-12}}, \Delta \text{LVK}_{\text{SS-12}}, \text{ and } \Delta \text{MAP}_{\text{SS-12}} \) were not different between T2D and Control groups (P= 0.204, P=0.347 and P=0.823 respectively).

\[
\begin{array}{c}
\text{(A)} & \text{(B)} & \text{(C)} \\
\end{array}
\]

\( \Delta \text{LBF}_{\text{SS-12}} \), \( \Delta \text{LVK}_{\text{SS-12}} \), and \( \Delta \text{MAP}_{\text{SS-12}} \) were not different between T2D and Control groups (P= 0.204, P=0.347 and P=0.823 respectively).

\textbf{Figure 8-7.} Change in (A) LBF, (B) LVK and (C) MAP from overall resting baseline to 12 kg steady state.

Data are means ± SD; symbols are individual participant data (grey bars and closed symbols = Control; white bars and open symbols = T2D).

8.4.3.3 Change in hemodynamic parameters over time

8.4.3.3.1 Control group

In the Control group, there was a main effect of Time for \( \Delta \text{LBF} \) (P<0.001) such that \( \Delta \text{LBF}_{\text{SS}} \) was greater than both \( \Delta \text{LBF}_{\text{ON}} \) and \( \Delta \text{LBF}_{\text{PIR}} \) (both P<0.001), but \( \Delta \text{LBF}_{\text{PIR}} \) and \( \Delta \text{LBF}_{\text{ON}} \) were not different (P=0.962). Likewise for \( \Delta \text{LVK} \), \( \Delta \text{LVK}_{\text{SS}} \) was greater than both \( \Delta \text{LVK}_{\text{ON}} \) and \( \Delta \text{LVK}_{\text{PIR}} \) (both P<0.001), but \( \Delta \text{LVK}_{\text{PIR}} \) and \( \Delta \text{LVK}_{\text{ON}} \) were not different (P=0.970). In contrast, \( \Delta \text{MAP} \) was not different across PIR, ON and SS (P=0.123), but was greater at rest-to-6 kg vs 6-to-12 kg (P=0.002).

8.4.3.3.2 T2D group

In the T2D group, for \( \Delta \text{LBF} \) there was a significant Time x Workload interaction (P=0.003), such that \( \Delta \text{LBF}_{\text{SS}} \) was significantly greater at 12 kg vs 6 kg (P=0.003) but was not different between workloads at \( \Delta \text{LBF}_{\text{PIR}} \) (P=0.173) or \( \Delta \text{LBF}_{\text{ON}} \) (P=0.839). For both workloads,
ΔLBF_{SS} was greater than both ΔLBF_{PIR} and ΔLBF_{ON} (all P<0.05), but ΔLBF_{PIR} and ΔLBF_{ON} were not different (P>0.05). Similarly for ΔLVK, there was a significant Time x Workload interaction (P=0.023), with a tendency for ΔLVK_{SS} to be greater at 12 kg vs 6 kg (P=0.059), but no differences between workloads at ΔLVK_{PIR} (P=0.339) or ΔLVK_{ON} (P=0.900). At both workloads, ΔLVK_{SS} was greater than ΔLVK_{PIR} and ΔLVK_{ON} (all P<0.05), but ΔLVK_{PIR} and ΔLVK_{ON} were not different from one another (both P>0.05). For ΔMAP, there was a main effect of Time (P=0.003) and a main effect of Workload (P<0.001), such that ΔMAP_{SS} tended to be greater than ΔMAP_{ON} (P=0.061), was significantly greater than ΔMAP_{PIR} (P=0.003), and was greater in rest-to-6 kg than in 6-to-12 kg (P<0.001).

8.4.3.4 Linear regressions

Within the T2D group, ΔLBF and HbA1c were not related at any point in the exercise transient (i.e. PIR, ON, SS) in either the rest-to-6 kg or 6-to-12 kg workload transition (all P>0.300; data not shown).

8.5 Discussion

The objective of this study was to test the hypothesis that T2D, when present in the characteristic constellation of co-morbidities and medications, slows the dynamic adjustment of exercising muscle blood flow and blunts the steady state, relative to co-morbidity- and non-T2D medication-matched Controls. The major findings of this study were the following: 1) the change from baseline to the peak initial LBF response (ΔLBF_{PIR}) was blunted in T2D relative to Control, irrespective of whether the transition was from rest-to-lower intensity or low-to-moderate intensity exercise; 2) regardless of group, the proportion of the total gain (i.e. at steady state) achieved with the peak initial response (％ΔLBF_{PIR,SS}) was significantly smaller in the 6-to-12 kg transition than the rest-to-6 kg transition; 3) the dynamic adjustment of LBF was not different between groups at either rest-to-exercise or low-to-moderate intensity exercise, as quantified via both the MRT and the change from baseline to discrete time points in the “on-transient” (ΔLBF_{ON}) of the response;
and 4) the change from baseline to steady state exercising LBF ($\Delta LBF_{SS}$) was not different between groups at either intensity [low (6 kg) or moderate (12 kg)]. These results demonstrate that persons with T2D and characteristic co-morbidities and medications exhibit an immediate amplitude impairment in the LBF response at the onset of exercise (or at the transition between exercise intensities) but that the overall dynamic adjustment and eventual steady state during low- and moderate-intensity submaximal exercise are not impaired in this population relative to matched Controls.

8.5.1 Dynamic adjustment of exercising LBF

With a step increase in exercise intensity, muscle blood flow increases in an exponential manner and reaches a plateau that is in proportion to the metabolic demand, such that steady state blood flow is linearly related to exercise intensity (10, 30). The initial rapid increase (phase I) is an immediate but incomplete response under “feed-forward” control that reaches a plateau in $\sim 5$-10 s (20). This increase may be followed by a transient reduction before a second slower feedback-mediated increase (phase II) begins (within $\sim 15$-30 s of the increase in contraction intensity) that adjusts blood flow to steady state during low- and moderate-intensity exercise (20, 34). During high-intensity exercise there is a third and very slow component (phase III) that begins around 1.5-2 min (34). Thus each phase of the response is governed by different mechanisms and, since each phase can impact myocellular oxygenation, both the initial adjustment and the eventual steady state are important with regards to a potential oxygen delivery impairment.

8.5.1.1 Initial response amplitude

In the present study, the change in LBF from baseline to the peak initial response (i.e. highest 1 s average in the first 10 s) was significantly reduced in T2D versus Control. This was due to a tendency for a blunted $\Delta LVK_{PIR}$ (with $\Delta MAP_{PIR}$ being similar between groups), and occurred irrespective of whether the transition was from rest-to-low or low-to-moderate intensity
exercise (see Figure 8-6). Mechanisms capable of contributing to the rapid initiatory response include mechanical compression of blood vessels (4), the muscle pump (46), venous emptying-mediated vasodilation (44), and rapid release of vasodilatory factors [K+, nitric oxide (NO), prostaglandins, and adenosine acting together] (7, 33).

Since our participants were matched for BMI (and presumably, crudely, leg size) and leg strength (MVC; Table 8-1) it is unlikely that there were any differences in mechanical compression or muscle pump function at the same absolute workloads. Although the evidence for venous-emptying mediated vasodilation is quite strong (44, 47), the mechanism by which emptying of congested veins evokes vasodilation is unknown and thus it is uncertain whether and/or how this might be impaired in T2D. With regard to rapid vasodilatory factors, at present it is also unknown how these mechanisms might be compromised in T2D. Impaired endothelial function and overall reduced bioavailability of NO is consistently observed in T2D (23), making reduced NO at the onset of exercise a potential candidate. Future investigation will be required to uncover the mechanistic basis for the blunted initial response.

The proportion of steady state LBF achieved with the PIR was not different between groups, but was significantly lower in the transition from 6-to-12 kg than from rest-to-6 kg. This is consistent with findings in young healthy persons in which the increase in exercising muscle blood flow was blunted when a work rate increase was initiated from exercise versus an increase of the same magnitude initiated from rest (35). This baseline-dependency may result from the absence of any additional contribution of the muscle pump and/or venous emptying-mediated vasodilation in the transition from low-to-moderate exercise intensity, since the muscle pump is already at its capacity for flow enhancement during low intensity contractions (18), and since venous-congestion mediated vasodilation occurs in proportion to the degree of venous congestion (47) which would not be anticipated to change during a workload transition.
8.5.1.2 Speed of adjustment and steady state

Despite the initial difference in the amplitude of the response, the overall speed of the LBF adjustment was not different between groups. This was confirmed by characterizing the dynamics in two different ways: the MRT and the average amplitude change from baseline to three time points during the “on-transient” (ON; 15, 45, 75 s). As previously observed (20), the on-transient was characterized by a rapid initial increase followed by a brief reduction and then a continued adjustment to steady state. Within both T2D and Control groups, the ∆LBF_PIR and ∆LBF_ON were not significantly different from each other in magnitude, signifying that the on-transient rapidly re-established flow following the brief reduction after the peak initiatory response (Figure 8-5 A, Figure 8-6 A). The change in LBF from baseline to steady state was not different between groups at either the low or moderate exercise intensity, and for the 12 kg workload this was true whether examined as two successive increments (i.e. rest-to-6 kg and 6-to-12 kg; Figure 8-6 Biii) or as the total change from rest to 12 kg steady state (Figure 8-7 A). This suggests that despite the initial disturbance to oxygen delivery in T2D, feedback control mechanisms are able to adjust LBF appropriately to steady state. Within the T2D group there was no relationship between the degree of glycemic control (i.e. as indicated by HbA1c) and ∆LBF at any time point (PIR, ON, SS).

8.5.2 Current understanding of potential T2D-mediated impairment

The present study is the first investigation of the impact of T2D on the dynamic adjustment of exercising muscle blood flow when accompanied by the typical constellation of co-morbidities and associated medications in this population. In the only other investigation of oxygen delivery dynamics in T2D, MacAnaney et al. (19) recruited a “healthier” cohort; only a small proportion of the T2D group also had dyslipidemia (33%) and hypertension (11%) as evidenced by medication use, and Controls were not matched for these characteristics. MacAnaney and colleagues (19) quantified leg vascular conductance during the transition from
rest to intermittent calf contractions (at 70% MVC), and found that the phase I amplitude tended to be ~20% lower in the T2D group (non-significant), in agreement with the present findings of a blunted ΔLVK PIR. They also observed that the time constant (tau) of the second phase of the LVK response was significantly greater in T2D versus Control, resulting in a greater MRT (median values of ~31 vs 16 s). Our findings of no difference in the MRT between T2D versus Control groups suggests that T2D in a constellation of co-morbidities still impacts the rapid initial response, but not the overall kinetics. The difference in findings between our study and that of MacAnaney et al. (19) may be due to the cohort examined, whereby the co-morbidity and medication status typical of this population already has an impairment effect that is not further compromised by T2D, as evidenced by the considerably slower MRT of LVK in our Control and T2D groups (~46 s) compared to theirs. In other words, it is possible that T2D exerts an independent effect in isolation, but that this effect is superseded by the greater combined influence of co-morbidities. Given that persons with “only” T2D represent a minority of patients, the practical utility of this suggestion is limited. Alternately, since Control participants in the previous study were not matched for co-morbid conditions, perhaps the finding of impairment should not be solely attributed to T2D per se. Our findings thus extend those of MacAnaney et al. by (a) evaluating LBF responses within the typical clustering of co-morbidities and medications in this population and matching Control participants for these characteristics to achieve ecological validity, (b) providing direct measures of LBF (in addition to LVK) kinetics, and (c) evaluating the kinetic response during both rest-to-exercise and exercise-to-exercise transitions.

With respect to steady state exercising LBF, investigations by Kingwell et al. (15) and Lalande et al. (17) are frequently cited as evidence for reduced steady state exercising LBF in persons with T2D, a finding which was not confirmed in the present study. However, Lalande et al. (17) enrolled only co-morbidity and medication-free participants, while Kingwell et al. (15)
included only two T2D participants who took anti-diabetic agents but completed testing after a 24 h drug-free period. As previously mentioned, persons with T2D typically take anti-diabetic agents in the management of the disease (16), as well as anti-hypertensive and anti-hyperlipidemic medications (3, 39). Importantly, anti-hypertensive and anti-diabetic agents are known to improve endothelial function (22, 26) and may increase exercising muscle blood flow (11, 50). Thus these medications may have contributed to the conserved LBF responses in the T2D group at steady state in the present study. Should these medications be responsible for the preserved LBF response, the implication is that in the real world persons with T2D (who are taking these medications) do not experience impairment that might otherwise be endogenous to the disease. Notably however, others have observed impaired blood flow in T2D when medications were continued during testing [only if HbA1c ≥ 8 (14)], or preserved blood flow when medications were discontinued (21). This suggests that differences in medication status cannot fully explain disparate findings across studies, and we cannot estimate the magnitude of effect the medications may have exerted in the present study.

8.5.3 Perspectives

The workloads employed in the present study represent low-to-moderate exercise intensities and reflect metabolic requirements encountered during activities of daily living (e.g. walking or climbing a flight of stairs). The current findings indicate that, while muscle oxygen delivery may be briefly blunted at the onset of exercise (or in the transition between exercise intensities) in persons with T2D in the context of co-morbidities and medications, the overall rate of adjustment and eventual steady state are not different from that in matched Control participants. The functional significance of the initial blunting of LBF is unknown, particularly since it is transient (i.e. occurring within the first 10 s of exercise, with unimpaired flow relative to Control restored shortly thereafter). Indeed, a recent investigation in our lab found no impairment to small muscle mass exercise tolerance in this population (unpublished
observations). However it remains possible that, when superimposing multiple transitions during activities of daily living, sequential impairments in the initiatory response could accrue to impact exercise tolerance. Future work will be required to ascertain the impact (if any) on exercise tolerance and to identify the mechanism(s) responsible for the attenuation of LBF at the onset of exercise.

8.5.4 Limitations

In the present study, a small muscle mass modality was utilized in order to isolate peripheral vascular contributions to exercising muscle blood flow. While no compromise to the overall speed or magnitude of the LBF adjustment was evident, it remains possible that a cardiac-mediated limitation to oxygen delivery could be present during whole body exercise. Findings cannot be generalized to the minority of persons with T2D who do not have co-morbidities and/or who are treated by diet and exercise alone. The present findings are also limited to men, although sex differences are unlikely given that \( \dot{V}O_2 \) kinetics have been shown to be similar in men and women with T2D (25). Similarly, results can only be generalized to the range of exercise intensities employed in the present study (i.e. low to moderate), although if anything impairment would be expected to be less likely during high intensity exercise since in a previous investigation \( \dot{V}O_2 \) kinetics were found to be slowed at workloads below but not above the lactate threshold in persons with T2D (32). Lastly, we assessed exercising hemodynamics during fasting conditions; LBF may have been differentially affected in the postprandial state since exercise hyperemia has been found to be reduced in insulin-resistant individuals when exercise is performed in hyperinsulinemic conditions (12).

8.5.5 Conclusions

This is the first study to systematically characterize the dynamic and steady state LBF responses during both rest-to-low intensity and low-to-moderate intensity exercise transitions in persons with T2D within the cluster of co-morbidities and medications that accompany this
disease. As anticipated, the initial amplitude of the LBF response was significantly blunted in T2D versus Control, but this was not intensity-dependent (i.e. the attenuation occurred irrespective of whether the transition was from a resting or exercising baseline). Contrary to our hypothesis however, there was no robust or consistent impact of T2D, on top of the co-morbidities and medications typical of this population, on the overall dynamic adjustment of LBF or the steady state levels ultimately achieved. These data demonstrate that impaired adjustment of oxygen delivery may have a limited contribution to exercise intolerance in persons with T2D, and that a reduction in exercising muscle oxygen delivery at steady state, beyond that which may already be present in the context of co-morbidities, is not an obligatory consequence of this disease. Future work will be required to uncover the mechanism(s) underlying the initial amplitude impairment and the impact (if any) on exercise tolerance.
8.6 References


40. **Stewart KJ.** Exercise Training and the Cardiovascular Consequences of Type 2 Diabetes and Hypertension: Plausible Mechanisms for Improving Cardiovascular Health. *JAMA* 288: 1622-1631, 2002.


Within the broad theme of cardiovascular health, disease and function, this dissertation had a dual focus: 1) Contributions to CVD: investigation of mechanisms linking two lifestyle factors (stress and fat intake) with the development of CVD, and 2) Cardiovascular consequences of T2D: investigation of exercising muscle oxygen delivery and exercise tolerance in persons with T2D. The primary findings of this work are as follows. For Focus 1, in young, healthy men: 1) Repeated mental stress tasks had no impact on postprandial lipemia following either a high- or low-fat meal; 2) Meal fat content did not significantly impact hemodynamic reactivity to, or recovery from, varied mental stress tasks over a 4 h postprandial period; and 3) Meal fat content did not affect FMD, but normalized FMD was depressed in the “stress-free” postprandial period (independent of meal type) relative to when this period was accompanied by mental stress. For Focus 2, within the typical constellation of co-morbidities and medications that accompany T2D: 1) Small muscle mass exercise tolerance ($fCF_{impulse}$) was not impaired in T2D relative to matched Controls; 2) Forearm muscle blood flow (and its determinants, FVK and MAP) did not differ between T2D and Control groups during exercise at $fCF_{impulse}$; and 3) While the initial amplitude of the LBF response in rest-to-exercise and exercise-to-exercise transitions was blunted in T2D versus Control, there was no robust or consistent impact of T2D on the overall dynamic adjustment of LBF or the steady state levels ultimately achieved.

9.1 **FOCUS 1. Contributions to CVD: Investigation of mechanisms linking stress and high fat meal consumption with the development of CVD**

9.1.1 **Focus 1 Key Findings and Implications**

Despite the persuasive rationale for predicting an interaction between stress and fat consumption, the findings of studies 1 and 2 (chapters 5 and 6) demonstrated that mental stress did not exaggerate the rise in blood lipid levels following fat consumption, high-fat meal ingestion did not amplify stress responsiveness, and endothelial function was not influenced by the type of meal (low or high in fat), but was modestly greater when the postprandial state was
accompanied by mental stress. These findings challenge the ideas that fat consumption augments cardiovascular risk via an impact on stress responsiveness, and that acute mental stress is universally detrimental to endothelial function.

In a recent meta-analysis of the prognostic value of FMD, a 1% change in FMD (assessed via the same methodology used herein) was found to be associated with a 9% change in risk of cardiovascular events (95% confidence interval: 4% to 13%) (13). Thus the difference in FMD between stress and control conditions in the present work (~1.3% greater in stress vs control; chapter 5), while small, was appreciable. Although the association reported in the meta-analysis reflects chronic FMD levels, it also signifies that transient perturbations of endothelial function, even in the order of magnitude observed in the present study, may contribute importantly to the atherosclerotic process if the vascular insults occur frequently or result in long durations of endothelial dysfunction. Thus in healthy persons, acute stress may confer protection from a negative impact of food consumption and, as reported in chapter 5, this may be due in part to a stress-induced reduction in plasma phosphorus levels.

It has been suggested that reductions in flow-mediated vasoactivity may be responsible for previous observations of fat-consumption-induced amplification of stress responsiveness (i.e. endothelial dysfunction resulting in a relatively more vasoconstricted state that evokes augmented blood pressure and TPR responses to stress) (6, 17). However, in addition to observing no impact of meal (high or low in fat) on endothelial function (chapter 5), previous findings of fat-induced-enhancement of stress responsiveness were not replicated in the present study at any point in the 4 hour postprandial period (chapter 6). Moreover, repeated mental stress tasks had no impact on postprandial lipemia following either a high- or low-fat meal. This suggests that an impact of fat intake on stress responsiveness, or inversely of stress responses on postprandial lipemia, may not necessarily play a role in increasing cardiovascular risk in this population.
Notably, traditional cardiovascular risk factors (including family history, obesity, smoking, T2D and dyslipidemia) do not fully predict CVD (8, 29), and efforts to improve risk prediction are needed (27). Given that risk factors and “unhealthy” behaviours occur in combination in real life, studying their interactions may be beneficial in this regard. While the relationships between stress and consumption of a high-fat diet and development of CVD are well-established (23, 26), the underlying mechanisms remain difficult to isolate. Since stress and fat-consumption commonly occur together in everyday life (19), it is reassuring that their combined experience may not interact in a way that is detrimental for cardiovascular health.

9.1.2 Focus 1 Strengths and Limitations

These studies were the first to examine: 1) the impact of high-fat meal consumption and repeated bouts of mental stress on vascular endothelial function, 2) the time-course of a fat-stress response interaction, and 3) the effect of fat consumption on an appropriate index of hemodynamic stress “recovery”. In addition to utilizing specific meals and stress tasks known to evoke substantial postprandial and stress responses, several metabolic (glucose, triglycerides, insulin, phosphorus), hemodynamic (HR, MAP, SBP, DBP, TPR, CO) and hormonal (cortisol, norepinephrine) parameters were quantified in order to comprehensively characterize the stimuli and to interrogate stimulus-response relationships.

The principal limitation of these findings was the homogeneity of the subject pool. The objective of this work was to characterize responses in healthy persons, and women were excluded to remove potential variability introduced by cyclic variations in hormones. Therefore, results cannot be generalized to women [for whom there is evidence of protection from the vascular insult of a high-fat meal (15)], or older or diseased populations [who may be more susceptible to prolonged stress-response activation (24), or who may have greater propensity for fat-induced impairment (3, 10)]. Given that susceptibility to stress- and/or fat-induced decreases in endothelial function have been shown to depend on individual characteristics [e.g. habitual
physical activity (21), and hostile affect (12)], we cannot exclude the possibility that the recruited participants represent a subset of the population who inherently have reduced vulnerability to stress- and/or fat-mediated impairments. We also did not utilize a fasting control condition, and thus it is unknown to what extent repeated episodes of mental stress in the absence of the postprandial state may have impacted endothelial function in this subject population, or how the magnitude and duration of stress responses in the fasted state may have compared.

9.1.3 Focus 1 Future Directions

The pathways through which fat consumption and metal stress may affect cardiovascular health and function are complex. The lack of demonstrated consistency in the impact of mental stress or fat consumption on endothelial function highlights the need to investigate the mechanisms underlying the interactions between these factors, and the moderating influences of individual characteristics. It remains possible that fat and stress could interact in a detrimental fashion given a longer combined exposure (e.g. during a week of exams, or over the course of a stressful life event); future work is required to investigate the potential differences with respect to acute versus chronic encounters with these stimuli. Given the potential for other meal components (e.g. phosphorus) to influence the outcomes of interest, the impact of fat should be studied both in isolation [e.g. “oral fat tolerance test” (5)] and in the context of ecologically valid meals. Future work in larger samples will be required to tease out “phenotypes” with respect to vulnerability to stress and dietary fat intake, and to determine whether results can be generalized to women, and older or clinical populations.

9.2 FOCUS 2. Cardiovascular consequences of T2D: investigation of exercising muscle oxygen delivery and exercise tolerance in persons with T2D

9.2.1 Focus 2 Key Findings and Implications

Collectively, the findings of studies 3 and 4 (in chapters 7 and 8) demonstrated that there was no independent impact of T2D, within the constellation of co-morbidities and medications
that typically accompany this disease, on small muscle mass exercising muscle blood flow (in terms of the rate of adjustment, submaximal or maximal steady state) or exercise tolerance ($f_{CF_{impulse}}$), relative to matched Controls. During step increases in exercise intensity (from a resting or exercising baseline), a brief LBF amplitude impairment was observed in T2D (i.e. within the first 10 s), and this was due to a tendency for a blunted LVK. The functional relevance of this transient attenuation is unknown however, and might be expected to emerge only when multiple intensity-transitions are superimposed. These findings challenge the assertions that T2D impairs exercising muscle oxygen delivery beyond that which may already be present in the context of co-morbidities and that peripheral vascular control impairment contributes to reduced exercise tolerance in this population.

Cardiovascular fitness and functional capacity naturally decline with age (<5% per decade with an active lifestyle, and 9%+ if sedentary); the average maximal capacity for men aged 50-70 (average age in the present studies) is ~9-10 METs (or ~36 ± 22 ml O$_2$/kg/min) (7). In contrast, the aerobic fitness of the participants in studies 3 and 4 was disproportionately reduced (maximum ~8 METs; ~24 ml O$_2$/kg/min), in agreement with previous findings in this subject population [e.g. average peak capacity of 8.0 ± 2.1 METs in a sample of 2,082 men with T2D (22)]. Whether this disproportionate decline is related to habitual inactivity, endogenous disease-related dysfunction, or both, is unknown. Irrespective of the underlying cause, the consequence of the decrement in maximal aerobic capacity is that many activities of daily living are performed at a high proportion of an individual’s functional reserve and thus require a substantial level of exertion (1). For example, simply walking requires ~3-5 METs, mowing the lawn or shoveling snow 5-6 METs, and carrying a load (such as groceries) up a flight of stairs can require upwards of 8-9 METs (2). Avoidance of these activities can lead to a self-perpetuating cycle of reduced physical activity and further reductions in functional capacity (1). While small muscle mass exercising muscle blood flow and exercise tolerance were not impaired in persons
with T2D in the context of co-morbidities and standard medications in the present studies, the possibility remains that this group as a whole has blunted peripheral vascular responsiveness and associated impaired small muscle mass exercise tolerance relative to healthy (i.e. co-morbidity-free) age-matched Controls, potentially contributing to the reduced whole-body functional capacity, and thus these factors could still be viable targets for intervention.

9.2.2 Focus 2 Strengths and Limitations

These studies were the first to investigate whether there is an independent impact of T2D on vascular responsiveness during exercise and associated small muscle mass exercise tolerance within the context of co-morbidities and medications that typically accompany this disease, thus achieving ecological and external validity. Together, these studies comprised a comprehensive characterization of exercising muscle oxygen delivery in this population, including assessment of responses during both submaximal (low and moderate intensity isometric knee-extension) and maximal effort (fCF_{impulse}) exercise, and in terms of both the dynamic adjustment (from resting and exercising baselines) and steady state. Utilization of small muscle mass modalities enabled isolation of peripheral vascular contributions to exercising muscle blood flow.

The primary limitation of these studies was their small sample sizes. Recruitment of “representative” persons from a clinical population, together with matched controls, is particularly challenging; it requires identification of eligible persons who have both a willingness and ability to participate. While strict inclusion and exclusion criteria enable control of potential confounding factors, this results in rejection of potentially appropriate participants. There was considerable heterogeneity in oxygen delivery and exercise tolerance responses in the present work, and the small sample sizes precluded sub-group analysis (e.g. division of participants into “impaired” vs “unimpaired” cohorts based on fCF_{impulse} values). However, the sample sizes do not prevent the important conclusions allowed by the present data: that considerable inter-individual variability exists, but that there are no unambiguous or uniform differences at the
group level in small muscle mass exercise hyperemia (LBF and FBF) and associated exercise tolerance (fCF\textsubscript{impulse}) responses.

The findings are also limited to men, and there is some evidence that women with T2D may have exaggerated exercise intolerance [e.g. greater reductions in \(\dot{V}O_2\text{peak}\) (22)], although this is consistent with typical sex differences in \(\dot{V}O_2\text{peak}\) (attributed to smaller muscle mass, lower hemoglobin and blood volume, and smaller stroke volume in women vs men) (7). The decision to enroll only men was primarily logistical; the use of baseline femoral artery diameter to quantify exercise hyperemia required that resting diameters be at least 0.79 cm (11), and on average men have larger femoral arteries than women (in proportion to larger body size) (11). \(\dot{V}O_2\) kinetics have been shown to be similar in men and women with T2D however (20), suggesting that sex differences in oxygen delivery within this population are unlikely.

9.2.3 Focus 2 Future Directions

Given the large inter-individual variability in exercise hyperemia and exercise tolerance responses, future studies in larger samples (including men and women) are necessary to more definitively establish the presence or absence of T2D-specific impairments, and to identify whether impaired and unimpaired cohorts exist within this population. Additional investigations are required to uncover the mechanism(s) responsible for the initial amplitude impairment in rest-to-exercise and exercise-to-exercise transitions in T2D, and to determine whether a functionally relevant impact on exercise tolerance accumulates with multiple workload transitions. Whether there exists a cardiac-mediated limitation to oxygen delivery during whole body exercise in T2D (due to increased sympathetic nervous system-mediated vasoconstriction to defend blood pressure secondary to reduced cardiac output, and/or augmented cardiac sympathetic afferent reflex activity) remains to be established.

It is standard practice to assess vascular responsiveness under fasting conditions (28). However, humans spend the majority of the time in the postprandial state (16), and thus exercise
is likely to occur superimposed on digestive, absorptive and anabolic metabolic processes. Given that persons with T2D have “postprandial dysmetabolism” (i.e. exaggerated and prolonged hyperglycemia and hyperlipidemia) (30), the postprandial state may be particularly relevant in this regard. Indeed, others have shown that exercise hyperemia is attenuated in obese, insulin-resistant individuals when exercise is performed in hyperinsulinemic conditions (14). Moreover, if fat intake impairs endothelial function (chapter 3), and if this lessens exercise hyperemia (18), then exaggerated postprandial lipemia in persons with T2D after consuming a meal could disproportionately reduce exercise hyperemia and exercise tolerance. Therefore future studies of vascular responses in non-fasting conditions are warranted in this population. Note that since there is wide variability in postprandial responses [both within healthy persons (9, 24) and those with T2D (4)], normalizing exercise hyperemia responses to changes in metabolic parameters in the postprandial period, and/or performing regression analysis, may control for inter-individual response differences, and provide insight into their underlying mechanisms.

9.3 Summary of PhD Research Experience

The candidate gained extensive research experience in the course of this dissertation. She reviewed the literature in diverse areas (both informally and formally for the generation of review papers), and based on the current state of knowledge formulated important research questions and designed and executed complex protocols to answer them. For instance, in the study described in chapters 5 and 6, a single experimental session lasted ~5 hours, with measurements taken at precise time points throughout. The candidate managed the projects from conception to completion, including: pilot work, experimental design, participant recruitment, data collection and analysis, and manuscript preparation.

These protocols required considerable innovation. For example, the candidate adapted stress tasks from the literature and created “stress programs” that were designed to minimize habituation over repeated stressful episodes, and co-developed the custom leg ergometer for
performance of isometric leg exercise. She pioneered the collaboration with personnel at the Cardiac Rehabilitation Centre at Hotel Dieu Hospital for purposes of participant recruitment and medical screening. She mastered several measurement and analysis techniques, including: venipuncture, blood and saliva sample preparation (centrifugation, separation into aliquots) and analysis (enzyme-linked immunosorbent assays, use of cone-plate viscometer and blood gas analyzer), and Doppler and Echo ultrasound of the brachial and femoral arteries (including application in a challenging population characterized by abdominal adiposity). In addition, studies 3 and 4 (chapters 7 and 8) were conducted as part of a larger protocol (not included in this dissertation) that involved a double-blind placebo-controlled intervention trial, for which the candidate coordinated all associated logistics (e.g. dosing protocol, sourcing and procuring materials, double-blind group assignment).

During data collection, the candidate coordinated and directed teams of up to 6 individuals, including a research nurse, “stress panel” members, computer operator, and personnel for technical or measurement assistance. Throughout data collection she established a positive rapport with study participants and maintained 100% retention over multiple laboratory visits and ~5 hours (studies 3 and 4) and ~22 hours (studies 1 and 2) of invasive data collection per participant. The candidate completed complex data analysis, including data filtering, curve-fitting, and statistics such as mixed-design 2- and 3-way repeated measures analyses of variance and bootstrapping. All data included in this dissertation are original data collected by the candidate. Finally, the candidate participated in dissemination of knowledge via manuscript publication and presentation of findings as a guest lecturer in undergraduate classes as well as at national and international academic conferences.

9.4 Summary and Conclusions

The findings of this dissertation challenge the assertions that mental stress and fat consumption are universally detrimental, and that T2D, on top of the typical constellation of co-
morbidities and medications, has an impact on exercising muscle blood flow and exercise tolerance. The specific conclusions are as follows. In young, healthy men: 1) Repeated mental stress tasks had no impact on postprandial lipemia in the 4 h period following a meal (high or low in fat); 2) Meal fat content did not significantly impact hemodynamic reactivity to, or recovery from, varied mental stress tasks over a 4 h postprandial period; and 3) Meal fat content did not affect FMD, but endothelial function was modestly greater when the postprandial state was accompanied by mental stress. Within the typical constellation of co-morbidities and medications that accompany T2D: 1) Small muscle mass exercise tolerance ($f_{CF_{impulse}}$) was not impaired in T2D relative to matched Controls; 2) During exercise at $f_{CF_{impulse}}$, forearm muscle blood flow (and its determinants, FVK and MAP) did not differ between T2D and Control groups; and 3) While the initial amplitude of LBF in response to step increases in exercise intensity was attenuated in T2D versus Control, there was no robust or consistent impact of T2D on the overall dynamic adjustment of LBF or the steady state levels ultimately achieved in rest-to-exercise or exercise-to-exercise transitions. This work is an important step in the exploration of contributions to, and consequences of, impaired cardiovascular health and function.
9.5 References


The impact of acute mental stress on post-prandial lipemia and endothelial function

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

June 9, 2010

Dr. K.E. Pyke
School of Kinesiology and Health Studies
28 Division Street
Queen's University

Dear Dr. Pyke,

Study Title: The impact of acute mental stress on post-prandial lipemia and endothelial function
Co-Investigators: Ms. Veronica Poltras

The members of the Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board have examined the protocol (April 2010) and the revised consent form (Version 2 - 10/06/2010) for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair's signature below. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study;

➢ Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. (see http://www.queensu.ca/vpr/reb.htm).

➢ Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

➢ Reporting of Complaints: Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

➢ Annual Renewal: Prior to the expiration of your approval (which is one year from the date of the Chair’s signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

[Signature]
Chair, Research Ethics Board

Date: June 10, 2010

ORIGINAL TO INVESTIGATOR - COPY TO DEPARTMENT HEAD - COPY TO HOSPITALS - FILE COPY

Study Code: PHE-103-10

➢ Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete
QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards as defined by the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S. DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number: #FWA00004184
#IRB00001173

Current 2010 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board

Dr. A.F. Clark
Emeritus Professor, Department of Biochemistry, Faculty of Health Sciences, Queen's University (Chair)

Dr. H. Abdollah
Professor, Department of Medicine, Queen's University

Dr. M. Evans
Community Member

Dr. S. Irving
Psychologist, Providence Care, St. Mary's of the Lake Hospital Site

Dr. L. Keeping-Burke
Assistant Professor, School of Nursing, Queen's University

Dr. J. Low
Emeritus Professor, Department of Obstetrics and Gynaecology, Queen's University and Kingston General Hospital

Ms. D. Morales
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Emeritus Professor, Department of Pharmacology & Toxicology, Queen's University

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Assistant Professor, Department of Anesthesiology, Queen's University

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WHO Professor in Psychosomatic Medicine and Psychopharmacology
Professor of Psychiatry and Pharmacology
Chair and Head, Division of Psychopharmacology, Queen's University
Director & Chief of Psychiatry, Academic Unit, Quinte Health Care, Belleville General Hospital

Dr. E. Tsai
Associate Professor, Department of Pediatrics and Office of Bioethics, Queen's University

Rev. J. Warren
Community Member

Ms. K. Weisbaum
LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)

Dr. S. Wood
Director, Office of Research Services (Ex-Officio)
Dysfunction of Exercising Muscle Oxygen Delivery and Utilization in Type II Diabetes

QUEEN'S UNIVERSITY HEALTH SCIENCES AND AFFILIATED TEACHING HOSPITALS
RESEARCH ETHICS BOARD ANNUAL RENEWAL

Queen's University, in accordance with the "Tri-Council Policy Statement 2, 2010" prepared by the
Interagency Advisory Panel on Research Ethics for the Canadian Institutes of Health Research, Natural
Sciences and Engineering Research Council of Canada and Social Sciences and Humanities Research Council
of Canada requires that research projects involving human participants be reviewed annually to determine their
acceptability on ethical grounds.

A Research Ethics Board composed of:

Dr. A.F. Clark, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University
(Chair)
Dr. H. Abdollah, Professor, Department of Medicine, Queen's University
Dr. C. Cline, Assistant Professor, Department of Medicine, Director, Office of Bioethics, Queen's University,
Clinical Ethicist, Kingston General Hospital
Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University
Dr. M. Evans, Community Member
Ms. J. Hudac, Community Member
Dr. B. Kislevsky, Professor, School of Nursing, Departments of Psychology and Obstetrics and Gynaecology,
Queen's University
Dr. J. MacKenzie, Pediatric Geneticist, Department of Paediatrics, Queen's University
Mr. D. McNaughton, Community Member
Ms. P. Newman, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy
Services, Kingston General Hospital
Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate,
Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute
Dr. A. Singh, Professor, Department of Psychiatry, Queen's University
Ms. K. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)

has reviewed the request for renewal of Research Ethics Board approval for the project Dysfunction
of Exercising Muscle Oxygen Delivery and Utilization in Type II Diabetes as proposed by Dr. Michael E.
Tschakovsky of the School of Kinesiology & Health Studies, at Queen's University. The approval is
renewed for one year, effective November 03, 2013. If there are any further amendments or changes to the
protocol affecting the participants in this study, it is the responsibility of the principal investigator to notify the
Research Ethics Board. Any unexpected serious adverse event occurring locally must be reported within 2
working days or earlier if required by the study sponsor. All other adverse events must be reported within 15
days after becoming aware of the information.

[Signature]
Date: October 16, 2013
Chair, Health Sciences Research Ethics Board
Renewal 1[ ] Renewal 2[ ] Extension [X] Code# PHE-083-08 Romeo file# 6004990

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Appendix B

Comprehensive summary of studies that examined the effect of fat consumption on endothelial function in humans
<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Effect of FC on EF</th>
<th>Key Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al., 2001 (2)</td>
<td>7 M, 5 F with T2D, ~47 years; 5 M, 7 F healthy</td>
<td>FMD, oxidative stress (lipid-derived free radicals, lipid peroxidation</td>
<td>↓</td>
<td>FMD ↓ from baseline at 4 h postprandial (~6.3 to 4.7% in controls; ~2.7 to 1.5% in T2D); GTN-mediated vasodilation not changed</td>
</tr>
<tr>
<td></td>
<td>controls, ~43 years</td>
<td>products and glyceryl trinitrate (GTN)-mediated vasodilation were measured before and 4 and 8 h after a HFM</td>
<td></td>
<td>↓ FMD correlated inversely with fasting HDL $(r = -0.84)$ in both groups</td>
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<td></td>
<td></td>
<td>Meal: homogenized milk-shake (1,480 kcal, 80 g saturated fat)</td>
<td></td>
<td>Postprandial FMD did not correlate with AUC postprandial plasma TG</td>
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<td></td>
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<td>Oxidative stress ↑ postprandially in both groups</td>
</tr>
<tr>
<td>Anderson et al., 2006 (1)</td>
<td>14 M, 6 F with T2D; 54 ± 9 years</td>
<td>Following 2 days of placebo or vitamin C therapy (1 g/day; double-blind cross-over), FMD, lipid profiles, and oxidative stress (venous free radicals) were measured before and 4 and 8 h after a HFM</td>
<td>↓ less following vitamin C</td>
<td>Pre-treatment, FMD fell from ~1.3% at baseline to 0.22% at 4 h and 0.7% at 8 h following HFM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meal: homogenized milk-shake (1,480 kcal, 80 g saturated fat)</td>
<td></td>
<td>Placebo: no effect on FMD or oxidative stress</td>
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<td></td>
<td>Vitamin C: ↑ baseline FMD (to ~3.8%) and attenuated postprandial ↓ (still reduced to ~2.8% and 2.9% at 4 and 8 h); attenuated oxidative stress at 4 h</td>
</tr>
<tr>
<td>Ayer et al., 2010 (3)</td>
<td>7 M, 4 F, obese 7 M, 4 F, normal weight 32 ± 5 years</td>
<td>FMD was measured before and 3 h following a HFM</td>
<td>↔</td>
<td>FMD not different between obese and normal weight individuals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meal: slice of carrot cake and a milkshake (1,000 kcal, 60 g fat)</td>
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<tr>
<td>Bae et al., 2001 (4)</td>
<td>10 M, 10 F; healthy; 56 ± 6 years</td>
<td>FMD, serum TG, and oxidative stress (superoxide anion formation) were measured after an overnight fast and 2 h after a HFM or LFM (between-groups comparison)</td>
<td>↓</td>
<td>HFM: Serum TG and oxidative stress increased; FMD decreased from ~13.7 to 8.2% at 2 h after HFM</td>
</tr>
<tr>
<td></td>
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<td>Meals – HFM: rice, Korean barbecue, egg, milk, oil, mayonnaise, vegetables (803 kcal, 53.4 g fat); LFM: rice, vegetable soup, vegetables, orange juice, apple, kimchi (802 kcal, 3 g fat)</td>
<td></td>
<td>LFM: no significant changes in TG, oxidative stress, or FMD</td>
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<td></td>
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<td>ΔTG correlated negatively with ΔFMD $(r = -0.650)$, and positively with Δ oxidative stress production $(r = 0.798)$</td>
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<td></td>
<td>Δ oxidative stress production correlated negatively with ΔFMD $(r = -0.784)$</td>
</tr>
<tr>
<td>Bae et al., 2003 (5)</td>
<td>10 M; healthy; 26 ± 1 years</td>
<td>On separate days, subjects’ FMD, serum TG, and malondialdehydes (MDA; lipid oxidation products) were measured before and 2, 4 and 6 h after a HFM, LFM, or HFM + antioxidant vitamin E (800 IU)</td>
<td>↓ following LFM and HFM + vitamin E</td>
<td>HFM: FMD ↓ from baseline at 2 and 4 h (~13.1% to 6.6% and 7.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meals same as in Bae et al., 2001 (4)</td>
<td></td>
<td>LFM or HFM + Vitamin E: ↔ FMD</td>
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<tr>
<td></td>
<td></td>
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<td>HFM and LFM: Inverse correlation between ΔFMD and ΔTG at 2 h postprandial $(r = -0.54)$</td>
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<td>All meals: No significant change in serum MDA</td>
</tr>
<tr>
<td>Reference</td>
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</tbody>
</table>
| Berry et al., 2008 (6)    | 17 M; healthy; 27 ± 5 years | **FMD and oxidative stress (plasma 8-isoprostane F2α)** were measured before and 3 h after two fatty test meals (randomized crossover design) **Meals:** 2 muffins and a milkshake, containing 853 kcal, 50 g of fat: 1) stearic acid (SA; shea butter blend, saturated fatty acid, known to result in blunted postprandial increases in TG), 2) oleic acid (HO; high oleic sunflower oil, monounsaturated fatty acid) | ↓ following HO meal ↔ following SA meal | • Postprandial ↑ in plasma TG was lower (66% lower incremental AUC) following SA meal than HO meal  
• HO meal: oxidative stress ↑ and FMD ↓ (~7.3 to 4.3%)  
• SA meal: oxidative stress and FMD ↔ (FMD ~7.3 to 6.0%)  
• No correlation between ΔFMD and ΔTG at 3 h |
| Borucki et al., 2009 (7)  | 8 M, 7 F; healthy; 25 ± 2 years | **FMD and serum TG were determined before and hourly for 6 h after a HFM with/without 2.5 g L-arginine (crossover design) **Subset (n = 11): protocol repeated with 2.5 g phenylalanine and 5 g leucine (amino acids that evoke the same insulin response as arginine, but without providing the arginine substrate for NO synthesis)  
**Subset (n = 7): tested effect of 2.5 g L-arginine on FMD in the absence of the HFM  
**Meal:** whipping cream, 3 ml (1 g fat)/ kg body weight | ↓ ↔ with addition of L-arginine to HFM | • HFM alone: FMD ↓ from ~8% at baseline to ~4.25% and 4% at 2 and 3 h postprandial and not significantly different from baseline thereafter  
• HFM + L-arginine: no change in FMD over time; FMD greater than HFM alone at 2 and 3 h postprandial  
• Phenylalanine and leucine did not prevent ↓ in FMD post-HFM  
• L-arginine alone: No acute effect on FMD |
| Burton-Freeman et al., 2012 (8) | 13 M, 12 F; healthy; 27 ± 8 years | **FMD, inflammatory cytokines (IL-6) and lipids were measured before and 3.5 h after HFMs containing either processed tomato product or non-tomato alternative (crossover design)  
**Meals:** Tomato Meal: bagel with cream cheese; tomato paste; whole, rice, and coconut milk; corn oil; apple juice (848 kcal, 44 g fat). Control Meal: bagel with cream cheese; potato; fiber supplement; instant non-fat dry, whole, rice, and coconut milk; corn oil; salt, salt substitute; apple juice with Vitamin C; sugar (852 kcal, 43 g fat) | ↔ | • TG was significantly > following the Tomato Meal vs Control at 4 h postprandial  
• The postprandial ↑ in IL-6 was significantly attenuated in Tomato Meal vs Control  
• FMD did not change significantly in the postprandial period and was not different between meals |
<table>
<thead>
<tr>
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<th>Effect of PC on EF</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ceriello et al., 2002 (10)</td>
<td>22 M, 18 F with T2D</td>
<td>FMD, blood glucose and TG, and nitrotyrosine (NT; used to infer production of peroxynitrite, a product of ROS-inactivated NO) were measured before and hourly for 4 h after a HFM with/without an oral glucose tolerance test (OGGT; 75 g glucose)</td>
<td>↓ FMD; ↓ less following short- or long-term simvastatin treatment</td>
<td>HFM and OGGT alone ↓ FMD and ↑ NT (in control and T2D subjects); effects more pronounced when HFM + OGGT were combined</td>
</tr>
<tr>
<td></td>
<td>12 M, 8 F, healthy controls</td>
<td>T2D patients were retested after 3 to 6 days or 3 months of simvastatin treatment (40 mg/d, to assess effect of inhibition of oxidative stress in the short-term and of decreased postprandial hypertriglyceridemia in the long-term) → cross-over placebo-controlled design with 3-mo wash-out period between drug/placebo</td>
<td></td>
<td>Short-term simvastatin treatment: no effect on lipid parameters, but reduced effect on FMD and NT</td>
</tr>
<tr>
<td></td>
<td>54 ± 3 years</td>
<td>Meals: 1) HFM (whipping cream; 75 g fat, 700 kcal/m² body surface area); 2) OGGT; 3) HFM + OGGT</td>
<td></td>
<td>Long-term simvastatin treatment: lower increase in postprandial TG and NT, and attenuated reduction in FMD</td>
</tr>
<tr>
<td>Ceriello et al., 2005 (9)</td>
<td>12 M, 8 F with T2D</td>
<td>FMD, endothelium-independent dilation (sublingual nitroglycerine), blood glucose and TG, NT and inflammatory markers (C-reactive protein, ICAM-1, IL-6) were measured before and hourly for 4 h after a HFM, OGGT, or HFM+OGGT</td>
<td>↓ FMD; ↓ less following 5-7 day treatment with atorvastatin, irbesartan or both</td>
<td>HFM: ↓ FMD and ↑ NT and inflammatory markers at 1-4 h post-meal</td>
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<tr>
<td></td>
<td>12 M, 8 F, healthy controls</td>
<td>Tests were performed again after 5-7 days of treatment with 40 mg/day atorvastatin (statin to lower blood cholesterol), 300 mg/day irbesartan (ang II receptor inhibitor to lower blood pressure), both, or placebo (random treatment assignment, 2 week washout period between each treatment)</td>
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<td>OGGT; ↓ FMD and ↑ NT and inflammatory markers at 1-3 h post-meal</td>
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<tr>
<td></td>
<td>52 ± 3 years</td>
<td>Meals: 1) HFM alone (whipping cream; 75 g fat, 700 kcal/m² body surface area); 2) OGGT; 3) HFM + OGGT</td>
<td></td>
<td>HFM+OGGT: greater ↓ in FMD and ↑ in NT and inflammatory markers than either HFM/OGGT alone</td>
</tr>
<tr>
<td>Cortes et al., 2006 (11)</td>
<td>11 M, 1 F, with hypercholesterolemia, 45 ± 13 years;</td>
<td>FMD, blood lipids and glucose, and asymmetric dimethylarginine (ADMA) were measured before and 4 h after a HFM containing 25 ml olive oil or 40 g walnuts (randomized crossover design)</td>
<td>↓ FMD at 1 h post- OGTT</td>
<td>Short term atorvastatin treatment or irbesartan, alone or in combination: No effect on lipid parameters or blood pressure, but significant improvements in basal FMD and significant attenuation of postprandial ↓ FMD (greatest improvement with combination therapy)</td>
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<td></td>
<td>9 M, 3 F, healthy, 32 ± 8 years</td>
<td>Walnuts are a source of antioxidants, L-arginine, alpha-linolenic acid, and a plant n-3 polyunsaturated fatty acid</td>
<td>↓ FMD in control group, walnut; ↓ FMD both groups, olive oil ↑ hypercholesterolemic, walnut</td>
<td>Endothelium-independent vasodilation not affected during the studies</td>
</tr>
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<td></td>
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<td>Meals: ~1,200 kcal (63% fat), 80 g fat. White bread, salami, cheese, yogurt, and water ad libitum, with either 25 ml olive oil or 40 g walnuts.</td>
<td></td>
<td>FMD was worse after olive oil meal than walnut meal (4 h FMD for olive oil vs walnut meals: Control group, ~3.9% vs 7.7%; Hypercholesterolemic group, ~2.3% vs 3.6%)</td>
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<td>FMD ↔ from fasting to 4 h after walnut meal in Control group, whereas it ↑ in hypercholesterolemic group (~4.1% to 5.1%)</td>
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<td>Endothelium-independent dilation ↔ in either group or meal condition (method of assessment not stated)</td>
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<td></td>
<td>Fasting TG concentrations correlated inversely with baseline FMD (r = -0.324)</td>
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<td>Plasma ADMA concentrations ↔, and concentration of oxidized LDL ↓ after both meals</td>
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<tr>
<td>Reference</td>
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<td>Protocol</td>
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<td>Key Results</td>
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<tr>
<td>Djousse et al., 1999</td>
<td>7 M, 6 F; healthy; 32 ± 9 years</td>
<td>FMD, endothelial-independent vasodilation (sublingual nitroglycerine), and TG were assessed before and at 2, 4 and 6 h after a HFM with red wine or an isocaloric beverage on 2 separate days (crossover design)</td>
<td>↔ (non-significant trend for ↓ with/without red wine)</td>
<td>No differences between HFM + wine and HFM + control beverage conditions</td>
</tr>
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<td></td>
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<td>Meal: Scaled to provide 0.8 g fat and 15 kcal per kg of body weight; burger (Whopper) with cheese and French fries (Burger King), and 3 ml/kg red wine or coke (isocaloric).</td>
<td></td>
<td>TG ↑ 2- to 2.7-fold over baseline, with peak at 5 h after HFM (± 2.5 mmol/L)</td>
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<td>Non-significant trend for ↓ FMD following both meals (FMD at baseline and 2, 4, and 6 h postprandial, in HFM + control: -9.5, 7.9, 6.8 and 7.3%, and in HFM + wine: -8.0, 5.7, 6.4, and 6.9%)</td>
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<td></td>
<td>Vasodilator response to nitroglycerine not affected by HFM or either beverage</td>
</tr>
<tr>
<td>Evans et al., 2000 (13)</td>
<td>11 M, 9 F; mean 47.5 years (range 37 to 53)</td>
<td>FMD and oxidative stress were measured after fasting and 4 h after a HFM, before, and after 3 months of treatment (ciprofibrate or placebo)</td>
<td>↓</td>
<td>HFM pre-treatment: ↓ FMD from fasting to 4 h postprandial (~3.8% to ~1.8%)</td>
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<td>Ciprofibrate → a lipid-regulating agent (upregulates lipoprotein lipase activity and thus decreases circulating VLDL and TG while increasing HDL)</td>
<td></td>
<td>After ciprofibrate: ↑ FMD at fasting and 4 h post-meal (~4.8 and 3.4%); ↓ fasting and postprandial TG (3.1 and 6.6 mmol/L to 1.5 and 2.8 mmol/L); ↑ HDL; ↔ total and LDL cholesterol; ↑ in oxidative stress significantly attenuated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meal: liquid fatty meal with 80 g saturated fat (specific composition not provided)</td>
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<tr>
<td>Fahs et al., 2010 (14)</td>
<td>10 M, 10 F; healthy; 25 ± 3 years</td>
<td>FMD was measured before and 4 h after a HFM with either placebo or fish oil supplement (~1 g omega 3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA))</td>
<td>↓</td>
<td>HFM + placebo: ↓ FMD normalized for shear rate</td>
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<tr>
<td></td>
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<td>Meal: choice of – 1) Big Mac and large French Fries (1,042 kcal, 54 g fat), or 2) Quarter Pounder with cheese and large French Fries (1,011 kcal, 51 g fat)</td>
<td></td>
<td>HFM + fish oil supplement: FMD normalized for shear rate was not different from baseline post-meal</td>
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<td>The HFM had no effect on central blood pressure or arterial stiffness</td>
</tr>
<tr>
<td>Fard et al., 2000 (15)</td>
<td>34 M, 16 F; with T2D 62 ± 9 years</td>
<td>FMD, plasma ADMA and lipids were measured at baseline and 5 h after a HFM</td>
<td>↓</td>
<td>HFM: ↑ plasma ADMA, ↓ FMD (~7% to ~1%), ↑ plasma TG and VLDL, ↓ LDL and HDL, ↔ total cholesterol</td>
</tr>
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<td>Subset (n = 10): protocol was repeated on a separate day with a non-fat isocaloric meal</td>
<td></td>
<td>↑ in plasma ADMA following HFM was significantly and inversely related to the ↓ in FMD (r = -0.37)</td>
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<tr>
<td></td>
<td></td>
<td>Meals – HFM: heavy cream, ice cream, safflower oil, powdered whey protein, Lactaid (1,265 kcal, 105 g fat). Non-fat Meal: whey protein, skim milk, evaporated skim milk, syrup, sugar (1,265 kcal, 0 g fat).</td>
<td></td>
<td>Non-fat meal: no changes in FMD or ADMA</td>
</tr>
<tr>
<td>Reference</td>
<td>Participants</td>
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| Funada et al., 2002 (16) | 24 normolipidemic subjects (14 M, 10 F; 13 healthy and 11 with mild hypertension); 50 ± 10 years | - FMD and serum lipids and lipoproteins were assessed during fasting at 4 h after a HFM  
- Subjects were divided into 2 groups: “high responders” [postprandial remnant-like particle cholesterol (RLP-C) of >7.5 mg/dl, n = 8], and “normal responders” (postprandial RLP-C level ≤ 7.5 mg/dl, n = 16)  
**Meal**: 40 g cream / m² of body surface area; mean of 24 g of fat per meal (35% fat) | ↔ | - Following HFM, TG and RLP-C increased significantly in high responders (for high and normal responders respectively: TG, ~1.31 to 1.84 mmol/L and 1.21 to 1.31 mmol/L; RLP-C, ~5.8 to 9.1 mg/dl and 5.0 to 5.7 mg/dl)  
- Basal FMD significantly lower in high responders than normal responders (~4.3 vs 8.3%)  
- After HFM, FMD did not change significantly in either group (4 h postprandial values of ~4.2% and 8.6% in high and normal responders)  
- Change in RLP-C levels from fasting to 4 h post-HFM correlated significantly with basal FMD (\( r = -0.588 \)) |
| Gaenzer et al., 2001 (17) | 17 M; healthy; 36 ± 4 years | - FMD and blood lipids were measured after an overnight fast (08:00) and 4 (12:00) and 8 h (16:00) later either in a continued fasting state or following a HFM (randomized crossover design)  
**Meal**: heavy whipping cream; 730 kcal and 65 g fat per m² body surface area | ↓ | - FMD showed diurnal variation with higher values in noon and afternoon hours compared with morning  
- At 12:00, post-HFM FMD was lower than continued fasting  
- Postprandial FMD correlated inversely with magnitude of postprandial lipemia (\( r = -0.81 \) for 4 h and \( r = -0.57 \) for 8 h postprandial). No correlation between postprandial FMD and fasting TG levels.  
- In multivariate analysis, lipemia (8 h TG AUC) was associated with impaired postprandial FMD independent of fasting TG, LDL and HDL cholesterol, insulin, age and BMI  
- 4 h TG levels varied widely between ~0.79 to 4.78 mmol/L; mean ~2.5 mmol/L  
- A lipemia threshold of ~9.12-10.83 mmol/L·8 h TG AUC appears to define a degree of postprandial lipemia above which endothelial function is clearly impaired |
| Giannattasio et al., 2005 (18) | 10 M, 6 F with moderate hypertriglyceridemia and hypercholesterolemia; 7 M, healthy; 46 ± 3 years | - Radial artery FMD (hand occlusion) and reactive hyperemia (RH) were measured before and 6 h after a HFM in mild dyslipidemic hypertriglyceridemic subjects and healthy controls  
- Subset (\( n = 6 \) hypertriglyceridemic patients): protocol was repeated in continued fasting conditions (time-control group)  
**Meal**: 680 kcal/m² of body surface with 83% fat | ↓ | - In mild dyslipidemic hypertriglyceridemic subjects, HFM did not alter RH induced by 4-min hand ischemia, whereas it reduced FMD (increase in artery diameter of ~0.31 vs 0.13 mm for baseline vs 6 h postprandial)  
- ↓ in FMD (expressed as absolute diameter change) correlated with ↑ in serum TG post-HFM (\( r = 0.49 \))  
- No change in FMD in control and fasting time-control group |
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| Gokce et al., 2001 (19) | Protocol 1: 8 M, 6 F; healthy; 30 ± 9 years | • Protocol 1: FMD was measured before and 2, 4 and 6 h following a HFM or LFM  
• Protocol 2: To assess the effects of TG elevation in isolation from hyperinsulinemia, FMD, nitroglycerin-mediated dilation, and FBF were assessed before and after a 2-h infusion of a triglyceride emulsion (with or without heparin – to release lipoprotein lipase and increase circulating FFAs)  
• Protocol 3: Isolated rabbit aortic rings were exposed to the TG emulsion to test whether TG have a direct effect on conduit artery tone  
*Meals for Protocol 1 – HFM: scrambled eggs, butter, cheese, sugar, white bread, bacon (1,067 kcal, 56 g fat). LFM: apple sauce, oatmeal cereal, non-fat milk, white bread, decaffeinated coffee, sugar (1,042 kcal, 18 g fat)* | ↓ but baseline vessel size increased postprandially, with no change in peak diameter | • Protocol 1: FMD ↓ from baseline to 2 h postprandial (~14.7 to 10.6%), and remained depressed at 4 and 6 h; ↓ was associated with a 6% ↑ in baseline brachial artery diameter (~3.5 to 3.7 mm), but ↔ in arterial diameter during hyperemia. Serum TG and insulin ↑ post-HFM by 94% and 438% respectively (peak TG ~1.60 mmol/L at 4 h), and ΔFMD from baseline correlated with both ΔTG (r = -0.31) and Δinsulin (r = -0.33).  
• Protocol 2: TG emulsion increased serum TG 197% but had no effect on insulin. Brachial artery diameter increased 4% from ~3.68 to 3.81 mm, and forearm flow increased 36%, reflecting vasodilation of forearm resistance vessels. FMD and nitroglycerin-mediated dilation unaffected.  
• Protocol 3: TG emulsion had no direct dilator effect on rabbit aortic tissue *in vitro*. |
| Gudmundsson et al., 2000 (20) | 11M, 4 F; healthy; 29 ± 2 years | • Endothelial-dependent and -independent function in forearm resistance vessels were assessed via blood flow responses to local intra-arterial infusion of endothelial-dependent (Ach, bradykinin) and –independent (nitroprusside, verapamil) vasodilators 3 h after isocaloric HFM and LFMs on 2 separate days (crossover design)  
*Meals – HFM: English muffin, egg, bacon, cheese, hash brown, corn oil, salt, margarine, butter, pork sausage, decaffeinated coffee (~910 kcal, 50 g fat). LFM: Kellogg’s raisin bran, sugar, skim milk, non-fat milk powder, grape juice (~900 kcal, 4 g fat)* | ↔ (relative to LFM; no comparison to pre-meal function) | • Serum TG ↑ from ~1.26 to ~1.86 mmol/L 4 h after HFM (significantly larger ↑ than after LFM)  
• No differences in endothelium-dependent or –independent vascular function between HFM and LFM |
| Harris et al., 2012 (21) | 10 M, 15 F; healthy; 20-24 years | • FMD, shear rate, and 17β-estradiol were determined at baseline and 4 h after a HFM in young women during the menses (ME), follicular (FO), and luteal (L) phases of the menstrual cycle. Male control participants were studied once.  
*Meal: Egg McMuffin, Sausage McMuffin, 2 hash brown patties (940 kcal, 48 g fat)* | ↔ in women ↓ in men | • 17β-Estradiol was elevated during FO and L phases compared to ME phase  
• FMD was elevated in the FO and L phases (~12 and ~11% respectively vs 8% in ME), with no change in shear rate  
• Female postprandial FMD was unchanged from baseline throughout the menstrual cycle  
• Men had 17β-estradiol levels not different from women during ME; men had 50% ↓ in FMD postprandially (~6 to 3%)  
• Postprandial FMD response was not associated with concentrations of either 17β-estradiol or progesterone |
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| Johnson et al., 2011 (22) | 7 active, 7 inactive (4 M, 3 F per group); groups matched for BMI, age, sex; 23-33 years | • Plasma TG, antioxidant activity (superoxide dismutase activity; SOD), oxidative stress (thiobarbituric reactive substances; TBARS) and FMD were measured at baseline and 4 h after a HFM, in active and inactive adults  
• Active (ACT) = participating in physical activity 3+ times/week for at least 30 min/session at an intensity of moderate or greater  
• Inactive (INA) = less than 30 min of moderate physical activity/week  
Meal: Egg McMuffin, Sausage McMuffin, 2 hash brown patties (McDonald’s) and water ad libitum (~940 kcal, 48 g fat) | ↓ in INA  ↔ in ACT | • TG increased significantly following HFM in INA and ACT (~4 vs 8 and 2 vs 3 mmol/L respectively, groups not significantly different)  
• Baseline oxidative stress (TBARS) was greater in ACT than INA, and remained unchanged following HFM in both groups  
• ACT exhibited greater antioxidant activity than INA at baseline and following HFM  
• Postprandial FMD was decreased in INA (~9 vs 6%), but was unchanged in ACT (~8 vs 9%)  
• No significant correlations among % ∆ from baseline in TG, TBARS, SOD, FMD (groups pooled) |
| Koulouris et al., 2010 (23) | 18 M, 3 F; healthy; 33 ± 11 years                | • Serum lipoproteins and FMD were assessed before and 2 and 4 h after one of 4 meals containing 1,000 kcal and 50 g of fat each, with different fat sources: Meal A) saturated fat (yellow cheese), Meal B) monounsaturated fat (olive oil), Meal C) polyunsaturated fat (margarine), Meal D) same as meal A but with addition of 250 ml red wine  
Meals – A) cheese, boiled vegetable marrow, potatoes, bread, apple; B) olive oil 2% yogurt, egg white, boiled vegetable marrow, bread, apple; C) margarine, 2% yogurt, egg white, boiled vegetable marrow, bread, apple; D) same as A but with addition of red wine | ↓ saturated fat ↔ mono- and polyunsaturated ↓ saturated fat + red wine, but baseline diameter increased | • All 4 meals significantly raised plasma TG  
• Meal A (saturated fat): FMD ↓ at 2 and 4 h after (~16.5% to 13.4% and 12.3% respectively)  
• Meals B and C (monounsaturated and polyunsaturated fat): no effect on FMD  
• Meal D (saturated fat + red wine): FMD ↓ 2 h postmeal, and returned to a level not significantly different from baseline at 4 h (14.3% to 8.6% to 12.6%), however, basal artery diameter was increased at 2 h postprandially (3.7 to 4.2 mm) and calculated reduction in FMD may be mathematical artifact |
| Lee et al., 2002 (24) | 12 healthy                                       | • FMD was assessed at baseline and 2 and 4 h after a HFM (with/without Vitamin E)  
Meal: | ↓ ↔ HFM + vitamin E | • FMD was decreased after HFM (baseline ~13.1% vs 7.7% and 7.3% at 2 and 4 h postprandial)  
• Decreases in FMD following HFM consumption were reversed by vitamin E treatment |
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<td>Lin <em>et al.</em>, 2008 (25)</td>
<td>14 M; healthy, 22 ± 1 years</td>
<td>• Subjects were divided into 2 groups: 1) HFM + placebo (Control group), 2) HFM + 15 g oral L-arginine (L-arginine group)</td>
<td>↓ in Control group ↓ less in L-arginine group</td>
<td>• Serum TG levels increased significantly 2 h after HFM in both groups (~0.96 to 1.46 mmol/L)</td>
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<td>• FMD, von Willebrand factor (vWF; procoagulant molecule that increases in response to endothelial cell damage), p-Selectin (cell adhesion molecule on surfaces of activated endothelial cells), and glutathione peroxidise (GSH-Px; antioxidant enzyme) were measured before and 2 h after the meal</td>
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<td>• Control group: significant ↓ in GSH-Px and FMD after HFM (FMD: ~10.5 to 6.8%)</td>
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<td>Meal: cited as being same as in Tsai <em>et al.</em>, (52); 900 kcal, 50 g fat</td>
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<td>• L-arginine group: small but significant ↓ FMD after HFM (10.3 vs 9.3%); GSH-Px not changed</td>
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<td>Liu <em>et al.</em>, 2002 (26)</td>
<td>74 patients with coronary artery disease (CAD); 50 control subjects; (overall, 100 M, 24 F; mean age 57 ± 8 years; 64 with hypertension, 54 were smokers)</td>
<td>• Serum TG, total cholesterol, LDL, and HDL were measured in the fasting state and 2, 4, 5, and 7 h after a HFM with or without 2 g of Vitamin C (between-subjects design)</td>
<td>↓ ↔ in HFM + Vitamin C</td>
<td>• Significant ↑ in P-selectin and vWF after HFM in both groups</td>
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<td>• FMD and nitroglycerin-induced vasodilation were assessed at baseline and 4 h postprandial</td>
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<td>Meal: based on meal by Vogel <em>et al.</em>, (55), but modified according to Chinese dietary habits (800 kcal, 50 g fat)</td>
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<td>Fasting FMD and nitroglycerin-induced dilation were impaired in patients with CAD relative to control subjects</td>
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<td>• Postprandial FMD significantly reduced in both subjects with/without-CAD, while nitroglycerine-induced dilation unchanged</td>
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<td>• FMD not significantly different following HFM + Vitamin C, although mild tendency toward improvement and aggravation in CAD and control groups respectively</td>
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<td>• Decrement of postprandial FMD correlated positively with increment of 2 h TG concentration in patients without Vitamin C (n = 62, r = 0.545)</td>
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<td>Maggi <em>et al.</em>, 2004 (27)</td>
<td>15 M; moderately dyslipidemic; 49 ± 12 years</td>
<td>• FMD, TG, RLP-C were measured before and 2, 4, 6, and 8 h after a HFM</td>
<td>↓</td>
<td>TG levels peaked between 4-6 h postprandial (~5.5 mmol/L) and decreased at 8 h (~4.3 mmol/L)</td>
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<td>Meal: 693 kcal (85% fat) per m² body surface</td>
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<td>• Significant ↓ FMD at 6 h postprandial (from ~16% to 11.5%)</td>
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<td>• Steady ↑ in RLP-C up to 6 h and ↓ at 8 h</td>
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<td>• Fasting TG and RLP-C correlated significantly with FMD at baseline</td>
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<td>• ↓ in FMD at 6 h correlated significantly with the AUC of TG (r = 0.53)</td>
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<td>• AUC postprandial RLP-C correlated with the ↓ in FMD (r = 0.66)</td>
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<td>Marchesi et al., 2000 (29)</td>
<td>10 M; healthy, 23 ± 2 years</td>
<td>• FMD and serum lipid profile were assessed before and 2, 4, 6 and 8 h after a HFM&lt;br&gt;&lt;br&gt;&lt;i&gt;Meal:&lt;/i&gt; whipping cream, liquid chocolate, non-fat dry milk (700 kcal, 65 g fat per m² body surface)</td>
<td>↓</td>
<td>• TG peaked at 4 h postprandial and decreased thereafter (~0.6 fasting to 1.3 mmol/L at 4 h)&lt;br&gt;• FMD fell significantly from 14.5 ± 6.6% fasting to 3.5 ± 1.5% and 4.0 ± 2.2 % at 2 and 4 h, and subsequently returned to basal values&lt;br&gt;• Strong inverse correlation between AUC postprandial TG (i.e. after subtraction of baseline TG) and AUC of ΔFMD post-HFM (r = -0.70)</td>
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<td>Marchesi et al., 2003 (28)</td>
<td>7 M, 3 F; hypertriglyceridemic; 47 ± 7 years</td>
<td>• FMD, and circulating levels of intercellular adhesion molecule (ICAM) and vascular cellular adhesion molecule (VCAM) were measured before, and 2, 4, 6 and 8 h after a HFM&lt;br&gt;&lt;br&gt;• Measurements were made before and after 3-months of treatment with fenofibrate (drug used in treatment of hypertriglyceridemia; 200 mg/day)&lt;br&gt;&lt;br&gt;&lt;i&gt;Meal:&lt;/i&gt; same as in Marchesi et al., 2000 (29)</td>
<td>↓</td>
<td>• Pre-treatment: FMD decreased and ICAM and VCAM increased from baseline at 2, 4 and 6 h postprandial&lt;br&gt;• Post-treatment: In fasting conditions, FMD increased and ICAM and VCAM decreased significantly relative to pre-treatment. Postprandially, FMD decreased and ICAM and VCAM increased significantly.</td>
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<td>Muntwyler et al., 2001 (30)</td>
<td>12 M; healthy; 30 ± 2 years</td>
<td>• Endothelium-dependent (Ach) and –independent (SNP) vascular function in the forearm vascular bed were assessed before and 4 h after a HFM&lt;br&gt;&lt;br&gt;&lt;i&gt;Meal:&lt;/i&gt; dairy cream, milk powder, sacharose, chocolate powder, 700 kcal/m² body surface area (83% fat)</td>
<td>↔</td>
<td>• Baseline flow was higher after HFM than during fasting&lt;br&gt;• Before and after HFM, no significant differences in Ach- or SNP-induced vasodilation&lt;br&gt;• No significant correlation between ↑ in postprandial TG-rich lipoproteins and Δ in Ach-induced vasodilation</td>
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<td>Nagashima et al., 2011 (31)</td>
<td>24 M; obese; 40 ± 9 years in placebo group, 48 ± 9 in pitavastatin group</td>
<td>• Subjects were randomly assigned to receive pitavastatin (2 mg/day; drug used to lower cholesterol levels) or placebo for 2 weeks&lt;br&gt;&lt;br&gt;• Pre- and post-treatment, FMD and lipid profile were assessed before and 4 h after a HFM&lt;br&gt;&lt;br&gt;&lt;i&gt;Meal:&lt;/i&gt; oral fat tolerance test cream (70 g of cream; 240 kcal, 35% fat, ~25 g fat)</td>
<td>↓ pre-treatment and in placebo group ↔ in pitavastatin group</td>
<td>• Pre-treatment: ↑ serum TG level (~2.20 to 4.18 mmol/L) and ↓ FMD (~10.7% to 9.5%) post-HFM in both groups&lt;br&gt;• Post-treatment, placebo group: no changes from pre-treatment&lt;br&gt;• Post-treatment, pitavastatin group: attenuated ↑ in postprandial TG (+2.07 vs +0.91 mmol/L); ↓ in postprandial FMD completely abolished (-1.1 vs +0.1%)&lt;br&gt;• Correlation between Δ in postprandial TG and Δ in postprandial FMD after 2 weeks of statin treatment (r = -0.737)</td>
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| Ng et al., 2001 (32)      | 10 M; healthy; 22 ± 2 years | ● FMD and endothelium-independent (GTN) dilation were assessed before and 4 h after a “local” high-fat dish (LHF; high in saturated fat from coconut milk), a western high-fat meal (WHF; McDonalds) and a low-fat meal (LFM), on separate days | ↓                  | • Baseline artery diameter and blood flow, and the ↑ in flow after cuff deflation, were similar in all trials  
• Significant ↓ in FMD after WHF and LHF compared to LFM  
• FMD not different between LHF and WHF  
• GTN-induced dilation not different before and after LFM, WHF or LHF meals |
| Nicholls et al., 2006 (33)| 8 M, 6 F; healthy; 29 ± 2 years | ● FMD and reactive hyperemia were assessed before and 3 and 6 h after isocaloric HFMs containing either 1) Polyunsaturated fat, or 2) Saturated fat, on 2 separate occasions  
*Meals:* carrot cake and milkshake, containing 1 g of fat/kg body weight (coconut and safflower oil for saturated and polyunsaturated meals) | ↓ saturated ↔ polyunsaturated (trend toward ↓) | • Similar ↑ in plasma TG, insulin, and nonesterified fatty acids after both meals  
• FMD decreased 3 h after the saturated meal (~6.9% to 4.7%), and tended to ↓ 3 h after polyunsaturated meal (5.2% to 4.3%, not significant), while hyperemic flow significantly increased at 3 h after both meals |
| Ong et al., 1999 (34)    | 16 M; healthy; 30 ± 5 years | ● FMD and endothelium-independent (glyceryl trinitrate) responses were measured before and 3 h after a LFM or HFM (crossover design)  
*Meals:* muffin and milkshake ~765 kcal, 50 g or 5 g fat for HFM and LFM respectively | ↔ relative to baseline ↓ relative to LFM | • Plasma TG concentrations increased significantly after HFM but not LFM (mean 2.4 mmol/L and 1.3 mmol/L)  
• FMD after HFM was significantly less than after LFM (1.2% vs 4.3%) |
| Padilla et al., 2006 (35) | 5 M, 3 F; healthy; 26 ± 1 years | ● Brachial artery vasodilation was assessed at baseline and 4 h after a HFM or LFM, with an active hyperemic stimulus (5 min of rhythmic handgrip exercise; HFM-A, LFM-A), or a reactive hyperemic stimulus (5 min of forearm occlusion; HFM-R, LFM-R) (crossover design)  
*Meal:* same as in Vogel et al., 1997 (55); ~900 kcal, and 50 g or 0 g fat | ↓ in HFM-R compared with LFM-R ↔ in HFM-A vs LFM-A | • No difference in brachial artery vasodilation between LFM-A and HFM-A (~5.75 vs 6.39%), but a significant ↓ in vasodilation in the HFM-R treatment compared with LFM-R (~4.29 vs 7.18%) |
| Padilla et al., 2006 (36) | 5 M; 3 F; healthy; 26 ± 1 years | ● 3 treatment conditions: LFM alone, HFM alone, and 1 session of aerobic exercise presented 2 h after a HFM (HFM+EX; 45-min continuous treadmill walking at 60% VO2peak)  
● FMD measured at baseline and 4 h post-meal for each treatment condition (crossover design)  
*Meals:*-- same as Vogel et al., 1997 (55); ~945 kcal, and 48 g or 0 g fat | ↔ relative to baseline ↓ relative to LFM ↑ in HFM+EX | • FMD significantly elevated from baseline following HFM-EX (~5.6 to 8.7%), but unchanged following LFM (~6.2 to 7.2%) and HFM (~5.7 to 4.3%)  
• FMDs at 4 h following LFM (~7.2%) and HFM-EX (~8.7%) were significantly higher than following HFM (~4.3%) |
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<td>Plotnick et al., 1997 (37)</td>
<td>7 M, 13 F; healthy; 37 ± 9 years</td>
<td>FMD was measured before and hourly for 6 h after a HFM, LFM, or HFM + vitamins (1 g Vitamin C, 800 IU Vitamin E) (randomized crossover design)</td>
<td>↓ ↔ in LFM, HFM + vitamins, or LFM + vitamins</td>
<td>Mean serum TG levels rose from ~1.1 preprandially to 1.7 and 1.6 mmol/L at 2 and 4 h following HFM (similar after HFM + vitamins, and no Δ with LFM)</td>
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<td>Subgroup (n = 10): LFM with the same vitamin pre-treatment (LFM + vitamins)</td>
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<td>HFM: ↓ FMD from 20 ± 8% before to 12 ± 6, 10 ± 6 and 8 ± 9% at 2, 3, and 4 h post-meal</td>
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<td>Meals – HFM: Egg McMuffin, Sausage McMuffin, 2 hash brown patties, non-coffeeinated beverage (~900 kcal, 50 g fat); LFM: Frosted Flakes, skim milk, orange juice (~900 kcal, 0 g fat)</td>
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<td>LFM, HFM + vitamins, LFM + vitamins: ↔ FMD</td>
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<td>↓ ↔ in LFM, HFM + vitamins, or LFM + vitamins</td>
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<td>ΔFMD after LFM and HFM correlated inversely with 2-h postprandial ΔTG (r = -0.54)</td>
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<tr>
<td>Plotnick et al., 2003 (38)</td>
<td>14 M, 24 F; healthy; 36 ± 10 years</td>
<td>FMD was measured before and 3 h after a HFM, before and after being randomized to 4 weeks of supplementation with: 1) a powdered fruit and vegetable juice concentrate [Juice Plus (JP)] with a supplement of nutritional antioxidants and herbal extracts [Vineyard (V)], 2) JP alone, 3) placebo (between-groups design)</td>
<td>↓ ↔ vs baseline after 4 weeks of JP or JP + V (trend for ↓)</td>
<td>FMD reduced after HFM consumption (e.g. ~20% to 12% in placebo group at baseline)</td>
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<td>Serum nitrate/nitrite concentrations were measured at baseline and at 4 weeks</td>
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<td>4 weeks of JP or JP-V blunted detrimental effect of HFM; placebo had no effect</td>
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<td>Meal: same as in Plotnick et al., 1997 (37)</td>
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<td>In subjects treated with supplements, concentrations of serum nitrate/nitrite increased from 78 ± 39 to 114 ± 62 µm/L</td>
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<td>Raitakari et al., 2000 (39)</td>
<td>7 M, 5 F; healthy; 33 ± 7 years</td>
<td>Resting FBF, post-ischemic FBF, and FMD were measured before and 3 and 6 h after a meal rich in a) saturated fatty acids or b) monounsaturated fatty acids</td>
<td>↔ (although shear stimulus for FMD was increased)</td>
<td>Baseline brachial artery diameter and FBF, and post-ischemic hyperemia increased after both HFMs</td>
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<td>Meals: sausage, muffins, hash browns cooked in 61 g of fresh tallow fat, 1,030 kcal. Meal 1: 48% saturated, 40% monounsaturated, 7.4% polyunsaturated, 4.6% trans fatty acids. Meal 2: 10%, 85%, 5%, 0% respectively.</td>
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<td>Trend toward decrease in FMD: before vs 3 h post-meals 1 and 2 respectively, -4.2 to 3.2%, -5.2 to 3.8%</td>
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<td></td>
<td>↑ in resting FBF correlated with ↑ in postprandial insulin (r = 0.80) and triglyceride (r = 0.77) levels</td>
</tr>
<tr>
<td>Rudolph et al., 2007 (40)</td>
<td>10 M, 14 F; healthy; 32 ± 11 years</td>
<td>FMD, serum TG and ADMA concentrations were assessed before and 2 and 4 h after 3 fast-food meals, chosen to represent 1) one of the world’s most frequently consumed fast-food meals, 2) an isocaloric vegetarian fast-food component, 3) a lower-fat and higher vitamin fast-food content (crossover design)</td>
<td>↓</td>
<td>Similar ↑ in serum TG after each meal (peak at 4 h ~1.11-1.40 mmol/L)</td>
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<td>Meals – 1) beef burger (Big Mac), fries, ketchup, Sprite (1,245 kcal, 49 g fat); 2) vegetarian burger, fries, ketchup, Sprite (1216 kcal, 49 g fat); 3) vegetarian burger, salad, fruit, yogurt, orange juice (containing ~200 mg vitamin C) (1,057 kcal, 31 g fat)</td>
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<td>Significant ↓ FMD after all 3 meals; no difference between meals</td>
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<td>For baseline, 2 h and 4 h: Meal 1: ~9.7, 7.5 and 6.2%; Meal 2: ~9.2, 7.1 and 6.3%; Meal 3: ~8.8, 6.2, 6.8%</td>
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<td>Postprandial baseline diameter increased vs fasting (no difference between meals; range of increase within a meal ~0.2 mm)</td>
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<td>Postprandial ΔTG did not correlate with Δ baseline diameter or FMD</td>
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<td>ADMA concentrations did not change postprandially</td>
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| Rueda-Clausen et al., 2007 (41) | 10 M; healthy; 21 ± 2 years | On 9 separate occasions, subjects’ FMD and blood glucose and lipids were measured before and 3 h after a potato soup meal containing one of 3 different vegetable oils (olive, soybean, palm), either fresh or at one of 2 different deep-fry levels (80 and 160 min of deep-frying) | ↓ | All meals resulted in similar acute FMD ↓ (~11% to ~7%) and postprandial ↑ in TG, independent of type of oil ingested and its deep-fry level  
No correlation between ↓ in FMD and postprandial TG ↑ |
| Meals: potato soup with vegetable oil (~600 kcal, 60 ml oil) | | | | |
| Sarabi et al., 2001 (42) | 5 M, 5 F; healthy; 20-25 years | Endothelium-dependent and –independent vasodilation were assessed by local infusion of MCh and SNP respectively in the brachial artery, before and 1 and 2 h after a mixed meal  
Subset (n = 2 M, 2 F): fasting time control condition  
Subset (n = 3 M, 3 F): isocaloric fat-free meal (anhydrous dextrose solution) | ↓ | Endothelium-dependent vasodilation decreased at 1 h postprandial, but returned to fasting level at 2 h; no change in endothelium-independent dilation  
At 1 h the serum FFA level was inversely related to endothelium-dependent vasodilation (r = -0.74), although there were no significant net changes in FFA levels  
No changes in endothelium-dependent vasodilation were seen during control protocols |
| Meal: rice, minced meat sauce, vegetables; 900 kcal for M and 700 kcal for F, 34% fat | | | | |
| Schillaci et al., 2001 (43) | 10 F; active; healthy; 23 ± 2 years  
(compared to M in Marchesi et al., 2000) | Study conducted in conjunction with that of Marchesi et al., 2000 (29); FMD and serum lipid profile were assessed before and 2, 4, 6 and 8 h after a HFM | ↔ in F  
↓ in M | TG increased postprandially from baseline (~0.69 mmol/L), with peak at 4 h (~1.14 mmol/L)  
FMD unchanged from ~14-15% at fasting to postprandial in F |
| Meal: whipping cream, liquid chocolate, non-fat dry milk (65 g fat and 700 kcal per m² body surface) | | | | |
| Schinkovitz et al., 2001 (44) | 6 M, 5 F  
healthy; 32 ± 2 years | Resistance vessel endothelial function was assessed via RH before and hourly for 8 h following a HFM  
Subset (n = 3 M, 3 F): fasting time control condition | ↓ | TG increased following ingestion of HFM (fasting vs peak at 4 h postprandial: ~1.05 vs 1.94 mmol/L)  
Peak RH transiently reduced 2 h following HFM, and not altered over time in time control group  
HFM did not influence total plasma antioxidant capacity or plasma peroxides  
No correlation between peak RH and peak TG or AUC TG |
<p>| Meal: rolls, butter, cheese, water (1,200 kcal, 90 g fat) | | | | |</p>
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| Sejda et al., 2002 (45) | 6 M, 5 F; healthy; 24 ± 5 years | • FMD was assessed before and 3 and 6 h after an acute HFM, after a 4-week low-fat diet and after a 4-week high fat diet (randomized cross-over design)  
• Fat composition of diets: Low-fat diet, 26%; High-fat diet, 44.5% (isocaloric)  
*Acute HFM:* cream cake, doughnut, cocoa, water, coffee, sugar-free beverages (~14 kcal/kg, 44.8% fat) | ↔ | • Greater total cholesterol content after high-fat diet than low-fat diet, but no differences in baseline TG  
• Greater baseline FMD after high-fat diet than low-fat diet (~5.3% vs 3.1%)  
• Postprandial FMD not affected by acute HFM, regardless of preceding dietary regimen  
• Nonsignificant trend for an ↑ in FMD postprandially after both diet regimens, possibly reflecting diurnal variation |
| Shimabukuro et al., 2007 (46) | 6 M, 6 F; healthy; 36 ± 1 year | • Peak FBF response during RH, and total RH flow were measured as indices of resistance vessel endothelial function, before and 2 and 4 h after ingestion of: 1) a high-CHO meal (300 kcal, 100% CHO), 2) a HFM (30 g fat/m², 35% fat), or 3) a standard test meal (478 kcal; 16.4% protein, 32.7% fat, 50.4% CHO) (crossover design) | ↓ | • Peak and total RH were unchanged after high-CHO and standard test meals, but decreased 2 and 4 h after HFM  
• After HFM, decreases in peak and total RH were correlated with the increase in plasma FFA (r = -0.535 and r = -0.486 for peak and total RH respectively) |
| Silvestre et al., 2008 (47) | 12 M; healthy; 22 ± 3 years | • FMD and lipemic variables were assessed before and at 2, 4 and 6 h after a HFM, with no exercise (NoEx) or exercise performed 16 h (EX-16) or 4 h (EX-4) before ingestion of the meal (crossover design)  
• The exercise session consisted of 6 resistance exercises and 30 min of treadmill running  
*Meal:* whipping cream and sugar-free pudding (13 kcal, 1.4 g of fat/kg) | ↔ | • Compared with NoEx, there were significant decreases in TG AUC and insulin AUC during EX-16 and EX-4  
• EX-4 resulted in significantly larger fasting arterial diameter than EX-16 and NoEx, but there were no other significant effects on endothelial function  
• All FMDs (pre- and post-prandial) were small (range: 1.4% to 2.8%)  
• No correlations between lipemic variables and FMD for any trials |
| Sodre et al., 2011 (48) | 17 M; healthy; 26 ± 4 years | • FMD, plasma ROS, lipids, glucose, and insulin, were measured before and 2, 4, 6, and 8 h after a HFM  
*Meal:* lactose-free powdered milk (40 g fat / m² body surface) | ↓ | • FMD impaired 8 h after HFM (~23 vs 13%)  
• Reduced ROS generation observed in postprandial period |
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| Steer et al., 2003 (49)    | 26 M and F; 24 ± 3 years | • Endothelium-dependent and independent vasodilation were assessed as FBF during administration of Mch (Mch-FBF) and sodium nitroprusside (SNP-FBF) at baseline and 1 and 2 h after either an ordinary Western meal (34% fat), or isocaloric meals with low-fat (20%) or minimal fat (3%) (between-groups design)  
  • An endothelial function index (EFI) was calculated as the Mch-FBF/SNP-FBF ratio. Meals: 700 and 900 kcal for subjects < and > 70 kg respectively. 34% and 20% fat meals: beef, tomato sauce, rice, vegetables, bread, butter, water. 3% fat meal: cod, tomato sauce, rice, beans, vegetables, bread, carbohydrate beverage. | ↓ after 34% fat meal  
↔ after 20% fat meal  
↑ after 3% fat meal | • Both Mch-FBF and the EFI decreased at 1 h after 34% fat meal, but approached fasting levels after 2 h  
• Mch-FBF and EFI did not change significantly in group consuming the 20% fat meal, but increased in 3% fat group  
• SNP-FBF not significantly affected by any of the meals  
• No correlation between change in Mch-FBF, SNP or EFI and change in TG in total sample |
| Strey et al., 2004 (50)    | 19 F with T2D; 58 ± 8 years, postmenopausal | • FBF was measured by strain gauge plethysmography during rest, during Ach infusion and post-ischemia in the fasting state, and again 3 h after a mixed meal  
  • In T2D participants, this testing was done before and 3 months after intensive glucose regulation  
  Meal: croissants, ham, cheese, polyunsaturated margarine, bread, tomatoes, eggs, water (660 kcal; 41 g fat) | ↔ | • Endothelium-dependent vasodilation impaired in T2D group relative to control, and improved following 3 month glucose regulation intervention (reduction in HbA1c of 0.96%)  
• Postprandial increases in glucose, insulin, TG were greater in T2D relative to control  
• Resting FBF increased in all groups post-HFM  
• No difference in fasting and postprandial endothelium-dependent vasodilation before and after improved glucose regulation in either group |
| Tentolouris et al., 2008 (51) | 21 M, 12 F with T2D; 58 ± 9 years | • FMD was measured before and 2, 4 and 6 h following consumption of a meal rich in: 1) monounsaturated or 2) saturated fatty acids  
  Meals – 1) white bread, 33 g extra-virgin olive oil; 2) white bread, 40 g butter; both with ~560 kcal and 36 g total fat | ↓ in saturated fat meal  
↔ in monounsaturated fat meal | • Similar changes in plasma glucose, insulin, lipid concentrations, and total plasma antioxidant capacity following both meals  
• Saturated fat meal: FMD decreased with peak impairment at 4 h (~6.9 to 1.1% for baseline vs 4 h)  
• No differences between meals or within meal conditions over time in: baseline artery diameter or baseline blood flow, peak blood flow |
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| Tsai et al., 2004 (52) | 16 M; healthy; 30 ± 5 years | • FMD, GSH-Px (antioxidant enzyme), and urinary excretion of oxidative modification products (marker of oxidative stress; 8-epi-prostaglandin F2α, 8-PGF2α) were measured before and 2, 4 and 6 h after a standard HFM. *Meal: same as in Vogel et al., 1997 (55); 900 kcal, 50 g fat*. | ↓ | • Serum TG increased and FMD decreased after HFM (peak impairment from ~9.5% to ~5.5% at 2 h postprandial and still impaired at 4 and 6 h postprandial)  
|                    |              |                                                                             |                    | • GSH-Px decreased 2 h after HFM; 8-PGF2α increased 4 h after HFM                                                                                                                                 |
| Tushuizen et al., 2006 (53) | 17 M, healthy, 25 ± 3 years | • Subjects were studied during 2 randomized visits:  
1) 2 HFM meals (one as breakfast and the other 4 h later as lunch), 2) fasting  
• Before and every 2 h after the first meal, FMD, MDA and oxidized LDL (oxLDL)/LDL cholesterol ratio were measured.  
*Meals – Breakfast: Egg McMuffin, croissant, butter, marmalade, milk, cream (~800 kcal, 50 g fat).  
Lunch: Quarterpounder, croissant, butter, milk (~800 kcal, 50 g fat).* | ↓ | |                                                                                                 |
|                     |              |                                                                             | reached significance after 2nd HFM | • Postprandial plasma glucose and TG concentrations increased significantly, especially after the second meal (baseline to peak, ~4.8 to 5.4 mmol/L and 0.8 to 1.7 mmol/L for glucose and TG respectively)  
• FMD significantly impaired after the second meal (6.9% to 2.7% for fasting vs 2 h-post second meal)  
• Markedly elevated (oxLDL)/LDL cholesterol ratio and MDA concentrations after the second meal  
• FMD unchanged over time in fasting condition                                                                 |
| Tyldum et al., 2009 (54) | 8 M; healthy, 42 ± 4 years | • FMD was assessed before and 30 min, 2 h and 4 h after a HFM preceded (16-18 h) by rest, a single bout of continuous moderate intensity exercise (CME) and high intensity interval exercise (HIIE)  
• CME: walking continuously for 47 min on treadmill at 60-70% HRmax  
• HIIE: 10 min warm-up at 50-60% HRmax, 4 x 4 min at 85-95% HRmax, 3 min active recovery (50-60% HRmax) in between and 5 min cool-down (isocaloric protocol)  
*Meal: vegetarain mozzarella pizza (~910 kcal, 48 g fat).* | ↓ | • Before HFM, similar initial brachial artery diameters in all trials, but after HFM basal diameter decreased in control and CME trials  
• Prior to HFM, both CME and HIIE improved FMD measured 16-18 h post-exercise, whereas FMD unaltered in control trial  
• CME did not completely protect from HFM-induced reduction in FMD (~7% at pre-exercise baseline to 6% 4 h postprandial), but the postprandial fall in FMD was significantly less than in the control trial (~7% at pre-exercise baseline to 4% 4 h postprandial)  
• In HIIE, FMD increased from pre-exercise to post-exercise, and was unchanged following HFM (i.e. greater postprandial than initial baseline)  
• Antioxidant status strongly correlated with FMD (*r = 0.9*) |

↓ denotes a decrease; ↔ denotes no change; ↓↓ less if preceded by CME if preceded by HIIE
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| Vogel *et al.*, 1997 (55) | 5 M, 5 F; healthy; 39 ± 10 years                                             | • FMD, serum lipoproteins and glucose were measured before and hourly for 6 h after single isocaloric HFM or LFM (crossover design) | ↓                  | • Serum TG increased from ~1.2 mmol/L preprandially to ~1.68 mmol/L 2 h after HFM  
• FMD significantly decreased from ~21% to ~11% at 2 and 3 h and 10% at 4 h after HFM; no changes in lipoproteins or FMD observed after LFM  
• Fasting LDL cholesterol correlated inversely (r = -0.47) with fasting FMD, but TG level did not  
• Mean change in postprandial FMD at 2, 3, and 4 h correlated with change in 2-h serum TG (r = -0.51)  
• In subset, no change in nitroglycerin-vasoactivity from baseline to 3 h postprandial |
| Vogel *et al.*, 2000 (56) | 5 M, 5 F; healthy; 28 to 56 years                                            | • On separate days, subjects’ FMD was measured before and 3 h after eating 1 of 5 meals (900 kcal and 50 g fat): 1) olive oil and bread, 2) olive oil, bread and vitamins C and E, 3) olive oil, bread and foods containing antioxidants (balsamic vinegar, salad), 4) canola oil and bread, 5) salmon and crackers  
• Meals were chosen to contain different fat sources and antioxidants (meals 2 and 3). | Depends on content of meal | • All 5 meals significantly raised serum TG, but did not change other lipoproteins or glucose at 3 h postprandial  
• Meal 1: reduced FMD (~14.3 to 9.9%), and inverse correlation between postprandial changes in serum TG and FMD (r = -0.47)  
• No significant change in FMD following remaining 4 meals |
| Volek *et al.*, 2008 (57) | 16 M, 14 F; healthy; 30 ± 8 years                                            | • Subjects participated in 2 testing days, each preceded by 3 weeks of supplementation with either 2 g/day of L-Carnitine or placebo, with a 3-5 week washout period between trials (double-blind, placebo-controlled, crossover design)  
• FMD was measured before and 1.5, 3, and 4.5 h after consumption of a HFM  
• IL-6, TNF-alpha, and MDA were measured as inflammatory and oxidative responses  
Meal: whipping cream, sugar-free instant pudding, macadamia nuts (908 kcal, 84% fat), with/without 2 g L-carnitine | ↓ in placebo relative to carnitine trial at 1.5 h post-HFM, but not relative to baseline | • Fasting FMD not different between carnitine and placebo trials after 3 weeks of supplementation (~6.6%)  
• FMD decreased to 5.8% at 1.5 h postprandial (time of approximate peak in TG) in placebo trial and increased to 7.7% in carnitine trial  
• Following HFM, postprandial lipemia and plasma IL-6 increased significantly (no effect of supplementation)  
• No significant postprandial changes or supplement effects for plasma TNF-alpha and MDA  
• No association between TG (expressed as AUC or values at any time point) and FMD |
| West *et al.*, 2005 (58) | 13 M, 5 F with T2D; 55 ± 2 years                                            | • FMD and TG were measured before and 4 h after 3 test meals rich in monounsaturated fatty acids (MUFA) and differing in the amount and type of omega-3 fatty acids  
Meal: 473 ml skim milk, ~625 kcal, 50 g fat from one of 3 blends of MUFA, ice and flavourings: 1) MUFA, 2) ALA+MUFA, with 3.3 g of α-linolenic acid (ALA), 3) DHA+MUFA, with 2.8 g eicosapentaenoic acid (EPA), 1.2 g docosahexaenoic acid (DHA), 0.2 g ALA | ↔ if low fasting TG  
↑ if high fasting TG | • FMD was unchanged or significantly increased from baseline at 4 h after meals with 3-5 g omega-3 fatty acids in subjects with low and high fasting TG respectively  
• After the MUFA meal, there was an inverse relationship between TG level and FMD (r = -0.50); this relationship was reversed following meals with omega-3 fatty acids (r = 0.49 for each of ALA+MUFA and DHA+MUFA meals) |
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<tbody>
<tr>
<td>Westphal et al., 2006 (60)</td>
<td>8 M, 8 F; healthy;</td>
<td>• FMD, TG, FFA, insulin, L-arginine, and ADMA were assessed before and</td>
<td>↓ for HFM alone</td>
<td>• FMD reduced following consumption of HFM, with peak reduction at 3 h postprandial (~8.5% at baseline to 4.9%, 3.6% and 4.1% after 2, 3 and 4 h respectively; not significantly different from baseline thereafter)</td>
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<td>21 ± 2 years</td>
<td>hourly for 8 h after consumption of: 1) HFM alone, 2) HFM + caseinate</td>
<td>↔ HFM + protein</td>
<td>• HFM + protein: no change in FMD postprandially</td>
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<td>protein, 3) HFM + soy protein</td>
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<td>• Addition of protein to HFM resulted in: ↓ TG and FFA, ↑ insulin, and ↑ arginine/ADMA ratio</td>
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<td><strong>Meals:</strong> 30% whipping cream, 1 g fat / kg body weight.</td>
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<td>Westphal and Luley 2011 (59)</td>
<td>2 M, 16 F; healthy;</td>
<td>• FMD, TG, and FFA were measured before and hourly for 6 h after</td>
<td>↓ for flavanol-poor HFM</td>
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<td></td>
<td>25 ± 3 years</td>
<td>consumption of a HFM with cocoa either rich in flavanols (918 mg) or</td>
<td>↓ less for flavanol-rich HFM</td>
<td>• Flavanol-poor HFM: FMD deteriorated over 4 h (~8.5% at baseline to ~6.5% at 1-3 h postprandial, 7.5% at 4 h and not significantly different from baseline at 6 h)</td>
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<td></td>
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<td>flavanol-poor HFM</td>
<td></td>
<td>• Flavanol-rich HFM: attenuated reduction in FMD in hours 2, 3 and 4 but did not abolish it completely (~8.75% at baseline to 7.5% at 1 h)</td>
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<td><strong>Meal:</strong> whipping cream and cocoa powder (1 g fat / kg body weight)</td>
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<td>• Postprandial TG and FFA not different between flavanol-rich and flavanol-poor HFMs</td>
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<tr>
<td>Williams et al., 1999 (61)</td>
<td>10 M; healthy;</td>
<td>• FMD and endothelium-independent dilation (GTN) were investigated</td>
<td>↓ for used cooking fat</td>
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<td>34-52 years</td>
<td>before and 4 h after 3 test meals: 1) rich in cooking fat that had</td>
<td>↔ for unused cooking fat or LFM</td>
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<td>been used for deep frying in a fast food restaurant (64.4 g), 2) rich</td>
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<td>in unused cooking fat (64.4 g), and 3) a low fat meal without the added</td>
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<td>fat (18.4 g)</td>
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<td><strong>Meals:</strong> ice cream, skim milk, evaporated milk, yogurt,</td>
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<td>timmed apricots, egg yolk, egg white, chocolate flavor. Meals 1 and 2</td>
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<td>added 64.4 g of used and unused fat.</td>
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<tr>
<td>Williams et al., 2001 (62)</td>
<td>14 subjects</td>
<td>• FMD and GTN-induced dilation were assessed before and 4 h after meals</td>
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<td>rich in olive oil and safflower oil used hourly for deep-frying for 8 h</td>
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<td>and containing high levels of lipid oxidation products</td>
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<td>(double-blind crossover design)</td>
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<tr>
<td>Wilmink et al., 2000 (63)</td>
<td>10 M, 10 F; healthy;</td>
<td>• Subjects were randomized to 2 weeks of folic acid treatment (10 mg/day)</td>
<td>↓ placebo group</td>
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<td>23 ± 3 years</td>
<td>or placebo</td>
<td>↔ folic acid group</td>
<td>• Placebo: FMD decreased and MDA excretion increased after HFM</td>
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<td>• After the treatment, FMD, endothelium-independent dilation</td>
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<td>(sublingual nitroglycerin), and oxygen radical stress (urinary</td>
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<td>• Folic acid: FMD and MDA excretion were unaffected by HFM</td>
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<td>excretion of MDA) were assessed before and 4 h after a HFM</td>
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<td>• Response to nitroglycerin unaltered throughout the study</td>
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<td><strong>Meal:</strong> whipped cream (50 g fat/m² body surface area)</td>
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</table>
| Yunoki et al., 2011 (64) | 17 M, 3 F (9 with dyslipidemia); 38 ± 10 years | • Subjects were randomized to 4 weeks of either ezetimibe treatment (lipid-lowering drug that selectively inhibits cholesterol absorption; 10 mg/day) or control  
• Before and after 4 weeks of treatment, subjects’ lipid profiles and FMD were assessed during a fasting state and at 2, 4, 6, and 8 h after a HFM  
  *Meal: cookie (30 g fat/m² body surface area)* | ↓ pre-treatment  
↓ less post-treatment | • Pre-treatment: in all subjects, FMD declined from ~8.5% at baseline to ~6.0% at 4 h postprandial; maximum reduction in FMD significantly correlated with maximum increase in postprandial TG ($r = -0.499$)  
• Ezetimibe treatment for 4 weeks significantly suppressed postprandial elevation in TG (assessed as AUC) and remnant lipoprotein cholesterol, and attenuated the postprandial reduction in FMD (maximum reduction in FMD from ~2.6 to -1.2%)  
• No significant changes in control group |

Abbreviations: %fat = percentage of total energy from fat; Ach = acetylcholine; ACT = active; ADMA = asymmetric dimethylarginine; AUC = area under the curve; BMI = body mass index; CAD = coronary artery disease; CHO = carbohydrates; CME = continuous moderate exercise; EF = endothelial function; EFI = endothelial function index; EX = exercise; F = female; FO = follicular; FBF = forearm blood flow; FFA = free fatty acid; FMD = flow mediated dilation (measured in brachial artery unless otherwise stated); GSH-Px = glutathione peroxidise; GTN = glyceryl trinitrate; HDL = high density lipoprotein; HFM = high-fat meal; HIIE = high intensity interval exercise; HO = high oleic sunflower oil; ICAM = intercellular adhesion molecule; INA = inactive; IL-6 = interleukin-6; L = luteal; LBF = leg blood flow; LDL = low density lipoprotein; LHF = local high-fat meal; LFM = low-fat meal; M = male; Mch = methacholine chloride; MDA = malondialdehydes; ME = menses; NO = nitric oxide; NT = nitrotyrosine; OGTT = oral glucose tolerance test; oxLDL = oxidized low density lipoprotein; RH = reactive hyperemia; RLP-C = remnant-like particle cholesterol; ROS = reactive oxygen species; SA = stearic acid; SNP = sodium nitroprusside; SOD = superoxide dismutase; T2D = type 2 diabetes; TBARS = thiobarbituric reactive substances; TG = triglyceride; TNF-alpha = tumor necrosis factor-alpha; VCAM = vascular cellular adhesion molecule; VLDL = very low density lipoprotein; vWF = von Willebrand factor; WHF = western high-fat meal.
References for Appendix B


25. **Lin CC, Tsai WC, Chen JY, Li YH, Lin LJ and Chen JH.** Supplements of L-arginine attenuate the effects of high-fat meal on endothelial function and oxidative stress. *Int J Cardiol* 127: 337-341, 2008.


Appendix C

Seven-Day Physical Activity Recall Interview
Seven-Day Physical Activity Recall Instructions / Script

The following is a sample script for the seven-day physical activity recall (PAR) Interview, as administered in this study.

- Now we are going to do a physical activity (PA) questionnaire, where I ask you about your PA over the last 7 days. This is simply a recall of actual activities for the past week, and isn’t a history of what you “usually” do. It’s not a test, and your answers will not affect your participation in the study – we’re just interested in your physical activity levels.
- I’m going to start off by asking you some questions about the past week.
- Questions on page 1 of Seven-Day PAR

- Over the course of this interview, I’ll be asking questions about yesterday, and then working backwards through the previous 7 days.
- So first, let’s talk about the time you spent sleeping in the past week.
  - By “sleeping”, I mean the time you went to bed one night and the time that you got out of bed the next morning. You may not necessarily have been asleep the entire time you were in bed. You may have been reading, watching TV, or doing paperwork. Time spent in sexual activity is not counted as “sleep”.
  - Today is (i.e. Monday), so yesterday was (i.e. Sunday). What time did you go to bed (Sunday) night and get up (Monday) morning. Record to the nearest ¼ hour. Do this for each of the 7-d recall. Calculate total time spent sleeping after completing the interview. Did you have any naps on (Sunday)? Did you have any disruptions to your sleep – any times when you got out of bed for 15 minutes or more?
    - Repeat for all other days

- Now I’m going to ask you about physical activities done in the past 7 days. In talking about PA, we will classify activities into 3 categories:
  - The “moderate” category is similar to how you feel when you’re walking at a normal pace, walking as if you were going somewhere
  - The “very hard” category” is similar to how you feel when you are running
  - The “hard” category just falls in between ➔ in other words, if the activity seems harder than walking but not as strenuous as running, it should go in the hard category
- These cards give examples of some activities that fall into each of these categories (sample activities were shown).
- I’m going to ask you about the PAs you engaged in during three segments of the day, which includes morning, afternoon, and evening.
  - “Morning” is considered from the time you get up in the morning to the time you have lunch
  - “Afternoon” is from lunch to dinner
  - And “evening” is from dinner until the time you go to bed
  - NOTE: If a meal is skipped, “morning” is from the time a person wakes up to 12:00 pm, afternoon from 12:00-6:00 pm, and evening from 6 pm to bed.
- For this interview we are not considering light activities such as desk work, standing, light housework, strolling, and stop-and-go walking such as grocery shopping or window shopping.
- We are interested in occupational, household, and sports activities that make you feel similar to how you feel when you are walking at a normal pace.
- Remember that this is a recall of activities for the past week, not a history of what you usually do.
We’ll start with yesterday. Today is (i.e. Monday), so yesterday was (i.e. Sunday). Think about what you did in general yesterday morning. Did you do any PA (Sunday morning)? How long did you do that activity? How much of that time was spent standing still or taking breaks? Did that activity feel similar to how you feel when you are walking or running or is it somewhere in between? Did you do any PA (Sunday afternoon)? (Duration, intensity). Did you do any PA (Sunday evening)? (Duration, intensity).

If people are giving too much information, it is appropriate to ask “how much time in general?” – i.e. remind them they do not need to account for every minute of the day. For an activity to be counted, it must add up to at least 10 min in one intensity category for one segment of the day (round to 15 min).

1. **At the end of each day:** Are there any PAs you might have forgotten? Did you do any PA at work? Any other recreational or sport activities? Housework or gardening? Were there any other walks that you might have taken?
2. **On the last day of recall:** Take a moment to think back over the course of the week and think of any activities you may have forgotten.
3. **Last question:** The last question I’m going to ask you is, “Compared to your PA over the past 3 mo, was last week’s PA more, less, or about the same?”
4. Thank you.

Prompting questions (examples):
- What were you doing [day] morning?
- You said that you got up at 6 am. Did you go anywhere after that?
- Did you watch any particular TV show?
- What did you make for dinner?
- What did you do that evening?
- Did you take any walks that you may have overlooked?
- Did you do any vigorous home repair or gardening?
- Are there any activities that you are unsure about?

**Scoring**
10 min and 22 min are rounded to 15 min = 0.25
23 min and 37 min are rounded to 30 min = 0.5
38 min and 52 min are rounded to 45 min = 0.75
53 min and 67 min are rounded to 60 min = 1.0
68 min and 1 hr 22 min are rounded to 1hr 15 min = 1.25

**Special Cases**
If the last week was totally atypical (e.g., the subject was in the hospital or in bed, involved in a family or work crisis, or travelling) it is possible to go to the previous week for the survey.
Scoring Continued

**Table C-1. Scoring of 7-Day PAR**

<table>
<thead>
<tr>
<th>Category</th>
<th>MET range</th>
<th>Hours</th>
<th>MET Value</th>
<th>Total MET hr/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>1.0</td>
<td>?</td>
<td>×</td>
<td>1.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3.0-4.9</td>
<td>?</td>
<td>×</td>
<td>4.0</td>
</tr>
<tr>
<td>Hard</td>
<td>5.0-6.9</td>
<td>?</td>
<td>×</td>
<td>6.0</td>
</tr>
<tr>
<td>Very Hard</td>
<td>&gt;7.0</td>
<td>?</td>
<td>×</td>
<td>10.0</td>
</tr>
<tr>
<td>Remaining Hours (Light)</td>
<td>1.1-2.9</td>
<td>?</td>
<td>×</td>
<td>1.5</td>
</tr>
</tbody>
</table>

TOTAL MET hr/wk: __________

*Note:* This script was adapted from: Seven-Day Physical Activity Recall. *Medicine & Science in Sports & Exercise* 29: 1997.
Physical Activity Recall

1. Were you employed in the last seven days (paid or volunteer)?  □ YES  □ NO  → Go to question 4

2. How many days of the last seven did you work?  □ (round to nearest day)

3. How many total hours did you work in the last seven days?  □□ hours

4. What days of the week do you consider to be your weekend or non-work days?  For most people, this would be Saturday and Sunday, but it may be different for you.

□ Sunday  □ Monday  □ Tuesday  □ Wednesday  □ Thursday  □ Friday  □ Saturday

********************Explain Moderate, Hard, and Very Hard Intensity levels ********************

At the end of the interview:

5. Compared to your physical activity over the past three months, was last week’s physical activity more, less or about the same?

□ More
□ Less
□ About the same

Subject ID: ___________  Interviewer Initials: ___________
<table>
<thead>
<tr>
<th></th>
<th>Yesterday</th>
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</table>

**Rounding:** 10-22mins = .25hrs  23-37mins = .50hrs  38-52mins=.75hrs  53-1:07mins=1.0hrs  1:08-1:22= 1.25hrs

Subject ID: __________  Interviewer Initials: ___________
7-Day PAR: Interview Evaluation Form

Were there any problems with the 7-Day PAR interview? (circle one)
1. Yes 2. No

Explain:
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

Do you think this was a valid 7-Day PAR interview?
1. Yes 2. Maybe 3. No

Please list below any activities reported by the participant that you don’t know how to classify:
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

Other comments/concerns:
_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
Appendix D
Food Intake Record
In studies 1 and 2 (chapters 5 and 6), since the experiment involved a dietary manipulation, subjects were required to consume a meal that was “typical” for them the night before participation and to replicate this meal prior to each subsequent session in order to control for the potential confound of the preceding meal. The following “Food Intake Record” forms were provided to monitor and facilitate adherence.
Food Intake Record

Name: ____________________________

Date: _____________________________

Subject code: ______________________

Visit # and Condition: _____________

Please record all foods and beverages consumed from **6 pm onward** in the evening prior to your appointment at the Cardiovascular Stress Response Lab; this should include your supper (even if it is consumed at an earlier time) and any additional snacks and/or beverages consumed up **until 9 pm** (after which you must fast until the meal provided as part of the study). You do not need to consume foods of any specific nature, but simply foods that are “typical” for you. The contents of your meal will not affect your participation in the study. Please be as specific and detailed as possible in the table provided. If you need additional space, please use the back of this form. Remember, you will need to consume these same foods in identical quantities prior to each of your subsequent visits to the lab. You will be provided with a photocopy of this form following your first visit to assist you in replicating your food intake for your successive lab visits.

<table>
<thead>
<tr>
<th>Food or Beverage</th>
<th>Quantity (#, volume, weight etc.)</th>
<th>Time Consumed</th>
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<tbody>
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</table>
Please drink at least 8 glasses of water during the day prior to your experimental visit. To help you meet this target, please keep track of the volume of water you consume in the table below:

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Approximate Volume of Water</th>
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Appendix E

Stress Programs and Control Task
The mental stress tasks in studies 1 and 2 (chapters 5 and 6) involved mental arithmetic (three tasks, with one repeated) or a speech (five tasks, with one repeated). The stress tasks were administered in the following two “Stress Programs”, with the intention of interspersing the task types to provide a varied stimulus to minimize habituation. The order of the Stress Programs was counterbalanced across subjects. Speech tasks were audio recorded, and standard prompts were provided when needed. The stress tasks were administered by a designated researcher who was instructed to “be polite, but cold; [and to] not show any emotions toward the subjects” (Lupien et al. 1997). In 5 of the 10 subjects, two additional researchers who were unfamiliar to the subjects served as an audience in order to increase social evaluative threat (i.e. the perception that task performance could be negatively judged by others) (Dickerson and Kemeny 2004). All tasks were 10 min in duration, including instructions, preparation (speech tasks only) and speaking time. The scripts used for the specific tasks (and for the “control” task) follow; references refer to the sources from which the tasks were adapted.

**Stress Program 1:**

1. Shoplifting Accusation Speech Task (Ghiadoni et al. 2000)
2. Mental Arithmetic 1 (Sarabi and Lind, 2001)
3. Anger Interview (Gottdiener et al. 2003; Greeson et al. 2009)
4. Public Speaking Task (Biondi and Picardi, 1999)

**Stress Program 2:**

1. Mental Arithmetic 2 and Stroop Task (Harris et al. 2000; Biondi and Picardi, 1999)
2. Job Interview Speech Task (Kirschbaum et al. 1993)
3. Public Speaking Task (Biondi and Picardi, 1999)
4. Mental Arithmetic 1 (Sarabi and Lind, 2001)
Shoplifting Accusation Speech Task

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions. As you hear these instructions, it is important that you stay still at all times.

Imagine that you have been apprehended and detained under charges of shoplifting. The case that is being built against you is quite strong. A clerk has submitted a statement indicating that he saw you put some merchandise into your knapsack. When your personal belongings were searched they found the item in your possession. You know that you are not guilty, but the authorities are convinced that you committed the crime.

Imagine that I am a representative of the authorities. At the end of these instructions you will be given some time to prepare a statement to convince me, and a panel who I will bring into the room, of your innocence. At the end of the preparation period you will be told to begin to deliver your statement. You will be given 5 minutes to speak, and you must utilize the full 5 minutes. You will also be required to address the panel while you are speaking. Your statement will be audio recorded, and please present it as if this recording will be used in future legal proceedings pertaining to this matter. You must be as convincing as possible in order to sway the authorities towards believing that you are innocent. I will tell you when your 5 minute time period is over.

I will be here in the room to make sure everything goes smoothly, but do not ask any questions during your statement. We are not able to provide you with any feedback during the task. You may feel the urge to use your hands when you begin to speak, but again, please resist this temptation and remain as still as possible. At the end of these instructions you will be given some time to prepare, and I will let you know when to begin your statement. You may now prepare your statement.

Allow the subject to prepare for 3 minutes and 30 seconds and bring the panel into the room near to the end of the preparation period. Then, when timer reads 5:00, say, “You may now deliver your statement.”

At the end of the 5 minute statement, when timer reads 10:00, say, “You may stop, this task is over.”

Note: If the subject stops speaking for 10 seconds, prompt them with, “Please Continue.”
- If subject has eyes closed or is not looking at the panel, state: “please address the panel”
- Take notes on clipboard

Timing:

Reading of instructions: ~1 min 30 sec
Preparation Period: 3 min 30 sec
Speech: 5 min
Total Time: 10 min
Mental Arithmetic 1

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions. As you hear these instructions, it is important that you stay still at all times. You will now participate in a mental arithmetic challenge. You will be provided with a number from which you must continuously subtract 13. If there is an error in your arithmetic, or if you are taking too long to respond, a buzzer (like this) will sound and you must restart the challenge. Part-way through the challenge, you will be provided with another number from which you must continuously subtract 17. Again, if there is an error in your arithmetic, or if you are taking too long to respond, a buzzer will sound and you must restart the challenge. I will let you know when the mental arithmetic challenge is over.

At each highlighted number in the list: Regardless of whether the correct answer is given, press the buzzer and state, “Continue subtracting 13 from ____ [the next number on the list].”

At end of first counting period, when the timer reads 5:00: Press the buzzer and state, “Now continuously subtract 17, starting from 3,571.”
At end of second counting period, when the timer reads 10:00: Press the buzzer and say, “Please stop counting, this task is over.”

Note: Also press the buzzer if an incorrect answer is given, or if 10 seconds pass without an answer being given, and have the subject continue from the number they were at (repeat this for them).

- Bring “the panel” into the room with you at the beginning of the instruction period so that they can be present for the task
- State “Faster Please” at numbers with arrows, or if the subject is taking too long to respond

Timing:

Reading of Instructions: ~30 sec
1st counting period: 4 min 30 sec
Transition Instructions: 15 sec
2nd counting period: 4 minutes 45 sec
Total Time: 10 min
### Mental Arithmetic 1 Answer Key – Increments of 13

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<tbody>
<tr>
<td>9973</td>
<td>9323</td>
<td>8673</td>
<td>8023</td>
<td>← 7373</td>
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<tr>
<td>9960</td>
<td>9310</td>
<td>8660</td>
<td>← 8010</td>
<td>7360</td>
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<tr>
<td>9947</td>
<td>9297</td>
<td>← 8647</td>
<td>7997</td>
<td>7347</td>
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<td>9934</td>
<td>9284</td>
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<td>7334</td>
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<td>← 8517</td>
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<td>9154</td>
<td>← 8504</td>
<td>← 7854</td>
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<td>← 8491</td>
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<td>← 8465</td>
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<td>← 7802</td>
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<td>← 8348</td>
<td>← 7698</td>
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<td>9635</td>
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Anger Interview

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions. As you hear these instructions, it is important that you stay still at all times.

For this task, you will be given some time to recall a personal event that occurred during the past 3-4 months that made you extremely angry when it occurred, and that still arouses feelings of anger upon thinking about it. Please select an event that can be easily described. After you are given some time to think and prepare, you will be given 5 minutes to verbally describe the event, and you must utilize the full 5 minutes. Please focus on what it was about the particular situation or event that made you angry. When relating the event, please speak in a tone and volume that allows you to express your emotions, but remember that you must remain physically still for the study measurements. Your statement will be audio recorded, and you will be required to address a panel who I will bring into the room momentarily. You may now prepare.

Allow the subject to prepare for 4 minutes, and bring the panel into the room near to the end of the preparation period. Then, when the timer reads 5:00, say, “You may now begin.”

At the end of the 5 minute speech, when the timer reads 10:00, say, “You may stop; this task is over.”

Prompts if Necessary:

- Please continue.
- How did that make you feel when it happened?
- Why were you so angry?
- What was it about this particular situation/event that made you angry?
- Re-direct the subject if he is not focusing on the anger – use whatever comment(s) you deem appropriate
- If subject has eyes closed or is not looking at the panel, state: “please address the panel”

Timing:

Instructions: 1 min
Preparation Time: 4 min
Speaking Time: 5 min
Total Time: 10 min

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Public Speaking Task

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions.

As you hear these instructions, it is important that you stay still at all times. You are going to give a 5 minute speech as if you are in front of a large audience. Please choose a subject that is interesting to you. It can be any subject as long as you could lecture on it for 5 minutes in a professional way – so more like an academic lecture than just talking about your day. [The topic of your speech must be distinct from that of your first visit. That is, on your first visit your speech was about _____, so today your speech must NOT be related to _____ in any way.] You will be given 5 minutes to speak, and you must utilize the full 5 minutes. Your speech will be audio recorded, and you will be required to present your speech to a panel who I will bring into the room in a few minutes. Please address the panel during your speech, and also present it as if it will be broadcast to a panel of experts who are evaluating you. I will tell you when your 5 minute time period is over.

I will be here in the room to make sure everything goes smoothly, but do not ask any questions during the speech. We are not able to provide you with any feedback during the task. You may feel the urge to use your hands when you begin to speak, but again, please resist this temptation and remain as still as possible. At the end of these instructions you will be given some time to prepare, and I will let you know when to begin your speech. You may now prepare your speech.

Allow the subject to prepare for 3 minutes and 45 seconds and bring the panel into the room near to the end of the preparation period. Then, when timer reads 5:00, say, “You may now begin your speech.”

At the end of the 5 minute speech, when timer reads 10:00, say, “You may stop, this task is over.”

Note: If the subject stops speaking for 10 seconds, prompt them with, “Please Continue.”

• If subject has eyes closed or is not looking at the panel, state: “please address the panel”
• Take notes on clipboard

Timing:

Reading of instructions: ~1 min 15 sec
Preparation Period: 3 min 45 sec
Speech: 5 min
Total Time: 10 min
Mental Arithmetic 2 and Stroop Task

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions. As you hear these instructions, it is important that you stay still at all times. You will now participate in a 2-part mental challenge.

For the first part, you will be required to do some mental arithmetic. You will be given a 3 digit number. You must take the sum of the 3 digits, and then add this sum to the original 3 digit number. For example, if the number you are given is 111, then you would add 1 + 1 + 1 equals 3, 111 + 3 = 114. Do not do the arithmetic out loud; just state your final answer. If there is an error in your arithmetic, or if you are taking too long to respond, a buzzer (like this) will sound and you must resume the challenge.

For the second part of this challenge, you will be shown cards with the names of colours printed on them in coloured ink. You must state the colour of the ink, not the name of the colour that is written. For example, this card says “green” in yellow ink – so you are required to say “yellow”. I will let you know when the mental arithmetic challenge is over and when you will start the colour task.

You may now begin. Your first number is 523.

For the mental arithmetic task – sound the buzzer if a response is not given within 10 seconds.

After the mental arithmetic task, when the timer reads 8:00, state, “Now we will switch to the colour task.”

At the end of the Stroop task, when the timer reads 10:00, say, “You may stop; this task is over.”

- Bring “the panel” into the room with you at the beginning of the instruction period so that they can be present for the task
- State “Faster Please” at numbers with arrows, or if the subject is taking too long to respond

Timing:

Reading of instructions: 1 min
Mental Arithmetic: 7 min
Transition Instructions: 10 sec
Stroop Task: 1 min 50 sec
Total Time: 10 min
### Mental Arithmetic 2 Answer Key

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Job Interview Speech Task

Instructions:

Listen carefully to these instructions; you will not be given an opportunity to ask questions. As you hear these instructions, it is important that you stay still at all times.

Imagine that you are applying for a job. I am a leader in your field, and I have raised several concerns. The first is that you were fired from your last job and you will now have to convince me that you are still a good candidate. While reviewing your application, I also believe that you may not have all the skills required to do this job. I am skeptical about your level of experience. You must now convince me that you are the ideal candidate.

At the end of these instructions you will be given some time to prepare a statement to convince me, and a panel who I will bring into the room momentarily, that you are the right person for the job. At the end of the preparation period you will be told to begin to deliver your statement. You will be given 5 minutes to speak, and you must utilize the full 5 minutes. You will also be required to address the panel while you are speaking. Your statement will be audio recorded, and please present it as if it will be broadcast to a panel of experts who are evaluating you. You must be as convincing as possible in order to be hired. I will tell you when your 5 minute time period is over.

I will be here in the room to make sure everything goes smoothly, but do not talk to me or the other experimenters or ask any questions during your statement. We are not able to provide you with any feedback during the task. You may feel the urge to use your hands when you begin to speak, but again, please resist this temptation and remain as still as possible. At the end of these instructions you will be given some time to prepare, and I will let you know when to begin your statement. You may now prepare your statement.

Allow the subject to prepare for 3 minutes and 30 seconds and then, when the timer reads 5:00, say, “You may now deliver your statement.”

At the end of the 5 minute statement, when the timer reads 10:00, say, “You may stop; this task is over.”

Note: If the subject stops speaking for 10 seconds, prompt them with, “Please Continue.”
- If subject has eyes closed or is not looking at the panel, state: “please address the panel”
- Take notes on clipboard

Timing:

Reading of instructions: ~1 min 30 sec
Preparation Period: 3 min 30 sec
Speech: 5 min
Total Time: 10 min
Control Task

Instructions:

Please excuse the formality of these instructions. For this task, you will simply be asked to count upwards, out loud, from 1. Please count slowly and in a constant rhythm at a speaking volume that is normal for you. Each time you get to 100, please start over again at 1. Don’t worry if you lose count; just continue from where you think you were at. After these instructions, you will be given approximately 4 minutes of rest. After the 4 minute rest period, I will cue you and you will begin to count. In the meantime, you can just relax.

At end of 4 minute period: “You may now begin to count.”
At end of task: “Thank you, please stop counting, this task is over. You can just relax again.”

Timing:

Reading of instructions: ~30 sec
Rest period: 4 min 30 sec
Counting period: 5 min
Total Time: 10 min
References for Appendix E


Appendix F

Exponential model used to investigate leg blood flow and leg vascular conductance responses in Chapter 8
Figure F-1. Schematic representation of the exponential model used to investigate the leg blood flow (LBF) and leg vascular conductance (LVK) responses to isometric knee-extension exercise transitions (rest-to-6 kg and 6-to-12 kg workloads). Abbreviations: G = gain; τ = “tau”, time constant; TD = time delay; 1, 2 and 3 phases of the response. Adapted from Saunders et al., 2005.

Reference:
Appendix G

Sample recruitment form for studies 3 and 4 in Chapters 7 and 8
Patient Name: ____________________________________________

Cardiologist Section – Please fill out regarding this patient.

Sex: □ male  □ female

Type 2 Diabetes: □ yes  □ no

BMI: _______kg/m²

Baseline level on GXT: _______METs

Stage 3 (or more advanced) renal disease: □ yes  □ no

Current smoker: □ yes  □ no

(If previous smoker, date quit (if known): _____year _____month).

Medication Status:

The patient is taking a β-blocker: □ yes  □ no

• If yes: I □ DO  □ DO NOT approve withdrawal of this medication
  ____________________________ (name) for up to 15 days

The patient is taking a PPI: □ yes  □ no

• If yes: I □ DO  □ DO NOT approve withdrawal of this medication
  ____________________________ (name) for up to 15 days

The patient is taking a PDE5 inhibitor: □ yes  □ no

• If yes: I □ DO  □ DO NOT approve withdrawal of this medication
  ____________________________ (name) for up to 15 days

Comments: _______________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

Physician Name: _________________________________________________

Physician Signature: _____________________________________________

Date: _________________________
Physiotherapist Section

This section can be left blank if the cardiologist has identified that one or more of the following applies to the patient:

☐ Has stage 3 (or more advanced) renal disease,
☐ Currently smokes, and/or
☐ Is taking a β-blocker, PPI or PDE5 inhibitor and withdrawal of this medication is NOT approved.

Otherwise, please (check boxes when complete):

☐ Inform the patient that he/she may be eligible to participate in a study that is being conducted in collaboration with Queen’s University
☐ Provide the patient with an information package (brochure and envelope)
☐ Give a brief description of the study:
  o This study is seeking to identify factors that make exercise feel more difficult for some people than others, and to investigate a possible intervention that may help to make exercise feel easier.
  o More specific information about the study can be found in the brochure information package.
☐ Obtain permission for the individual’s name to be emailed to the research team at Queen’s and for a researcher to be in contact via phone and/or email if he/she is identified as being eligible to participate. (Indicate that this does not mean the individual is obligated to participate, but that he/she will simply receive more information.)
  o Permission Granted: ☐ yes ☐ no

Comments: ____________________________________________________________
________________________________________________________________________
________________________________________________________________________

Physiotherapist Name: _________________________________________________
Physiotherapist Signature: _____________________________________________
Date: _________________________

Thank You! 😊

Please contact Veronica Poitras, PhD Candidate and Co-Investigator, with any questions or concerns at:

613-533-6000 x78425 or v.poitras@queensu.ca