Postprandial Metabolism and Inflammation: Novel Insights Focusing on True-to-Life Application

by

Sam R. Emerson

B.S., Oklahoma State University, 2012
M.S., Kansas State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics, and Health
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Abstract

The aims of this dissertation were to provide innovative, applicable insights regarding the impact of single-meal consumption on metabolic and inflammatory responses in the acute post-meal ("postprandial") period. In Chapter 2, the connection between large postprandial glucose and triglyceride (TG) fluxes and cardiovascular disease (CVD) risk were reviewed. A new marker of metabolic status, Metabolic Load Index (MLI), calculated by adding glucose and TG, was proposed based on several considerations: 1) independent associations between postprandial glucose and TG with CVD risk, although the substrates are considered to increase risk through similar mechanisms; 2) postprandial glucose and TG responses are interrelated; and 3) meals consumed in daily life typically contain both carbohydrate and fat. MLI may be useful in characterizing metabolic status/risk in both clinical and research settings. Chapter 3 was a systematic review with the purpose of objectively describing postprandial responses (i.e. magnitude and timing) to a high-fat meal (HFM) in five commonly assessed inflammatory markers: interleukin (IL)-6, C-reactive protein (CRP), tumor necrosis factor (TNF)-α, IL-1β, and IL-8. IL-6 increased in >70% of studies, starting at ~1.4 pg/mL pre-meal and peaking at ~2.9 pg/mL ~6 hours post-HFM. Other markers (CRP, TNF-α, IL-1β, and IL-8) did not change after the HFM in the majority of studies. These findings suggest that IL-6 is an inflammatory marker that routinely increases following HFM consumption. Future postprandial studies should further investigate IL-6, as well as explore novel markers of inflammation. In Chapter 4, we compared the metabolic and inflammatory responses to a HFM (17 kcal/kg, 60% fat), representative of meals used in previous postprandial studies, to two meal trials that were more reflective of typical eating patterns: a moderate-fat meal (MFM; 8.5 kcal/kg, 30% fat), and a biphasic meal (BPM), in which the MFM was consumed twice, three hours apart. The HFM elicited a greater

total area-under-the-curve (tAUC) TG response (1348.8 ± 783.7 mg/dL x 6 hrs) compared to the MFM (765.8 ± 486.8 mg/dL x 6 hrs; $p = 0.0005$) and the BPM (951.8 ± 787.7 mg/dL x 6 hrs; $p = 0.03$), but the MFM and BPM were not different ($p = 0.72$). It appears that the large postprandial TG response observed in previous studies may not be representative of the daily metabolic challenge for many individuals. Chapter 5 assessed the impact of both aging and chronic physical activity level on postprandial metabolic responses by comparing three groups: younger active (YA), older active (OA), and older inactive (OI) adults. The TG tAUC response was lower in YA (407.9 ± 115.1 mg/dL x 6 hr) compared to OA (625.6 ± 169.0 mg/dL x 6 hr; $p = 0.02$) and OI (961.2 ± 363.6 mg/dL x 6 hr; $p = 0.0002$), while the OA group TG tAUC was lower than OI ($p = 0.02$). Thus, it is likely that both aging and chronic physical activity level impact the postprandial metabolic response. This series of projects provides needed clarification regarding the postprandial metabolic and inflammatory responses to single-meal intake, particularly in the context of real-life application.

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Approved by:

Major Professor
Sara K. Rosenkranz

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Dedication

I dedicate this dissertation to my wife, **Emily**. Not only were you an encouragement throughout the entire dissertation process, but you were also an integral part of the research team. I am very grateful for your support.

Maybe after reading this 171-page document, you will decide to do one yourself.

Preface

All of the projects (Chapters 2-5) comprising this dissertation have been published in peer-reviewed academic journals.

Project 1 (Chapter 2) was published in the *British Journal of Nutrition*:

Emerson SR, Haub MD, Teeman CS, Kurti SP, Rosenkranz SK. Summation of blood glucose and TAG to characterise the 'metabolic load index'. British Journal of Nutrition. 2016 Nov;116(9):1553-63.

Project 2 (Chapter 3) was published in *Advances in Nutrition*:

Emerson SR, Kurti SP, Harms CA, Haub MD, Melgarejo T, Logan C, Rosenkranz SK. Magnitude and Timing of the Postprandial Inflammatory Response to a High-Fat Meal in Healthy Adults: A Systematic Review. Advances in Nutrition: An International Review Journal. 2017 Mar 1;8(2):213-25.

Project 3 (Chapter 4) was published in *Current Developments in Nutrition*:

Emerson SR, Kurti SP, Teeman CS, Emerson EM, Cull BJ, Haub MD, Rosenkranz SK. Realistic Test-Meal Protocols Lead to Blunted Postprandial Lipemia but Similar Inflammatory Responses Compared to a Standard High-fat Meal. Current Developments in Nutrition. 2017 Jan 1:cdn-116.

Project 4 (Chapter 5) was published in the *Journal of Nutrition, Health and Aging*:

Emerson SR, Kurti SP, Emerson EM, Cull BJ, Casey K, Haub MD, and Rosenkranz SK. Postprandial Metabolic Responses Differ by Age Group and Physical Activity Level. The Journal of Nutrition Health and Aging (in press).

Chapter 1 - Introduction

Rationale for Postprandial Assessment

Dietary intake has been well-established as a lifestyle factor that can modify health status (1). Diets high in saturated fat, refined carbohydrates, red meat, and processed foods; and low in fiber, fruits, vegetables, nuts, and legumes are considered to increase risk for certain chronic diseases, such as cardiovascular disease (CVD) and cancer (1). Additionally, there is evidence that consumption of single meals can modify health status via physiological responses occurring in the human body in the transient period following meal consumption (2,3). Indeed, the following outcomes associated with CVD risk have been shown to occur in the acute hours after a single high-fat meal (HFM): increased inflammation (4), impaired endothelial function (5), elevated low-grade endotoxemia (6), increased adhesion molecules (7), and elevated blood coagulation/thrombotic factors (8,9,10). Findings suggest a strong positive association between the magnitude and duration of the post-meal (postprandial) response with risk for cardiovascular events (11). Based on data for typical eating patterns of individuals in Western society, it is likely that most individuals spend the majority of their day in a "postprandial state" (12), which can be considered the acute period following meal consumption in which substrate clearance mechanisms are active, attempting to return metabolic levels to baseline/homeostasis. Thus, assessing the features of the postprandial response to single-meal consumption is crucial to understanding the metabolic state of individuals in daily living.

Triglycerides in the Postprandial Period

Triglycerides (TG) are a type of lipid composed of a glycerol backbone and three fatty acids. Due to their hydrophobic nature, TG circulate in the blood within lipoproteins, primarily chylomicrons, very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein

cholesterol (LDL-C). Many studies have confirmed that TG increase in the postprandial period following a HFM, typically peaking approximately four hours after the meal and returning to baseline around eight hours post-HFM (11,13). There is a general methodology for assessing the postprandial TG response ("postprandial lipemia"). An individual will 1) fast for 10-12 hours prior to their appointment; 2) have a baseline/fasting blood draw conducted; 3) eat a HFM; and 4) remain in the lab for 6-8 hours (without eating) while blood samples are drawn periodically to characterize the postprandial TG response. Clinical studies have revealed that prolonged and/or large increases in magnitude in TG following a HFM are associated with increased risk for CVD. (11). In addition, while it is equivocal whether fasting TG levels are a strong predictor of CVD risk (2), the preponderance of evidence points to postprandial/non-fasting TG as a strong independent predictor of CVD risk (14).

Glucose in the Postprandial Period

Glucose is also a metabolic substrate that increases in circulation following meal consumption, as long as the meal contains sufficient carbohydrate. However, glucose fluxes occur more rapidly as compared to TG, generally peaking and returning to near baseline within two hours in healthy individuals following a glucose challenge (15). Evidence suggests that a large postprandial glucose response is associated with both CVD (16) and type 2 diabetes (17). Similarly to TG, it appears that postprandial glycemia is a better predictor of chronic disease risk than fasting glucose values (16). There is even evidence indicating that >30% of people diagnosed with type 2 diabetes, determined via postprandial glucose challenge, present with normal fasting glucose levels (18). Clearly, with regard to both TG and glucose, there is considerable merit to assessment of postprandial metabolic responses to a single meal or challenge, as postprandial values better predict risk as compared to fasting values.

Inflammatory Markers in the Postprandial Period

Deleterious physiological changes in the postprandial period are not limited to circulating energy available in the form of TG and glucose. Inflammation is thought to be involved in the development of several chronic diseases, including CVD (19). In the past decade, many studies have tested markers of inflammation pre- and post-HFM (20). Inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and IL-8, have been shown to be positively associated with CVD risk (21- 25). While there are numerous markers of inflammation, each with slightly different physiological functioning, many exert their detrimental effects via participating in, and further propagating, the inflammatory cascade that is a hallmark of the atherosclerotic lesion process (19). Thus, testing the postprandial responses of pro-inflammatory cytokines and other molecules has the potential to further our understanding of the connection between dietary intake and chronic disease risk. Several previous studies have reported increases in commonly-assessed markers of inflammation in the postprandial period following HFM consumption, including CRP, TNF-α, IL-6, and IL-1β (26). The inflammatory response, in addition to lipemic and glycemic responses, points to the potential deleterious physiological outcomes that can occur in response to intake of a single HFM.

Gaps in Current Knowledge

Several issues and questions remain unaddressed in understanding the relationship between single-meal intake and the acute metabolic and inflammatory responses that are linked to chronic disease risk. First, while large TG and glucose fluxes have been shown to be independently associated with elevated chronic disease risk, and these substrates increase risk through similar physiological mechanisms, no effort has been made to include TG and glucose in a single metabolic index representative of the total metabolic challenge (energy availability) faced by the

body. This is problematic, since it has been revealed that addition of carbohydrate to a HFM can blunt the postprandial lipemic response (27), and addition of fat to a high-carbohydrate meal can blunt the glycemic response (28). Thus, the postprandial responses of glucose and TG are interdependent. Additionally, meals consumed in daily living typically contain a mixed macronutrient distribution. Based on these reasons, it may be valuable to develop a comprehensive index of metabolic challenge in the postprandial period that considers both TG and glucose. Second, postprandial inflammation has been assessed in many studies, but there is no consensus with regard to how the most commonly assessed markers respond, in terms of magnitude and timing, to a HFM. From both clinical and research perspectives, it would be valuable to synthesize the results of previous investigations in order to determine which markers of inflammation are the most likely to respond, and the characteristics of those responses, following the consumption of a HFM. Next, a common issue with many previous studies investigating postprandial responses has been the utilization of test meal protocols that are not realistic or "true-to-life". For instance, it is not uncommon for participants to eat a very large meal (e.g., ~1500 kcal, 60% fat), then not eat again for 6-8 hours while their response is monitored (29). Some studies will also simply use a laboratory-derived liquid test meal, rather than mixed meals (30). These study design features weaken the practical applicability of the findings from postprandial investigations. Studies investigating the postprandial metabolic and inflammatory responses to realistic meal challenges are warranted. Finally, older adults have been sparsely tested in the postprandial literature. As older adults are at increased risk for CVD (31), as well as a growing segment of the population (32), more research focusing on the postprandial response in older adults, including the lifestyle factors that may affect the response, would be very useful. Overall, given the connection between the postprandial response and CVD

risk, this dissertation aimed to fill some of the gaps in knowledge regarding postprandial responses to single-meals in hopes of furthering our understanding of this important lifestyle factor.

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Chapter 2 - Summation of Blood Glucose and Triglycerides to Characterise the "Metabolic Load Index"

Abstract

Research points to postprandial glucose and triglyceride measures as preferable assessments of cardiovascular risk as compared to fasting values. Although elevated postprandial glycemic and lipemic responses are thought to substantially increase chronic disease risk, postprandial glycemia and lipemia have historically only been considered separately. However, carbohydrates and fats can generally "compete" for clearance from the stomach, small intestine, bloodstream, and within the peripheral cell. Further, there are previous data demonstrating that the addition of carbohydrate to a high-fat meal blunts the postprandial lipemic response and the addition of fat to a high-carbohydrate meal blunts the postprandial glycemic response. Thus, postprandial glycemia and lipemia are interrelated. The purpose of this brief review is twofold: first, to review the current evidence implicating postprandial glycemia and lipemia in chronic disease risk, and second, to examine the possible utility of a single postprandial glycemic and lipemic summative value, which will be referred to as the metabolic load index. The potential benefits of the metabolic load index extend to the clinician, patient, and researcher.

Introduction

Meal consumption, particularly meals that are high in fat, processed sugars, and total calories, results in transient elevations in blood glucose and lipids. These elevations, termed postprandial glycemia and lipemia, respectively, have both been linked to the progression of cardiovascular disease (CVD), primarily through a resultant rise in oxidative stress, inflammation, and endothelial dysfunction (1,2). Thus, exaggerated postprandial glycemia and lipemia, if

experienced several times per day, represent a clear metabolic challenge to homeostasis. Individuals in Western society are thought to spend most of their waking hours in the postprandial state (3), and thus in a near-constant state of postprandial challenge which could be amplified to prolonged dysmetabolism in the case of individuals at risk for metabolic disease. Hence, recent research has been devoted to better understand how the body, in health as well as disease, handles these metabolic excursions. Typically postprandial glycemic and lipemic responses are characterized by measuring blood glucose and triglycerides serially for a given amount of time after a meal, and from these data, calculating area under the curve (AUC). While these methods are effective in characterizing the glycemic and lipemic responses independently, previous efforts have not considered them simultaneously. However, meals typically contain both carbohydrate and fat, as well as some protein; thus it may be worthwhile to consider the summative metabolic response of both carbohydrate and lipids, as opposed to simply focusing on just one or the other. This brief review will first consider postprandial glycemia and lipemia independently in relation to metabolic status and CVD risk, followed by a discussion of the potential utility of a postprandial metabolic load index that combines glycemia and lipemia together, indicating total circulating energy availability.

Blood Glucose and Metabolic Status/Risk

Fasting Blood Glucose, HbA_{1c}, and Cardiovascular Risk

Assessment of long-term glucose control via hemoglobin A1c (HbA1c) or fasting glucose levels are commonly used as valuations of cardiovascular and diabetic risk (4). This practice is based on several epidemiological studies linking HbA1c levels or impaired fasting glucose to heart or vascular disease.(5,6) For example, the United Kingdom Prospective Diabetes Study found that mean HbA1c over time in the cohort was a valuable predictor of ischemic heart disease (5).

Specifically, the data showed that for every 1% increase in HbA1c there was a ~10% increase in heart disease risk (5). With regard to fasting glucose levels, a meta-analysis of nearly 700,000 people found that fasting blood glucose was modestly and non-linearly associated with vascular disease in diabetic individuals (6). Finally, an epidemiological study in US adolescents also found a link between impaired fasting glucose and CVD risk factors, including high fasting insulin, total and low-density lipoprotein (LDL) cholesterol, triglycerides, systolic blood pressure, and low high-density lipoprotein (HDL) cholesterol (7). Thus, fasting glucose and HbA1c measures are considered by some to be valuable screening tools for type 2 diabetes and indeed are commonly used in clinical environments. However, it is interesting to note that early in the development of type 2 diabetes, postprandial glucose tolerance plays a large role, whereas fasting glucose plays a more substantial role later in the disease process (8). In other words, derangements in fasting blood glucose take longer to develop, whereas impairments in post-challenge glucose tolerance could be observed earlier.

Post-challenge/postprandial Blood Glucose and Cardiovascular Risk: Epidemiological Studies

In contrast to impaired fasting glucose, there are substantial data suggesting impaired glucose tolerance should be preferentially considered when assessing chronic disease risk (9,10). To support this, Tominaga et al. (9) found that survival rates in frank diabetes and impaired glucose tolerance were significantly lower than individuals with impaired fasting glucose, and further concluded that impaired glucose tolerance was a risk factor for CVD, but impaired fasting glucose was not. This finding was also supported by Blake et al. (10) who followed 937 nondiabetic individuals for nearly ten years and found that impaired glucose tolerance was phenotypically different from impaired fasting glucose, and is associated with increased presence

of CVD risk factors. Further, the authors reported that individuals with impaired fasting glucose had CVD risk factors that were similar to individuals with normal glucose tolerance, further weakening the apparent utility of fasting glucose as a measure of CVD risk (10).

A common way to assess glucose tolerance is the oral glucose tolerance test (OGTT), in which a plasma glucose measurement is made 2 hours after the ingestion of a standard glucose load, typically 75 or 100 g (11). There are numerous studies linking 2-hour glucose levels following an OGTT with CVD risk (12,13,14). The Chicago Heart Study found that 2-hour post-challenge glucose predicted all-cause mortality in a cohort of 11,554 white men and 666 black men (13). Similarly, results from the DECODE study indicated that high blood glucose following an OGTT was associated with increased risk of death, independent of fasting glucose (14). Finally, the Helsinki Policeman Study, Paris Prospective Study, and the Whitehall Study all found that all-cause and coronary heart disease mortality was predicted by 2-hour post-challenge glucose following an OGTT (15,16,17,18).

There are certainly drawbacks to the use of the OGTT to assess glucose tolerance and subsequent CVD risk. Consumption of 75-100 g of pure glucose rarely happens in daily life, and thus does not perfectly simulate the real-life glucose challenges faced daily by many people. Further, the extent of glycemia experienced following a meal is dependent on the entire contents of the test meal challenge. Hence, to better understand how the body tolerates glucose, it is important to challenge the metabolic system with a test meal that is representative of that experienced during daily living, such as a mixed meal. For this reason, some studies have utilized test meals that include fat and protein when assessing the glycemic response and subsequent CVD risk (19,20). Similar to studies using OGTTs, the Diabetes Intervention Study found that the postprandial glycemic response to a mixed meal was a significant predictor of coronary heart disease (19).

There is evidence that the glucose response seen following an OGTT is very similar to a mixed meal (in this case, consisting of wafers, canola oil, and honey), which suggests that the OGTT may have utility in assessing glucose tolerance, despite its apparent drawbacks (20). Thus, the glucose response to an OGTT may potentially be used as a proxy for, but is not identical to, true-to-life postprandial glycemia (2).

Post-challenge/postprandial Blood Glucose and Cardiovascular Risk: Intervention Studies

An important intervention study connecting postprandial/post-challenge hyperglycemia to CVD risk is the STOP-NIDDM trial (21). Results from the STOP-NIDDM trial show that treatment of individuals with impaired glucose tolerance with acarbose, an α-glucosidase inhibitor that specifically reduces post-challenge glycemia, is associated with a substantial (36%) reduction in the likelihood of progressing to overt diabetes (21). Additionally, acarbose treatment was associated with a 34% risk reduction of developing hypertension and a 49% risk reduction for developing cardiovascular events (21). Finally, in a subgroup of participants in which carotid intima media thickness was measured, treatment with acarbose was associated with a significantly attenuated progression of intima media thickness compared to the control group (22). This work was subsequently supported by a meta-analysis that indicated a significant reduction in cardiovascular events following acarbose treatment in diabetic individuals, even after consideration of other risk factors (23).

Another important intervention study by Esposito et al. (24) investigated the effects of two insulin secretagogues (compounds that promote insulin secretion) repaglinide and glyburide, which have differing effectiveness on hyperglycemia (with repaglinide being more effective), on surrogate measures of atherosclerosis. After one year, 52% of diabetic patients receiving

repaglinide and 18% of participants receiving glyburide displayed carotid intima media thickness regression (24). Further, C-reactive protein (CRP) and interleukin-6 (IL-6), both markers of systemic inflammation, decreased more in the repaglinide group than the glyburide group (24). These findings suggest a direct link between postprandial/post-challenge glucose intolerance and CVD.

Mechanisms Behind Blood Glucose and Cardiovascular Risk

How may post-challenge hyperglycemia and CVD be mechanistically linked? First and foremost, glucose intolerance is typically a feature of insulin resistance (25). Insulin resistance may result in deterioration of metabolic homeostasis in terms of both glucose and circulating free fatty acids (25). Not only do type 2 diabetes patients experience impaired clearance of glucose by insulin, they are also resistant to insulin-induced suppression of free fatty acid release (26). If not controlled, the elevated free fatty acid levels will precipitate elevated hepatic glucose production (27). The increased hepatic glucose production in combination with reduced insulin-stimulated glucose uptake produces a spike in blood glucose levels (25). The elevation of both fasting and postprandial glycemia results in numerous adverse outcomes.

To start, diabetic individuals typically experience endothelial dysfunction early in the disease process (28). Specifically, the vasodilatory response is not only diminished in diabetics but is also related to glycemic control (28), which is supported by findings from in vivo studies demonstrating that hyperglycemic spikes induce endothelial dysfunction in both normal and diabetic subjects (29,30). Similarly, there are data showing that during the postprandial period in diabetic participants, decreased flow-mediated dilation is negatively associated with the extent of postprandial glycemia (31). Additionally, rapid upsurges of glucose result in nonreversible glycosylation of proteins (32). These advanced glycosylated end-products bind to endothelial

smooth muscle receptors, precipitating increased endothelial permeability and vascular smooth muscle cell proliferation (2,32).

Additional avenues by which postprandial hyperglycemia may be mechanistically linked to CVD are increased blood coagulation and/or thrombosis. When hyperglycemia is induced experimentally, there is an increase in platelet aggregation (33), fragments of prothrombin (34), and a shortening of the half-life of fibrinogen (35). There also may be an immune and inflammatory component to postprandial hyperglycemia. Increases in TNF-α and IL-6 have been shown to occur both in the context of postprandial hyperglycemia (36) and with a hyperglycemic clamp (37). In addition, circulating levels of intracellular adhesion molecule-1 (ICAM-1) have been demonstrated to increase substantially following post-challenge glycemia, suggesting activation of one of the most proximal steps of the atheromatous lesion process (38). Finally, LDL oxidation is known to increase in type 2 diabetic patients after meal consumption and is inversely proportional to degree of metabolic control (39,40). Collectively, these findings provide support for the notion of direct metabolic and physiologic links between postprandial/post-challenge hyperglycemia and CVD risk.

Triglycerides and Metabolic Status/Risk

Fasting Triglycerides and Cardiovascular Disease Risk

Similar to fasting blood glucose, elevated fasting triglyceride levels are typically thought to be a risk factor for CVD. However, the data supporting this relationship are somewhat equivocal. It appears that, unlike HDL-C and LDL-C for which there are strong, consistent relationships with cardiovascular risk (41), the data do not clearly reveal fasting triglycerides to be an independent risk factor for CVD. For example, the relationship between triglyceride levels and cardiovascular risk, although significant in univariate analyses, sometimes disappears or weakens in

multivariate analyses that control for HDL-C (42). In addition, measurement of triglycerides, clinically or experimentally, can fluctuate greatly based on recent dietary composition, physical activity, or weight status (42,43). However, some evidence does point to the utility of measuring fasting triglycerides in order to assess cardiovascular risk (44,45). First, the Copenhagen Male Study, which featured an 8-year follow-up of nearly 3,000 middle- to older-aged men, found that men in the middle and highest tertile groups of fasting triglyceride levels had an increased risk for ischemic heart disease, as well as a risk gradient based on triglyceride levels, even after being stratified for HDL-C (44). Intriguingly, the middle and highest tertiles of triglyceride levels displayed relative risks for ischemic heart disease of 1.5 and 2.2 respectively, compared to the lowest tertile, even after consideration of LDL-C, HDL-C, physical activity, hypertension, type 2 diabetes, smoking, body mass index, and age (44). Further, a meta-analysis that included 46,413 men and 10,864 women across 17 population-based, prospective studies found that elevated triglyceride levels were associated with a ~30% increase in CVD risk in men and ~75% increase in women (45). While controlling for HDL and LDL cholesterol indicated a lowered associated risk between triglycerides and CVD, having elevated triglyceride levels was still a statistically significant risk factor.

Postprandial Triglycerides and Cardiovascular Disease Risk

While the clinical and experimental utility of measuring fasting triglycerides remains equivocal, there is substantial evidence suggesting that the transient, extensive rise in blood lipids (namely, triglycerides) following a meal can have direct atherosclerotic effects (46). Thus, postprandial lipemia is considered by many to be an independent CVD risk factor (2,47). First, whereas the relationship between fasting triglyceride levels and cardiovascular risk can be confounded by HDL-C levels (42), there are data demonstrating that as triglycerides increase in the postprandial

period there is a related decrease in certain HDL-C subfractions (48). Furthermore, postprandial levels of certain triglyceride-rich lipoprotein subfractions (particularly, small chylomicron remnants) were found to be correlated with coronary lesion progression in 32 post-infarction men, even after controlling for HDL-C and dense LDL apolipoprotein B (49). In a landmark study by Patsch et al. (46), 40 control subjects, and 61 subjects with severe coronary artery disease (CAD), completed meal tolerance tests in order to assess postprandial lipemia and CAD risk. Measures of postprandial lipemia (peak and AUC values) were significantly greater in the CAD patients than in the controls (46). Furthermore, postprandial lipemic values predicted the presence or absence of CAD with 68% accuracy by logistic regression analysis (46). Although not synonymous with postprandial triglycerides, non-fasting triglycerides still provide a way to examine the blood lipid levels that a person may experience in daily life, and are more practical for large-scale studies. In a prospective study of 26,509 initially healthy women, fasting and non-fasting triglycerides were assessed over ~11 years of follow-up and hazard ratios for incident cardiovascular events were determined (50). Although both baseline fasting and non-fasting triglyceride levels correlated with cardiac risk factors and markers of insulin resistance, the relationship disappeared with regard to fasting triglyceride values after covarying total cholesterol, HDL-C, and measures of insulin resistance (50). On the other hand, the relationship between non-fasting triglycerides and cardiac events remained robust even in the fully adjusted model (increasing tertiles of non-fasting triglyceride levels: 1 [reference tertile], 1.44, and 1.98), thus supporting the use of non-fasting (or postprandial) triglyceride measures over fasting triglyceride measures when predicting cardiovascular risk (50).

Mechanisms Behind Triglycerides and Cardiovascular Risk

The primary mechanism explaining how elevated triglyceride levels, be it fasting, non-fasting, or postprandial, induce CVD is the subendothelial retention of lipoproteins, which initiates atherogenesis (51). Circulating chylomicrons and large very low-density lipoproteins (VLDL) molecules, which are rich in triglycerides, are too big to penetrate the arterial wall, but smaller dense VLDL and other remnant molecules can enter the arterial wall and bind to proteoglycans (52). Presence of these lipoprotein and remnant molecules, which can be elevated during acute postprandial lipemia, initiates a local inflammatory response that includes macrophages and T-cells that promote subsequent lesion development (51). This hypothesis is in line with studies demonstrating that the extent of coronary blockage and subsequent CVD risk is associated with the degree of postprandial lipemia following a high-fat meal (46,49). In addition, there are also data suggesting that carotid intima media thickness is significantly positively associated with the postprandial lipemic response in both healthy and diabetic individuals (53,54). Further, many studies have provided evidence suggesting that markers of inflammation significantly increase in the presence of postprandial lipemia, including plasma IL-8, neutrophil counts, ICAM-1, and TNF-α (36,55,56).

Another avenue by which lipemia may increase cardiovascular risk is impaired endothelial function. It has been shown that endothelial function, measured as brachial artery blood flow, can be impaired in response to a high-fat meal, providing a potential mechanistic link between postprandial lipemia and heart disease, independent of cholesterol (57). Interestingly, a study by Erridge et al. (58) found a significant increase in circulating bacterial endotoxin following a high-fat meal, suggesting that low-grade endotoxemia may contribute to the inflammatory state that occurs after a high-fat meal, and may explain the endothelial dysfunction that occurs during

postprandial lipemia. As a whole, evidence suggests several overlapping mechanisms by which elevated triglyceride levels, fasting or postprandial, may lead to increased CVD risk.

Similarities between Glycemia and Lipemia

Since postprandial glucose and lipids both appear to fluctuate from homeostasis on a daily basis in normal living, and more importantly these aberrant responses appear to be strongly linked to cardiovascular risk, it may be worth considering them in unison, compared to independently as is currently common practice. However, in order to understand the ways in which glycemia and lipemia are linked, it is vital to discuss and recognize the overlapping metabolic pathways shared between dietary carbohydrate and lipids.

Uptake into Peripheral Cells

Circulating blood glucose is taken up by human cells via facilitated diffusion across the plasma membrane. There are at least six known glucose transporters (GLUTs) that perform this task (59). GLUT4 is the most widely acknowledged, as it is expressed on adipose cells, skeletal muscle cells, cardiac muscle cells and other insulin sensitive cells (60). As insulin binds to its receptor, GLUT4 is translocated to the plasma membrane and glucose enters the cell (60).
There are two primary ways in which lipid (specifically, fatty acids) can become available for energy use in the cytosol of a given cell. One option is for fatty acids to diffuse across the plasma membrane of the cell, similar to carbohydrate. These incoming fatty acids are either: 1) freed from triglycerides in lipoproteins by the action of lipoprotein lipase (LPL); 2) circulating bound to albumin; or 3) circulating as free fatty acids. The other option for fatty acids to become available in the cytosol is liberation from intracellular lipid pools. Regardless of how they arrive in the cytosol, fatty acids are typically found linked to fatty acid binding protein (FABP) (61).

In discussing the uptake of fat and carbohydrate into peripheral cells, it is worth considering the shared relationship of both glucose and fatty acids with the hormone insulin. As discussed previously (see Mechanisms behind blood glucose and cardiovascular risk), insulin works to both allow entrance of glucose into peripheral cells as well as suppress the release of free fatty acids into circulation. Thus, insulin clearly plays a major role in the regulation of circulating glucose and fatty acids. Further, as elevations in circulating free fatty acids can precipitate augmented hepatic glucose production (27), perturbations in the level of one substrate can affect the other. Therefore, based on the data there is a relationship between an individual's circulating levels of insulin, glucose, and fatty acids, and consequently derangement of one substrate or mechanism (e.g. insulin sensitivity) can produce a downward metabolic spiral (25).

Another relevant consideration when discussing uptake of carbohydrate and lipid is the effect of dietary fats on insulin resistance. Evidence suggests that increased levels of fatty acids, both in the diet and in circulation, can play a role in development of insulin resistance (62). The level of fatty acids in the diet and circulation, as well as a the type, i.e. the proportion of saturated fatty acids relative to unsaturated fatty acids, will be reflected in the fatty acid composition of peripheral cell membranes (63). Higher levels of saturated fatty acids in cell membranes appear to substantially hinder insulin action (62). In addition, as Randle and colleagues (64) first suggested in 1963, elevated fatty acids likely compete with glucose for clearance via oxidation, resulting in elevated blood glucose, and thus high fatty acid intake can be directly responsible for the body's inability to clear glucose. Over time this could lead to severely impaired glucose disposal and overt diabetes. It also appears that high levels of myocellular lipid can impair insulin sensitivity (65,66). As the traditional Western diet is typically high in fat, especially

saturated fat, these findings represent additional ways in which dietary lipid and carbohydrate intake are interconnected in the determination of chronic disease risk.

Major Metabolic Pathways

The initial step towards obtaining energy from glucose present in the cytosol is glycolysis, followed by the Kreb's cycle, and the electron transport chain (see reviews: references 67 & 68). Glycolysis is a set of ten reactions by which glucose is converted to two molecules of a pyruvate, with two adenosine triphosphate (ATP) molecules and one hydrogenated nicotinide adenine dinucleotide (NADH) molecule produced in the process. The newly generated pyruvate can have one of several different destinations, including entering the mitochondria and being converted into acetyl coenzyme A (CoA) via pyruvate dehydrogenase, or being converted to alanine or other amino acids, lactic acid, oxaloacetate, or back to glucose.

In order to obtain energy from the fatty acids located in the cytosol, the final destination is the Kreb's cycle as well, although there is a longer and more complex path. The process of fatty acid oxidation, known as beta-oxidation, is a four step process that results in the products flavin adenine dinucleotide (FADH2), NADH + H+, and acetyl CoA being produced. The acetyl CoA produced via beta-oxidation can either travel to the liver to form ketone bodies or condense with oxaloacetate to form citrate and enter the citric acid cycle. (It should be noted that odd-chain fatty acids and unsaturated fatty acids require additional steps and/or enzymes compared to even-chain saturated fatty acids, which were discussed here for simplicity.)

Acetyl CoA, whether its original source was lipid, carbohydrate, or otherwise, enters the citric acid cycle, also known as the Kreb's cycle, where it condenses with oxaloacetate to form citrate. Eight subsequent reactions occur, after which oxaloacetate is again present, thus making the process cyclic. During one round of the citric acid cycle, two carbon dioxide molecules, three

NADH+ molecules, one FADH2 molecule, and one ATP molecule are generated. NADH+ and FADH2 can then transfer their electrons to the electron transport chain, through which oxidative phosphorylation produces more ATP.

An Overwhelmed Pathway

It is important to consider the postprandial glycemic and lipemic responses together because they ultimately share similar destinations or outlets. Although details vary depending on the physiologic site, carbohydrates and fats can generally "compete" for clearance from the stomach, small intestine, bloodstream, and within the peripheral cell. Specifically with regard to the latter, both carbohydrates and lipids are largely converted to acetyl CoA and enter the citric acid cycle and the electron transport chain, as previously discussed. Consumption of large-volume, calorie-dense, and easily-digestible food precipitates substantial spikes in free fatty acids, triglycerides, and blood glucose. As the fat and carbohydrate compete for clearance, the excessive upsurge of metabolic energy overwhelms the metabolic capacity of the mitochondria within adipose and skeletal muscle cells, as well as all other metabolically active cells (69). Fatty acids and glucose swamp the citric acid cycle, which promotes the excess production of NADH+ that outperforms the electron transport chain (2). The buildup of NADH+ raises the proton gradient within the mitochondria, promoting the transfer of single electrons to oxygen, generating free radicals that include the superoxide anion (2,70,71). The elevated production of reactive oxygen species will subsequently oxidize LDL particles that have penetrated the endothelium and entered the subendothelial space (72,73). Oxidized LDL particles lead to an inflammatory cascade in the following ways: 1) increased expression of cellular adhesion molecules, which promote attachment and penetration of immune cells to the endothelium (74); 2) elevated release of pro-inflammatory cytokines (75); 3) activation of endothelial and smooth muscle cells (76); and 4)

increased uptake of the oxidized LDL particles by M2 macophages, triggering additional release of pro-inflammatory cytokines (73). Thus, the subendothelial penetration of lipoproteins and their subsequent oxidation by reactive oxygen species – both downstream results of the energy excess in circulation – can precipitate increased inflammation, sympathetic tone, and vasoconstriction, as well as thrombogenecity and advanced glycation end-products (AGE's) – all of which can increase an individual's CVD risk (2,69,77,78). So, since both glucose and fatty acids can "team up" to overwhelm the metabolic machinery of the body and produce the above response, it does not seem unreasonable to consider the glycemic and lipemic responses in the postprandial period together.

Summing Glucose and Triglycerides to Characterize the "Metabolic Load Index"

Given the potential utility and value in assessing postprandial glycemia and lipemia in unison, we suggest a summative index that we have termed "metabolic load index" (MLI). To create a MLI score, the clinician or scientist could simply combine the values of circulating blood glucose and triglycerides:

$$\text{MLI (mg/dL)} = \text{TG (mg/dL)} + \text{Glu (mg/dL)}$$

For instance, if considering a single time point, glucose and triglycerides could be summed to determine the metabolic load index in mg/dL for that point in time. The principle would remain the same if AUC was being considered: simply sum the triglyceride and glucose AUC values. In this way, the researcher and clinician could get an idea of the overall metabolic load, or challenge, that the patient or participant is experiencing as well as the volume of circulating available energy, either at that specific time point or over time (such as following a meal).

Figure 2 demonstrates MLI utilizing published data from our laboratory, which displays hourly changes in glucose, triglycerides, and MLI following a high-fat meal (79). The test meal consisted of ice cream and whipping cream (1 g/kg body weight carbohydrate and 1 g/kg body weight fat, ~60% fat, 20% carbohydrate). Metabolic load index is the sum of triglycerides and glucose at each time point. It is interesting to note that, since the meal contained ~60% fat, the MLI closely follows the change in triglycerides over time, most likely due to the large contribution of fat to the total calories of the meal. Similarly, as the carbohydrate load in the meal was relatively low, the blood glucose response was marginal and negligibly impacted the MLI during the postprandial period. This test meal was high in fat and few people would likely deem the meal "healthy". However, if blood glucose response was the main/only outcome of interest, the conclusion after consumption of such a meal could be that metabolic disturbance was minimal, although this is clearly not the case when the triglyceride and MLI responses are considered together. Thus, clinically, assessment of MLI could provide a measurement of metabolic challenge or disturbance without biasing towards glucose or triglycerides alone. Further, consideration of MLI may be more relevant when determining metabolic responses to mixed meals that are more typically consumed in daily living, as compared to an all-lipid or all-carbohydrate meal, as is used in an OGTT.

To support this notion, there is evidence indicating that postprandial fat and carbohydrate metabolism are not independent processes and alterations in one can affect the other. An early study elucidating this relationship was conducted by Albrink and colleagues (80) in 1958 in which a 60 g fat meal was consumed with and without 100-250 g of added glucose. The authors found that the addition of glucose either diminished or completely abolished the postprandial rise in triglycerides, depending on the amount of added glucose (80). In a related study several years

later, Mann et al. (81) found that postprandial lipemia was cleared more slowly following a meal that contained a glucose load compared to a meal that contained sucrose. These early findings have been supported by several more recent studies demonstrating similar results (82,83,84). In a randomized crossover study, Westphal et al. (84) investigated the effect of adding 75 g of glucose to a high-fat meal compared to a high-fat meal without the added glucose on triglycerides, VLDL, and chylomicrons. The addition of glucose to the meal resulted in a delay in the triglyceride response, as well as a lower peak (84). There was also a 42% reduction in incremental AUC for the meal with glucose compared to the fatty meal alone. Finally, there was a delayed chylomicron response and a blunted VLDL response. A similar investigation by Knuth et al. (83) demonstrated a reduction in postprandial plasma triglycerides in women following a fat meal containing additional carbohydrate compared to a fat meal alone. Collectively, these studies suggest that the addition of carbohydrate to a meal and the resultant glycemic response alters the postprandial rise in triglycerides. The interrelationship between postprandial glycemia and lipemia also holds when adding fat to a high-carbohydrate meal (85,86). In an investigation by Collier and O'Dea (85), when 50 g fat (as butter) was added to 50 g carbohydrate (as potato), the postprandial glucose response was significantly blunted compared to ingestion of potato alone. This mitigation was likely a result of delayed glucose absorption in the small intestine, consequent to a fat-induced repression of gastric emptying. Together, these findings suggest that postprandial glucose and lipid metabolism are not independent processes – rather they are interrelated, and consequently it would be valuable to consider them together.

There may also be merit in considering an energy-adjusted version of the MLI. Lipid typically yields ~9 kcal/kg and carbohydrate yields ~4 kcal/kg. In an attempt to consider the amount of circulating energy in the bloodstream at a given time, there may be utility in adjusting the

metabolic load index equation to reflect the difference in energy yield between carbohydrate and fat, which could be accomplished via an "Adjusted Metabolic Load Index", or A-MLI:

$$\text{AMLI (in kcal/dL)} = (\text{TG (in mg/dL)} \times 0.001 \text{ g/mg} \times 9 \text{ kcal/g}) + (\text{Glu (in mg/dL)} \times 0.001 \text{ g/mg} \times 4 \text{ kcal/g})$$

For instance, if an individual was found to have triglyceride levels of 120 mg/dL and glucose levels of 90 mg/dL, their A-MLI would be 1.44 kcal/dL. Thus, the A-MLI would provide an equation through which to quantify or estimate the available circulating energy from fat and carbohydrate. However, there are some issues with the A-MLI, which include biasing towards triglycerides and presenting somewhat unusual units (kcal/dL). Nevertheless, the A-MLI may prove to be a valuable clinical tool in assessing the metabolic state. Research comparing MLI and A-MLI with regard to prediction of and association with health outcomes would be valuable.

Utility of the Metabolic Load Index

What are some ways or instances in which utilization of the MLI would be beneficial or enhance our understanding of an individual's metabolic status? First and most clearly, use of the (MLI) could better reveal the degree of metabolic challenge that a person is experiencing either while fasting or after a meal. This utility would be particularly applicable to patient populations, as it has already been previously discussed how fluctuations in postprandial glycemia and lipemia can be cardiovascular risk factors. Specifically, it may be valuable to develop a "cut point" for fasting MLI, such as 200 mg/dL, since postprandial assessments may be too cumbersome in certain clinical settings. Particular patient populations for which assessing MLI would be valuable might include individuals with diagnosed cardiovascular disease or type 2 diabetes. Since metabolism of lipids and carbohydrates is so interrelated, consideration of the glycemic or lipemic response together would be valuable.

Metabolic load index would also be useful in terms of standardizing responses across studies investigating postprandial metabolism. One of the most difficult aspects of compiling and comparing studies either investigating postprandial lipemia or glycemia (when a mixed meal is used) is the heterogeneity of test meal challenges that are utilized in the investigations. While differences in the amount of total energy (and perhaps participants' body mass) would still need to be considered, MLI may help in comparing across studies that use meals of different macronutrient distribution but similar caloric value.

A current area of debate is the ideal proportion of macronutrients that should be consumed in a healthy diet. If the MLI response is similar between diets of different macronutrient distribution but similar caloric load, it may make the focus on select macronutrient profiles less prevalent.

Considerations of the Metabolic Load Index

Given the novelty of the MLI, there are certainly considerations to be made. First, it has been shown that dietary fructose leads to different postprandial metabolic responses than dietary glucose (87). Specifically, fructose consumption (compared to glucose) as part of a mixed meal will lead to 1) a greater triglyceride response, particularly in the latter stages of a postprandial assessment; 2) a comparatively negligible glucose response; and 3) a much more tempered insulin response (87). However, we assert that the different metabolic responses of glucose and fructose do not represent a weakness of MLI, but rather a strength. Considering the different metabolic consequences of glucose and fructose, the outcomes of interest are nevertheless the same: circulating glucose and triglycerides, the two markers measured in the MLI. Thus, while fructose may lead to a greater triglyceride response and a lesser glucose response compared to dietary glucose (87), this will be captured in the MLI. If only considering the blood glucose response, one could surmise that fructose is better to include in a mixed meal because there is a

much less blood glucose response. However, utilizing the MLI, it can be seen that fructose still has substantial metabolic effects – though it more drastically alters the other component of the MLI equation, triglycerides. Thus, we assert that a strength of MLI is that it "throws a broader net". In other words, even though many nutrients clearly have differing metabolic effects, such as glucose and fructose, MLI is better equipped than blood glucose or triglyceride assessment alone to capture the metabolic challenge that the body is experiencing.

It would be valuable to determine an optimal postprandial time point to assess MLI. For instance, it appears that triglycerides measured two and four hours after meal consumption have the strongest association with cardiovascular events (50) and that triglycerides tend to peak four hours after a meal (88,89). Additionally, one study has found that measuring triglycerides four hours post-HFM is a suitable surrogate for longer and more involved postprandial lipemia assessments (90). On the other hand, as previously discussed, post-challenge glucose tolerance is typically assessed in the context of a two-hour OGTT (11) and two-hour glucose values are strongly related to CVD risk (12,13,14). Clearly, there are differences in the timing of postprandial glucose and triglyceride responses, and consequently the timing of assessment to best ascertain an individual's disease risk. Considering these differences, it is difficult to state an optimal, single time point at which to assess MLI in clinical and research settings. It would be very worthwhile for future studies to address this question using large datasets of postprandial metabolic markers and disease risk outcomes.

Similarly, a logical question is: which individuals would benefit most from ascertaining their fasting and postprandial MLI? An expert panel statement has suggested that postprandial triglyceride assessments are most valuable in individuals with fasting levels between 89-180 mg/dL (91). The rationale for this stance is that individuals with fasting triglycerides <89 mg/dL

will typically show a negligible postprandial response, while those with fasting triglycerides >180 mg/dL will present an excessively delayed and exaggerated postprandial lipemic response, such that there is little diagnostic utility (91). In line with this, there may be certain populations that could most benefit diagnostically from determination of MLI. It could be that individuals with very low or high fasting glucose and/or triglyceride levels may not benefit very much from the additional metabolic information provided via MLI. However, we are hesitant to state which individuals would and would not benefit from fasting and postprandial MLI assessment, given the current dearth of evidence to support a conclusion. Further, as addressed previously, with both glucose and triglycerides, postprandial values are typically better predictors of CVD risk compared fasting values. Thus, there is not compelling evidence suggesting that individuals with certain fasting MLI values would not benefit substantially from a postprandial MLI assessment. It would be advantageous for future research to further explore this concept.

Finally, it is likely that genetics influence the postprandial MLI response. This supposition is based on the notion that gene variation has been found to contribute to inter-individual differences in both postprandial lipemia and hyperglycemia. With regard to postprandial lipemia, the most heavily studied genetic regions have been the encoding of various apolipoprotein genes, such as *APOA1* (92) and *APOC3* (93), and genes encoding lipid metabolism enzymes, such as LPL (94) and HL (hepatic lipase) (95). A few areas of genetic contribution to hyperglycemia include adiponectin gene polymorphisms (96,97), telomere length (98), and *TCF7L2* variation (99), a transcription factor that is to date the most substantial genetic contributor to type 2 diabetes incidence (100). Clearly, as there are established genetic contributors to both postprandial lipemia and hyperglycemia, it is logical to assume genetic factors influence MLI as well.

Conclusion and Future Directions

It has been well-established that postprandial glycemia and lipemia are risk factors for CVD. Specifically, postprandial glycemia and lipemia have been linked to increased disease risk by way of oxidative stress, inflammation, and endothelial dysfunction, as well as other mechanisms. Although carbohydrate and lipids ultimately share similar clearing mechanisms, the fluctuations in these substrates in daily life, or experimentally following a test meal challenge, have historically only been considered separately. However, in light of evidence that postprandial glycemia and lipemia are not independent of one another, it does not seem unreasonable to consider both of these phenomena together in a single index. The concept of metabolic load index, which is the sum of blood glucose and triglycerides, is valuable in that it considers the total metabolic challenge that the body is experiencing, either at a single time point, or over time. In our opinion, the MLI carries significant potential clinical utility. It would be worthwhile for future investigations to assess MLI in the context of disease risk, investigating the relationship between MLI and markers of inflammation, oxidative stress, endothelial dysfunction, and other risk markers. However, currently we do not know whether adding the two outcomes in a one to one ratio is the most accurate way to predict risk when considering glucose and triglycerides together. Thus, future work should seek to derive the best equation for assessing glycemia and lipemia simultaneously by using data from clinical populations and longitudinal studies.

Figures

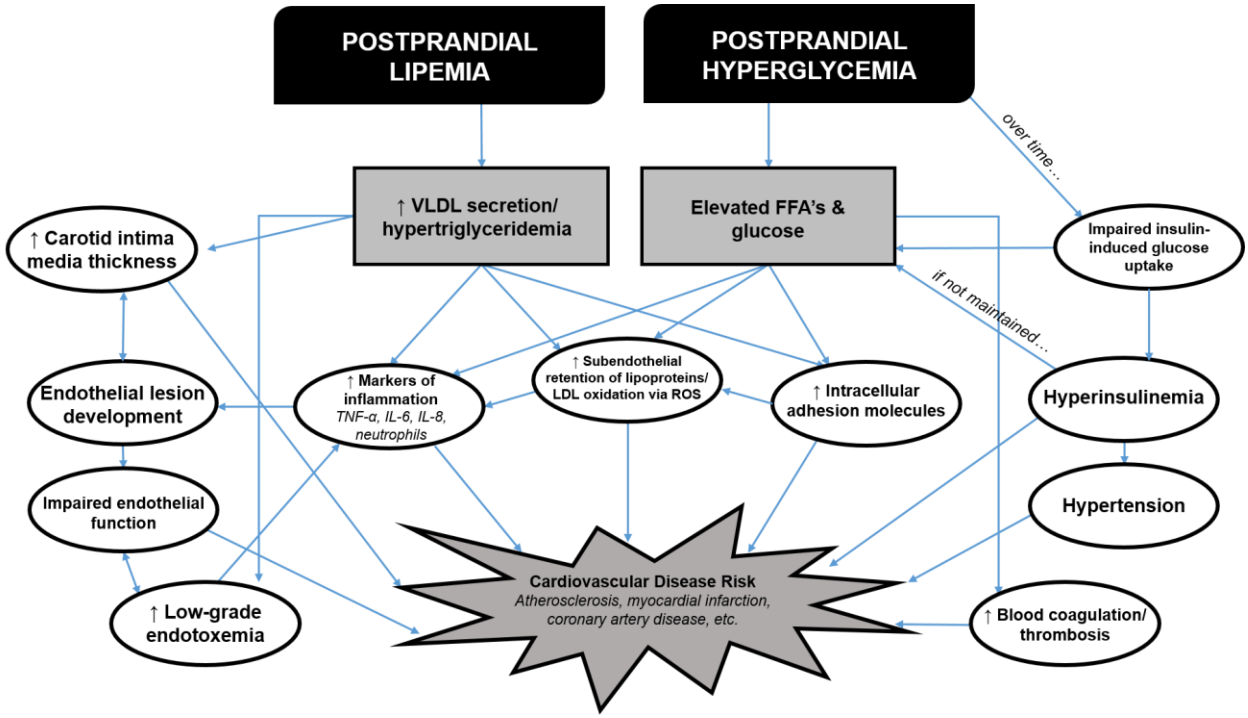

Figure 2-1. Postprandial lipemia, postprandial hyperglycemia, and cardiovascular disease risk.

Multiple potential mechanisms exist to explain the connection between postprandial dysmetabolism (glycemia and lipemia) and cardiovascular disease risk. Similar primary facets involved in both postprandial glycemia and lipemia are elevated markers of systemic inflammation, increased subendothelial retention of lipoproteins, and increased intracellular adhesion molecules. See *Mechanisms behind blood glucose and cardiovascular risk* and *Mechanisms behind triglycerides and cardiovascular risk* for more detail.

VLDL, very low-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FFA, free fatty acids; ROS, reactive oxygen species.

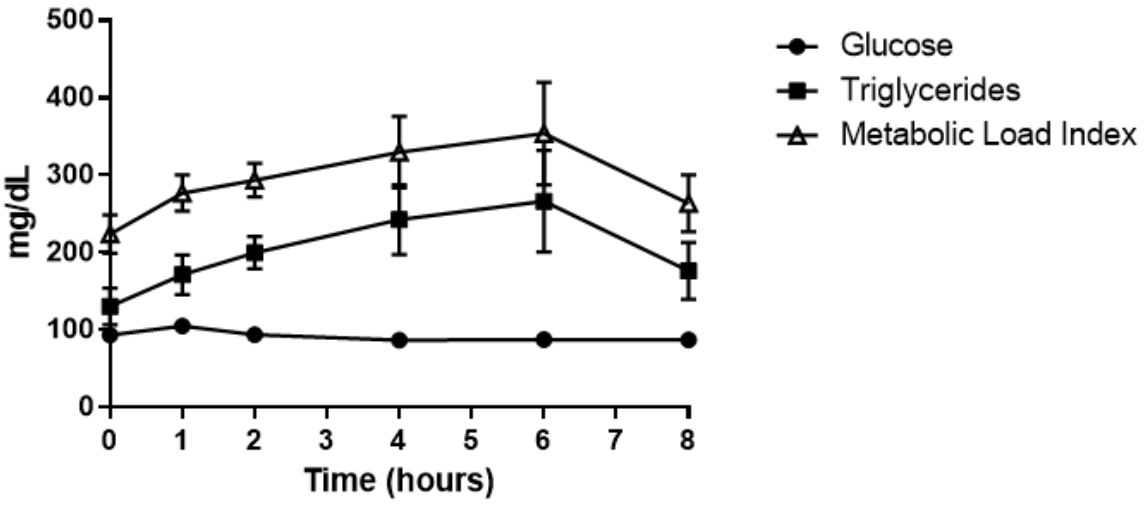

Figure 2-2. Hourly changes in glucose, triglycerides, and metabolic load following a high fat meal.

These data were taken from a previously published study in our laboratory.(79) This figure displays glucose (●), triglyceride (■), and metabolic load index (Δ) at baseline (time 0) and for eight hours during the postprandial period following consumption of a high-fat meal. With this data, fasting and postprandial metabolic load index can be determined by adding the triglyceride and glucose value at each time point. See *Part V – Summing glucose and triglycerides to characterize "Metabolic Load Index"* for more detail. Data are Mean ± SEM.

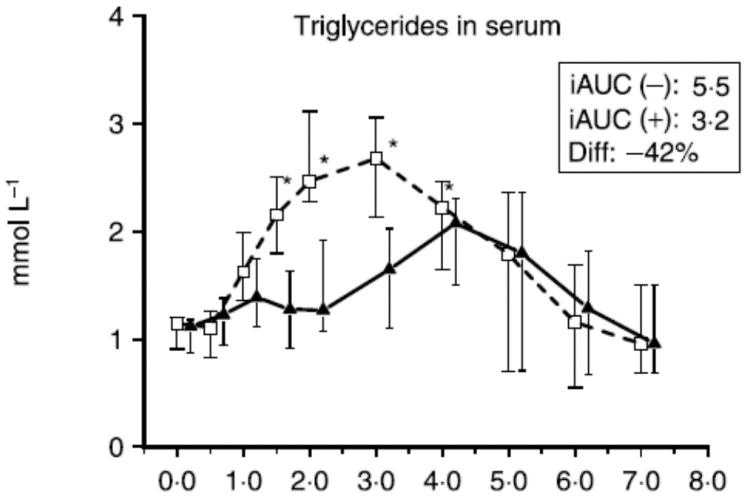

Figure 2-3. Postprandial triglyceride responses to a high fat meal with (▲) and without (□) 75 g of added glucose.

The triglyceride response is blunted in terms of both peak value and time to peak when glucose is added to the high fat meal. Incremental AUC is 42% lower in the fat meal with added glucose compared to the fat meal alone (see legend). Adapted with permission from Westphal *et al*. (84).

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Chapter 3 - Magnitude and Timing of the Postprandial Inflammatory Response to a High-fat Meal in Healthy Adults: A Systematic Review

Abstract

Research findings over the past several decades have revealed that inflammation is a prominent feature of many chronic diseases, with poor diet being one likely inflammatory stimulus. Specifically, a single high-fat meal (HFM) has been suggested to increase inflammation, although there is currently no consensus regarding the specific changes of many of the pro-inflammatory markers that are frequently assessed after a HFM. The aim of this systematic review was to objectively describe the postprandial timing and magnitude of changes of five common inflammatory markers: interleukin (IL)-6, C-reactive protein (CRP), tumor necrosis factor (TNF)-α, IL-1β, and IL-8. Ten relevant databases were searched, yielding 494 results, of which 47 articles met the pre-established inclusion criteria: 1) healthy men and women age 18-60 years; 2) consuming a single HFM (≥30% fat, ≥500 kcal); and 3) assessing relevant inflammatory markers post-meal for ≥2 hours. The only marker found to consistently change (increase) in the postprandial period was IL-6 – on average starting at a baseline of ~1.4 pg/mL and peaking at ~2.9 pg/mL approximately 6 hours post-HFM (an average relative change of ~100%). C-reactive protein, TNF-α, IL-1β, and IL-8 did not change significantly in 79% (23/29), 68% (19/28), 67% (2/3), and 75% (3/4) of included studies, respectively. We conclude that there is strong evidence that CRP and TNF-α are not responsive at the usual time scale observed in postprandial studies in healthy humans younger than age 60 years. However, future research should further investigate the role of IL-6 in the postprandial period, as it routinely increases,

even in healthy participants. We assert that the findings of this systematic review regarding markers of inflammation in the postprandial period will considerably aid in informing future research and advancing clinical knowledge.

Introduction

Cardiovascular disease (CVD) is widely recognized to be the leading cause of death in the United States and throughout Western society (1). Lifestyle factors that appear to increase risk for CVD include insufficient physical activity (2), obesity (3), and poor dietary habits (4). While the causal factors leading to the manifestation of CVD are certainly complex and numerous, it has become clear that a common feature of heart and vascular diseases is inflammation (5). Atherosclerotic lesions, a prominent feature of CVD, are a hotbed of inflammatory activity. Briefly, immune cells such as T-cells, macrophages, and mast cells will infiltrate into an atheromatous lesion, where they can: 1) promote prothrombotic factors, 2) cause the release of metalloproteinases and cysteine proteases that can reduce the stability of the atherosclerotic plaque, and 3) promote the release of pro-inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) (6). These acute markers of inflammation then travel to the liver, whereby they stimulate the increased release of chronic, low-grade markers of inflammation, such as C-reactive protein (CRP). Excessive inflammation is positively associated with type 2 diabetes, obesity, and coronary artery disease (7,8).

But what are the stimuli that jumpstart the deleterious inflammatory cascade? A commonly suggested inflammatory stimulus is a chronic high-fat diet. Indeed, when rodents are fed a high-fat diet, there is an increase in markers of inflammation both in adipose tissue and in systemic vasculature (9,10,11). Thus, a diet high in fat and overall energy may partly cause the elevated systemic inflammation that underpins cardiovascular disease, as well as insulin resistance, and is

associated with obesity (12). However, the effect of dietary consumption on inflammation may not be limited to chronic intake, but may be evident after consumption of a single meal.

To this end, numerous studies have been undertaken to investigate the effects of a single high-fat meal (HFM) on postprandial inflammation (see Table 1 and Supplemental Tables 1-4). Many studies have found a significant increase in markers of systemic inflammation following a HFM, while others have found no changes. Study design variables that could potentially affect relevant findings and consequently precipitate inter-investigation differences include meal size, meal composition, subject characteristics, previous acute exercise, postprandial period assessment length, and method of drawing blood. As a result, we are currently far from consensus with regard to the response features (i.e. timing and magnitude) following a HFM of even the most commonly assessed markers of inflammation. A synthesis of previous research investigating postprandial inflammation, with particular attention to the specific features of the response, would inform future research and advance clinical understanding.

Therefore, the purpose of this systematic review was to characterize the postprandial inflammatory response, in terms of magnitude and timing, to a HFM in healthy men and women (age 18-60) based on the consolidated findings of previous relevant investigations. The markers of inflammation included in the present review include IL-6, IL-1β, IL-8, TNF-α, and CRP, as these are frequently assessed inflammatory markers in the postprandial period.

Methods

Inclusion Criteria

In order to be incorporated into the present systematic review, there were multiple inclusion criteria that each study was required to meet. Individuals being assessed were to be human men or women of age 18-60 years. Participants had to be healthy and not diagnosed with any chronic

disease. Studies featuring overweight and obese participants were included in the analyses as long as they did not present with any other chronic disease. The study must have included a HFM challenge that provided at least 500 kcal of energy with at least 30% of the energy from fat. The study needed to feature a single meal, or studies with serial meals provided in the postprandial period were included if there were data included for time points prior to the second meal. Any data reported after a second meal were excluded from the present analyses. If a study contained multiple meal trials or subsets of participants, each meal or participant group that met the inclusion criteria was considered separately. If a study included an exercise session, only the control (no exercise) condition was included. In order to be included, each study must have assessed one or more of the previously stated markers of inflammation (IL-6, IL-1β, IL-8, TNF-α, and CRP), both at baseline (fasting) and in the postprandial period for at least 2 hours. Only data from full-text, peer-reviewed and published articles were included (i.e. data from conference proceedings, abstracts, and textbooks were not included). There were no restrictions on year of publication, but only English-language articles were included. If a study did not satisfy all of the aforementioned criteria, it was excluded from the present systematic review.

Search Strategy

Article searches occurred in January and February of 2016. Automatic searches recurred weekly throughout manuscript preparation in order to capture very recent publications. However, recurring automatic searches produced no new articles. Databases that were searched include: NCBI Pubmed, Scopus, Proquest Nursing and Allied Health, Web of Science, Cochrane Library, SpringerLink, SPORTdiscus, Health and Wellness Resource Center, Health Reference Center Academic, and PsycINFO. Key search terms were: *postprandial* or *post prandial*; *high fat meal*; *inflammat** or *cytokine* or *interleukin*; *healthy* or *normal*; and *humans* or *men* or *women*.

Appropriate search modifiers were used to exclude *children*, *elder**, *rodents*, *rats*, and *mice*. Articles retrieved during searching were imported into and catalogued using Refworks reference management software (ProQuest LLC, Ann Arbor, MI). The process and results of the systematic search are displayed in Figure 1. There were 494 total citation hits from all databases combined. Of these, 163 citations were eliminated as duplicates (75 duplicates were retained). Of the 331 citations that passed the duplicate check, 103 citations were eliminated on the grounds of not being full-text peer-reviewed research articles. The remaining 228 articles were assessed based on aforementioned criteria, in abstract-form only, by two independent reviewers (SRE and SPK). The reviewers then met to discuss inclusion/exclusion of each abstract and 156 articles were eliminated. Of the eliminated articles, 88 papers were eliminated due to not being postprandial studies assessing responses to a meal. The other 68 abstracts that were postprandial studies were eliminated due to not testing humans age 18-60 years (8 articles), participants presenting with a chronic disease (12 articles), not utilizing test meals that were at least 500 kcal and 30% fat (9 articles), and/or not testing one or more of the relevant inflammatory markers (51 articles). The remaining 72 articles were retrieved in full-text form and given thorough assessment by both reviewers. Finally, 25 articles were eliminated following full-text assessment, leaving 47 articles to be included in the final analyses. The reasons for the eliminated 25 full-text articles are detailed in Figure 1. Some abstracts and full-text articles were eliminated for not complying with multiple inclusion criteria.

Data Extraction

Information regarding the test meal (composition, fat and energy content), participants (number of participants, male/female ratio, and age and BMI of participants), blood draw method (cannula or repeated venipuncture), and length of postprandial assessment were extracted from each study.

In addition, for each inflammatory marker of interest (IL-6, IL-8, IL-1β, TNF-α, and CRP), the following information was extracted: whether or not the marker significantly changed from baseline, the fasting value, the time to peak or nadir (if applicable), and the peak or nadir value (if applicable). For many studies, all of the necessary information was not explicitly included in the manuscript. In these cases, authors were directly contacted in order to obtain the missing information. Many authors provided the missing information (see Acknowledgements), although some did not; thus, some data are missing from the present analyses.

Assessing Risk of Bias

A quality appraisal or risk of bias assessment was conducted for all 47 articles included in the analyses. All of the included studies utilized the same general study design, therefore traditional quality appraisal assessment tools were not applicable to the present systematic review. Consequently, we developed an internal validity checklist in order to assess the strength of each study. This tool included nine criteria based on different components of postprandial inflammation study design: control of diet, fasting quality control, control of exercise, sample size adequacy, postprandial period length, blood draw frequency, blood draw method, normalization of test meal, and proper processing of inflammatory marker analyses. Each study was assessed against the above criteria and given a score ranging from 0-9. Studies were scored separately by two independent reviewers (SRE and SPK), after which the reviewers met to confirm a final score for each study.

Statistical Analyses

Data analyses were performed using GraphPad Prism (Version 6.05; GraphPad Software, Inc; La Jolla, CA) and SPSS Statistics software (v.22; SPSS, Inc; IBM Corporation; Armonk, NY). The primary outcome measures in this review were mean baseline and peak values and the time-to-

peak value for each marker of inflammation (in some studies, certain inflammatory markers were found to decrease, in which case the nadir and time-to-nadir values were utilized). Fasting and peak values for each inflammatory marker were assessed for objective statistical outliers using the ROUT method (50), which utilizes nonlinear regression, in GraphPad Prism. The Q value (or maximum false discovery rate) was set at 1%. The ROUT method involves three steps. First, a robust nonlinear regression is employed to fit a curve that is not affected by outliers. Second, the residuals of the robust regression are assessed to determine whether or not there are any outliers. The third step of the ROUT method is simply removing the outliers from the dataset. Nine outliers (seven fasting values and two peak values) were removed from the IL-6 dataset, one outlier (a fasting value) was removed from the CRP dataset, and seven outliers (four fasting values and three peak values) were removed from the TNF-α dataset. The datasets for IL-8 and IL-1β were too small for outlier analyses, and thus all values were retained. For analyses of changes/differences between baseline and peak values, if there was no significant change post-meal for a given marker in a study, the baseline value was used as both the fasting value and the peak value in order to have a complete dataset and not bias the findings toward a significant change. (Many studies did not report a post-HFM value in the event of no statistically significant change. Thus, in the original analyses, in all studies that did not find a significant change after the meal, the baseline value was used as the post-meal value. However, a secondary analysis that included post-HFM values of studies that did not find a statistically significant change (when available) did not appreciably change the results. Also, the studies that did report a statistically non-significant post-meal value generally did not find a biologically significant change either.) A paired t-test was used to assess differences between mean baseline and peak IL-6 values from each study. This analysis was only performed for IL-6 because most studies found no significant

change in the other markers post-meal. Spearman rank correlations were used to assess relationships between percent change in IL-6 (from fasting to peak) and mean participant BMI, mean participant age, energy content (in kcal) of the test meal, and percent energy content from fat in the test meal. As BMI and percent change in IL-6 were not normally distributed, they were transformed using the Log-10 method. Data are presented as Mean ± SD. For all relevant analyses, the p-value was set at less than 0.05.

Results

Table 1 and Supplemental Tables 1-4 display the extracted information from each study separated by respective marker of inflammation. Some studies are represented in multiple tables, as they assessed more than one of the relevant markers of inflammation. For IL-6, 32 of 45 studies (~71%) found a significant increase in the marker following HFM consumption. Ten studies (~22%) found no change in IL-6 post-HFM, two studies (~4%) found a significant decrease, and one study did not report whether or not the marker changed. Of the 32 studies that found a significant increase, two used repeated venipuncture and thirty utilized a cannula. In studies that found a significant change post-HFM, the time to peak was 5.9 ± 2.0 hours. For twenty-two of the 34 studies that found a significant postprandial IL-6 change, the mean peak value occurred at the final time point assessed in the study protocol. Prior to removal of outliers, the baseline (fasting) and peak IL-6 values for all studies combined was 4.83 ± 8.02 pg/mL and 4.76 ± 6.87 pg/mL, respectively. Following outlier removal, the mean baseline and peak values were 1.37 ± 0.93 pg/mL and 2.85 ± 1.85 pg/mL, respectively. The mean percent change from baseline to peak (following outlier removal) was 153 ± 256 %. After removal of outliers, but including studies that found no change or a decrease in IL-6 post-HFM, there was a significant increase ($p < 0.0001$) in IL-6 from baseline to peak value. Figure 2A displays the IL-6 change

from fasting to peak for each individual study (following outlier removal) and the mean response.

Twenty-nine studies met the inclusion criteria and measured CRP in the postprandial period. Of these, 23 studies (~79%) found no change in CRP in the assessed postprandial period. Four studies (~14%) found an increase and two studies (~7%) found a decrease in CRP after the meal. Of the studies that found an increase, the peak occurred at 4.7 ± 2.3 hours post-HFM. The fasting value for CRP was 1.50 ± 1.15 mg/mL before outlier removal and 1.35 ± 0.86 mg/mL after outlier removal. Three of the four studies that found a significant increase in CRP post-HFM did not report the peak value; thus, a mean peak value has not been calculated. Figure 2B displays the mean and individual responses of CRP to a HFM for all studies.

TNF-α was assessed in 28 of the included studies. Five studies (~18%) found an increase, four studies (~14%) found a decrease, and nineteen studies (~68%) found no significant change in TNF-α from baseline during the postprandial period. Prior to removal of outliers, fasting and peak TNF-α values were 33.5 ± 143.5 pg/mL and 102.0 ± 242.0 pg/mL, respectively. After removal of outliers, the fasting and peak values were 2.76 ± 1.95 pg/mL and 2.85 ± 2.02 pg/mL, respectively. In the studies that found a significant post-meal TNF-α increase, the peak occurred at 5.5 ± 3.0 hours. In the studies that found a decrease, the nadir occurred at 7.5 ± 1.0 hours. Figure 2C provides a visual representation of change or stagnation of TNF-α following HFM intake for the studies that passed the outlier check.

With regard to IL-1β, three studies assessed this marker in the postprandial period. Two studies found no significant change from baseline during the postprandial period, while one study found a significant decrease. In this study, the mean nadir value occurred at four hours post-meal. Figure 2D displays fasting and postprandial values for the three studies that measured IL-1β.

Four studies measured IL-8 in the postprandial period. Of these, one study found a significant increase in IL-8 from baseline to peak and three studies found no change. The baseline value for IL-8 was 2.29 ± 0.36 pg/mL. In the one study that found a significant increase, a peak value of 3.26 ± 2.56 pg/mL was found four hours after the HFM. Figure 2E shows the mean and individuals values for IL-8 at baseline and post-meal for the four respective studies.

Figures 3A-D show correlations of the percent change in IL-6 from fasting to post-meal with different independent variables that could potentially affect the IL-6 response. There was a significant negative correlation ($\rho = -0.42$; $p = 0.02$) between the percent of energy from fat in the test meal and the percent change in IL-6 (Figure 3A). There was not a significant correlation ($\rho = 0.23$; $p = 0.26$) between the energy content of the test meal and the percent change in IL-6 (Figure 3B). Similarly, mean BMI showed no significant relationship ($\rho = -0.12$; $p = 0.55$) with the percent change in IL-6 (Figure 3C). Finally, there was not a significant negative correlation ($\rho = -0.28$; $p = 0.16$) between mean age of the participants and the percent change in IL-6 (Figure 3D).

The results of the quality appraisal for each study are displayed in Table 1 and Supplemental Tables 1-4. There was little variability in quality assessment scores among studies, ranging from 2.5 to 6.5 points out of a possible 9 points. Generally, no differences were observed between studies with regard to appraisal score and whether or not a postprandial inflammatory change was detected. Since the quality appraisal scores did not noticeably impact our primary outcomes, we did not adjust analyses to weight studies differently based on appraisal score. Further, as the research questions primarily involved calculation of baseline and peak means in inflammatory markers (as opposed to effect sizes of interventions, etc.), the present analyses did not fit well with traditional meta-analyses statistics conducted within systematic reviews.

Discussion

Main Findings

The purpose of this systematic review was to characterize the magnitude and timing of changes in markers of inflammation following HFM consumption, utilizing methodically selected research articles that met pre-established criteria. The primary findings were that: 1) very often, there was a postprandial increase in IL-6; 2) IL-6 typically peaked at approximately 2.9 pg/mL, or, more relatively, exhibited a ~100% increase from baseline, that typically occurred approximately 5.9 hours after the HFM; 3) TNF-α and CRP were assessed many times and yet very infrequently showed an increase post-HFM; 4) IL-8 and IL-1β have only rarely been assessed post-HFM in studies meeting our criteria; and 5) in studies that did assess IL-8 and IL-1β, although equivocal, the data suggested that these markers of inflammation did not significantly change after consumption of a HFM. We believe that these findings are likely to be instrumental in advancing our understanding of the immune and inflammatory status of healthy individuals before and after HFM intake, and will have utility in designing and interpreting future research.

Importance of Postprandial Metabolism

Why should we be concerned with the timing and magnitude of inflammatory cytokine responses following HFM ingestion? It is because substantial research points to the notion that persistent low-grade inflammation is an underlying factor in several high-mortality chronic diseases, and that diet can contribute to, or attenuate, that inflammation (5). It was previously thought that atherosclerosis was a lipid-storage disease (7). However, we have come to realize the vital role of inflammation in the etiology of vascular diseases (5,6,51). To be sure, lipids play a role in the disease process, as subendothelial penetration and retention of lipoproteins can serve as an

initiating event for the atherosclerotic cascade (52). However, once the lipoproteins are in the endothelium, oxidative stress and inflammation processes assume a prominent role (6,7). The lipoproteins are oxidized by reactive oxygen species, forming oxidized low-density lipoproteins (LDL). Oxidized LDL particles have several pro-inflammatory effects, including: 1) increased expression of adhesion molecules (such as vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1(ICAM-1)), which promote the adhesion and penetration of immune cells to the endothelium (53); 2) increased pro-inflammatory cytokine release (54); and 3) activation of smooth muscle and endothelial cells (55). In turn, the increased presence of immune cells, via the functions of adhesion molecules, further increases the oxidation of lipoproteins (7). Many oxidized LDL particles will be phagocytically ingested by macrophages. These macrophages, formerly monocytes, are present consequent to their recruitment by inflammatory mediators, such as cytokines, and the linkage and induction properties of adhesion molecules (6). When monocytes penetrate the endothelium, they differentiate into macrophages. Macrophages play a crucial role in the inflammatory processes that characterize atherosclerosis. Macrophages present cell surface receptors which, when activated, result in the increased production of many pro-inflammatory cytokines and adhesion molecules (56). Further, as macrophages become increasingly lipid-laden, as a result of oxidized lipoprotein phagocytosis, they will be laid down in the endothelium as foam cells, which are the hallmark cells of the atherosclerotic process and promote the progression of intima media thickness (6).

The increased production of pro-inflammatory cytokines throughout the process outlined above is important considering their physiological effects. First, cytokines act as intermediary messengers, recruiting immune cells such as monocytes, dendritic cells, and lymphocytes to the site of vascular damage, increasing the inflammatory tone of the endothelium (57). Further, it

appears that inflammatory cytokines promote the activation of vascular smooth muscle and increase vascular sympathetic tone. These effects are evidenced by increased systolic blood pressure, decreased flow-mediated dilation, reduced release of the vasodilator nitric oxide, and decreased nitroglycerin-induced vasodilation (58). There is, also, evidence that pro-inflammatory cytokines, such as IL-6 and TNF-α, directly increase insulin resistance in adipocytes (59). Finally, locally produced pro-inflammatory cytokines often travel to the liver and increase the production of acute phase response proteins, such as CRP and serum amyloid A (SAA) (6,12). CRP and SAA are strongly associated with chronic disease risk. Briefly, CRP levels have been shown to be predictive of, among other things, peripheral vascular disease (60), future cardiovascular events (61), and ischemic stroke and transient ischemic attack (62). Similarly, SAA levels have been shown to be associated with coronary artery disease and future cardiovascular events (63). However, evidence implicating markers of inflammation with CVD are not limited to acute phase response proteins. Elevated levels of IL-6 in circulation are associated with myocardial infarction (64), mortality (65), and type 2 diabetes (66). Evidence linking inflammatory marker levels to chronic disease also exists for TNF-α (67), IL-1β (68), and IL-8 (69). Clearly, there is a connection between elevated markers of systemic inflammation and the development of disease.

The connection between diet and systemic inflammatory tone has often been suggested (70,71). Several studies have investigated potential single-meal effects on markers of inflammation, with most studies utilizing HFMs. However, as evidenced by our findings, there is inconsistency and ambiguity between studies with regard to the "normal" inflammatory response to a HFM. A recent review (12) performed a similar systematic search (with slightly different inclusion/exclusion criteria) and compilation of findings, but the authors elected to not quantify

the collective response characteristics across studies. In the context of the equivocal nature of the postprandial inflammation research findings to date, as well as considering the physiologically important implications of altered inflammatory marker levels, the present systematic review was conducted to better inform future research studies, as well as advance our understanding regarding which inflammatory markers are responsive in the transient window following HFM intake.

Findings for Individual Inflammatory Markers

The present review found that the majority of studies detected an increase in IL-6 following consumption of a HFM, agreeing with the findings of Herieka and Erridge (12). The rise is generally robust, as the average relative increase is ~100% of the baseline value; thus, IL-6 is quite responsive to HFM intake. It has been suggested that detected increases in IL-6 in the postprandial period should be viewed with skepticism, since the process of cannulation has been shown to lead to increases in local IL-6 production (72). Indeed, it has been demonstrated that cannulation without HFM consumption can lead to increases in IL-6 that are similar to those seen after HFM intake (73). In the present review, the vast majority of articles (30/32) that found a significant increase in IL-6 post-HFM utilized the insertion of a cannula for repeated blood sampling. Thus, it is possible that in these studies some or all of the increase in IL-6 could be an artifact of a local inflammatory response to the cannula, as opposed to a systemic response to the meal. However, two important points should be considered. First, two studies in our analysis utilized repeated venipuncture for blood sampling and found significant postprandial increases in IL-6 (35,49). Second, one study (25) found both a significant increase in circulating IL-6 using a forearm cannula and an increase in muscle expression of IL-6 using a vastus lateralis biopsy, suggesting a systemic effect and not merely a local inflammatory response to the cannula.

Collectively, these considerations suggest that at least some, if not all, of the IL-6 response to a HFM can be credited to the meal intake, versus a mere local inflammatory response to cannulation. Regardless, as the majority of postprandial inflammation studies employ cannulation, our findings nonetheless quantify the timing and magnitude of the collective IL-6 response to cannulation and a HFM. As IL-6 is by far the most frequently assessed marker of inflammation in the postprandial period, and in consideration of its varied function in the progression of atherosclerosis, we assert that the findings of the present analyses have relevant clinical and research implications. Namely, elevated IL-6 levels have been linked to multiple clinical considerations. IL-6 levels have been found to be significantly associated with systolic and diastolic blood pressure, fasting insulin, and insulin sensitivity (74). A systematic review found that long-term elevated IL-6 levels are associated with coronary heart disease to a similar degree as most traditional risk factors (75). Finally, high IL-6 levels have been associated with mortality in a population-based study in older adults (65). Harris et al. (65) found that the individuals in the highest IL-6 quartile, and therefore presenting the highest mortality risk, had IL-6 levels >3.19 pg/mL. Interestingly, the present review found that IL-6 starts at ~1.4 pg/mL and peaks at ~3 pg/mL after a HFM in healthy adults below age 60. Thus, a single HFM can induce a considerable postprandial increase in which circulating IL-6 levels can approach clinically high levels, even in young healthy individuals. While more research is needed with regard to the clinical importance of acute IL-6 fluctuations, considering the established relationship between IL-6 levels and adverse health outcomes, these acute IL-6 fluxes likely represent an important physiological occurrence, similar to other postprandial excursions (e.g. triglycerides, glucose) that have been shown to be associated with negative health outcomes.

Based on our search results, CRP was the second most frequently assessed (29 studies) marker of inflammation in the postprandial period following HFM intake. As ~80% of these studies found no significant change in CRP in the assessed postprandial period, and considering that the remaining six studies that found a significant change were divided in their findings (i.e. four found a significant increase and two found a significant decrease), the evidence strongly suggests that CRP is not a responsive marker of inflammation in the typically assessed 4-8 hour postprandial period in healthy adults. This assertion is in agreement with our understanding of the physiological pathway that results in an increase in CRP. The main drivers behind an increase in circulating CRP are pro-inflammatory cytokines produced locally at the site of damage (e.g. the inflamed endothelium). These pro-inflammatory cytokines, especially IL-6, then travel to the liver and stimulate increased production of acute phase response proteins, such as CRP and SAA (6). The time-course by which this pathway occurs is considerably slower than those of locally-produced cytokines, as there is typically no detectable change in the first five hours following a stimulus. Instead, CRP will slowly rise and peak at approximately 24 hours post-stimulus (Note: these responses are typically experimentally described using an endotoxin model, not necessarily a HFM; 12). Thus, as CRP and other acute phase response proteins reflect the cumulative inflammatory response (i.e. include the amplification and stimulation of many locally produced inflammatory molecules and their subsequent stimulation of acute phase response proteins in the liver), as well as bearing in mind the delayed rise and fall of acute-response phase proteins, CRP is a particularly advantageous marker of chronic inflammation to assess both clinically and in research. However, for these same reasons, CRP is not a viable inflammatory marker to assess in the prototypical postprandial assessment study. In consideration of the delayed response of CRP post-stimulus, in combination with the findings of

the present review indicating that CRP shows no change in the vast majority of postprandial inflammation studies, we recommend that CRP no longer be assessed for postprandial changes in response to a HFM in healthy adults. To be sure, CRP retains its utility in assessing overall or baseline inflammatory status; however, it is simply unlikely to change in the 4-8 hours following ingestion of a HFM in healthy individuals younger than 60 years of age.

TNF-α has also been widely assessed in the postprandial period as an inflammatory marker that is thought to typically increase following HFM intake. Specifically, TNF-α, like IL-6, is believed to increase quickly in the post-stimulus period, peaking at approximately 2-3 hours, then returning quickly to baseline (12). However, the findings of the current review disagree with this notion in the context of a HFM. We found that, of the studies that met the pre-established inclusion criteria, ~70% (19 of 28 studies) found no significant change in TNF-α following a HFM. Similar to CRP, the remaining studies that did find a significant change were split in terms of detecting a significant increase (five studies) or decrease (four studies). TNF-α is primarily produced by macrophages, such as those that populate inflamed regions of the vascular endothelium (6,76). TNF-α is known to be an important mediator in both acute and sustained inflammation (76). Specifically, TNF-α can induce increased secretion of itself, as well as other pro-inflammatory cytokines, making it an important contributor to the amplifying nature of the inflammatory response (76). However, while it appears that TNF-α may be particularly responsive in an endotoxin model of inflammation, it is not very responsive to HFM intake. As 23 of 28 studies assessing postprandial TNF-α in the present review found either no change or a significant decrease following a HFM, it appears that TNF-α is either not sufficiently responsive to a HFM stimulus, or is too variable in its assessment, to be deemed a reliable marker of inflammation in the hours following HFM intake.

While the majority of included studies did not find a significant change in CRP or TNF-α, it is interesting that there was disagreement with regard to the directionality of the change in studies that did detect significant differences. This could possibly be driven by the composition of the test meal characteristics. With regard to meal composition, prior evidence suggests that type of fat (77), macronutrient distribution (35), and overall nutrient-density (78) of the meal can alter the postprandial inflammatory response. Nevertheless, there were no clear, common differences among studies that found an increase versus a decrease in CRP or TNF-α in the current review. Overall, due to the heterogeneity of study designs (especially test meal composition), this review is not well equipped to accurately identify the meal characteristics that induce inflammation. On the contrary, the goal of this review was to summarize the overall post-HFM inflammatory response. While there are potentially certain nuances and influential factors that likely affect the response, the data synthesized in the present systematic review strongly suggest that CRP and TNF-α do not typically change in the acute hours following HFM consumption.

The remaining markers of inflammation assessed in the present study, IL-1β and IL-8, were rarely measured in the acute postprandial period in healthy individuals (IL-1β, three studies; IL-8, four studies). Since few studies have analyzed these markers, we cannot make firm conclusions regarding their activity in the hours after a meal. However, our findings do not suggest that these markers robustly change following consumption of a HFM, as two studies found no change in IL-1β post-HFM and one found a significant decrease, and three studies found no change in IL-8 post-HFM and one found a significant increase. Despite being less-frequently assessed, IL-1β and IL-8 are both considered pro-inflammatory cytokines that play adverse pathophysiological roles in CVD development, recruiting immune cells to the site of vascular damage, as well as promoting increased production of other pro-inflammatory cytokines

(79). IL-8 is produced from a variety of cells, including monocytes, macrophages, T lymphocytes and endothelial cells (80), whereas IL-1β is produced primarily by activated macrophages. Similar to TNF-α, although IL-1β and IL-8 are produced locally at the site of damage, they do not appear to transiently and/or robustly change in the postprandial period following consumption of a HFM.

Strengths and Limitations

There are several strengths to the present systematic review. First, we used a robust systematic search of 10 relevant databases with a search strategy developed with the assistance of a librarian (CL). The relatively large number of citations found with the original search (494 citations), in addition to the number of duplicate citations found by multiple databases (164 duplicates eliminated), suggests that the search was comprehensive and that it is unlikely that many, if any, relevant articles were not captured with our systematic search. Next, our generally broad, yet clearly defined inclusion criteria ensure that our findings are applicable to many people, namely healthy males and females between the ages of 18 and 60, independent of geographic region and body weight status. Finally, a strength of this study lies in its research and clinical utility. This systematic review represents the first attempt to clearly quantify the specific changes in commonly assessed markers of inflammation in response to a HFM.

However, this review is not without limitations. As with any systematic review, it is possible that we may have missed one or more pertinent studies. In addition, not all of the studies that met our inclusion criteria provided all of the information needed to help answer our research question. While all of the authors whose papers were missing data were contacted in an effort to retrieve those data, and many authors complied and submitted their data to us (see Acknowledgments), not all responded, and consequently some studies are still missing important information, such as

peak and time to peak responses for an assessed cytokine. Next, the external validity of our findings are limited to healthy adults. Diseased individuals will typically present with a high systemic inflammatory tone, therefore the postprandial inflammatory response may be more dramatic in these populations. Additionally, it should be noted that most studies included in the present systematic review assessed postprandial inflammation for 4-8 hours following HFM intake. Thus, our review is not equipped to describe any inflammatory marker changes that could potentially occur outside of that typically utilized window of time. Next, an additional analysis regarding the relationship between the type of fat or meal in determining the postprandial inflammatory response would have been informative. However, due to the heterogeneity of test meals and the manner in which they are reported, this point was not possible for the present systematic review to address in a qualitative analysis. Qualitatively, though, there do not appear to be any noticeable trends between studies that found an increase in a marker and those that found no change, other than that most studies employ meals reflective of the Westernized diet: high in animal (saturated) fats, simple carbohydrates, processed foods, and kilocalories, and low in fruits, vegetables, whole grains, and fiber. Finally, a frequent consideration with postprandial metabolic and inflammatory response research is the use of test meals that are not necessarily representative of meals that individuals might consume during normal daily living. Consequently, this systematic review contained many studies with test meals that were quite large, energy-dense, and high in fat (Table 1 and Supplemental Tables 1-4). This point should be considered when interpreting and drawing conclusions from the present data.

Conclusion and Future Directions

This systematic review aimed to characterize the postprandial response of five commonly assessed markers of inflammation following the intake of a HFM. Our findings suggest that only

one of those five markers, IL-6, consistently increases in the 4-8 hours post-HFM. Specifically, IL-6 will, on average, start at a baseline of ~1.4 pg/mL and peak at ~2.9 pg/mL approximately six hours later. In relative terms, IL-6 will increase ~100% in response to a HFM. Of the potential independent variables considered, only percent fat in the test meal showed a significant (negative) correlation with the percent change in IL-6 post-HFM, although a linear regression model including age, BMI, percentage fat in the test meal, and energy content of the test meal was found to significantly predict the percent change in IL-6. With regard to CRP and TNF-α, these markers were found to be very commonly assessed in the postprandial period, although they very rarely show any change. IL-8 and IL-1β also infrequently changed following HFM consumption in healthy individuals, though these markers have only been assessed in a few studies. In light of these findings, we have several recommendations for future research: 1) we suggest that CRP and TNF-α no longer be assessed for postprandial changes in healthy individuals within the normal 6-8 hour postprandial time-course; 2) instead, there may be more merit in assessing other inflammatory markers, such as leukocyte-bound markers, in healthy individuals exposed to a HFM, as they may be more likely to display postprandial changes (12); 3) a similar review focusing on the postprandial inflammatory response of diseased individuals is warranted, as the results could very likely differ from the present review that focused on healthy individuals; and 4) further investigation into the specific role that IL-6 plays following HFM intake would be beneficial.

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Tables

ARTICLE (REF)	MEAL	BLOOD	S/P	MO	FAT	ENERGY	N	M/F	AGE	BMI	PPP	↑↓↔	TTP	FASTING	PEAK	QA
					%E	*kcal*			*y*	*kg/m²*	*hrs*		*hrs*	*IL-6, pg/mL*	*IL-6, pg/mL*	
Arjunan et al. 2013 - South Asian (13)	White bread, butter, cheese, milkshake	cannula	P	N	57	14.3 kcal/kg	10	10M/0F	22.3 ± 1.3	25.4 ± 2.5	9	↑	9	1.31 ± 1.11	3.47 ± 1.43	6
Arjunan et al. 2013 – European (13)	White bread, butter, cheese, milkshake	cannula	P	N	57	14.3 kcal/kg	10	10M/0F	23.2 ± 2.0	25.2 ± 1.6	9	↑	6	1.25 ± 0.80	2.65 ± 1.40	6
Arjunan et al. 2015 - South Asian (14)	White bread, butter, cheese, milkshake	cannula	P	N	57	14.3 kcal/kg	15	15M/0F	24.0 ± 3.0	25.4 ± 3.3	9	↑	9	0.80 (0.50 to 1.28)	6.39 ± 6.20	6
Arjunan et al. 2015 – European (14)	White bread, butter, cheese, milkshake	cannula	P	N	57	14.3 kcal/kg	14	14M/0F	22.0 ± 1.0	22.7 ± 2.2	9	↑	9	0.34 (0.21 to 0.55)	4.48 ± 4.90	6
Bidwell et al. 2014 (15)	Eggs, muffin, butter, sugary drink	cannula	P	N	40	600	22	11M/11F	M: 20.8 ± 0.7 F: 21.5 ± 0.9	M: 23.9 ± 0.9 F: 21.1 ± 0.5	6	↑	NS	NS	NS	6
Brandauer et al. 2013 (16)	Sugar, heavy cream, chocolate syrup, powdered milk	cannula	P	Y	84	1310 ± 34.1	10	10M/0F	27 ± 1	24.6 ± 0.7	4	↑	4	0.64 ± 0.39	0.97 ± .64	4.5
Burton-Freeman et al. 2012 (17)	Bagel, cream cheese, potato, milk, apple juice	cannula	P	N	46	852	25	13M/12W	27 ± 8	22 ± 2	6	↑	6	NS	NS	5
Caixas et al. 2008 – lean (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	23.0 (21.0 - 26.0)	20.3 (18.9 - 25.1)	6	↑	6	0.61 (0.56 - 2.37)	3.69 ± 1.13	4
Caixas et al. 2008 – obese (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	26.0 (23.0 - 27.0)	43.9 (32.8 - 48.0)	6	↑	6	1.75 (1.09 - 2.68)	5.04 ± 0.71	4
Campbell et al. 2006 (19)	Apple muffins, milk shake	venipuncture	S	N	41	976	15	15M/0F	28 ± 9	NS	6	↓	4	1.6 ± 0.3	1.3 ± 0.3	6.5
Delgado-Lista et al. 2011 (20)	NS	NS	P	N	60	NS	45	45M/0F	NS	NS	4	NS	NS	NS	NS	3.5
Drew et al. 2014 (21)	Turkey burger, white bread	cannula	P	N	50	600	16	16M/0F	45 ± 11	27.6 ± 5.3	6	↑	6	1.96 ± 1.70	3.35 ± 2.29	3.5
Ehlers et al. 2014 (22)	Hamburger, French fries	cannula	P	N	39	1106	6	6M/0F	44.3 ± 5.2	24.8 ± 2.5	8	↑	6	NS	NS	5
Esser et al. 2013 (23)	Milk shake (cream, sugar, water)	cannula	P	Y	85	954	20	20M/0F	22 ± 2	22.7 ± 2.4	6	↑	6	0.74 ± 0.24	1.38 ± 0.73	5
Gill et al. 2003 (24)	Whipping cream, fruit, cereal, nuts, chocolate	cannula	P	N	67	1075	8	8M/0F	27.8 ± 12.1	23.6 ± 1.0	6	↑	6	1.34 ± 1.16	6.93 ± 5.77	5
Gregerson et al. 2012 (25)	Cheese, eggs, oil, cream, white bread	cannula	P	Y	77	928	15	4M/11F	44 ± 3	26.3 ± 2.0	3	↑	3	0.87 ± 0.12	1.13 ± 0.1	3
Harrison et al. 2009 (26)	Croissants, butter, ice cream, chocolate, potato crisps	cannula	S	N	60	1450	8	8M/0F	26.9 ± 4.1	26.0 ± 3.6	6	↑	4	0.74 ± 0.45	3.13 ± 2.87	5.5
Jimenez-Gomez et al. 2009 (27)	Butter, wholemeal bread, hard-boiled egg, whole milk	venipuncture	P	Y	60	NS	20	20M/0F	NS	NS	9	↔	-	NS	-	6
Johnson et al. 2016 (28)	Ice cream, whipping cream	cannula	P	N	45	1360 - 2160	12	12M/0F	23.0 ± 3.2	24.5 ± 2.7	4	↔	-	29.8 ± 38.0	-	4
Kiecolt-Glaser et al. 2015 (29)	Eggs, turkey sausage, biscuits, gravy	cannula	S	N	60	930	86	43M/43W	38.22 ± 8.18	32.07 ± 5.83	7	↑	6.5	1.76 ± 4.03	4.34 ± 3.03	4.5
Kracmerova et al. 2014 (30)	Pork meat, egg, French fries, hazelnut spread, croissant	cannula	P	Y	47	1470	10	10M/0W	26.3 ± 1.04	23.11 ± 0.59	4	↑	4	0.899 ± 0.509	2.168 ± 0.44	3.5
Lundman et al. 2007 (31)	Pasta, chicken, peas, mayonaisse	venipuncture	P	Y	60	1000	26	26M/0F	51 ± 3	26.4 ± 3.3	6	↑	4	3.81 ± 3.49	NS	6.5
Madec et al. 2011 (32)	Butter, bread, ham	NS	P	Y	52	730	16	NS	NS	NS	6	↔	-	0.43 ± 0.27	-	4
Mariotti et al. 2015 (33)	Milk cream, sucrose, whey protein	cannula	P	N	70	1200	10	10M/0F	34 ± 9	30.2 ± 1.5	6	↔	-	3.66 ± 1.46	-	3.5
Miglio et al. 2013 (34)	Fried potatoes, eggs, cheese, bread rolls	cannula	P	Y	52	1416	15	13M/2F	45 ± 8	26.7 ± 1.9	8	↑	8	0.3 ± 0.3	0.97 ± 0.52	4
Nappo et al. 2002 (35)	Sausage, bread, egg, butter, olive oil	venipuncture	P	N	59	760	20	10M/10F	44 ± 5	26.8 ± 1.2	4	↑	2	1.9 ± 1.0	3.1 ± 1.0	5
Payette et al. 2009 – Men (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	39	39M/0F	44.0 ± 9.1	28.9 ± 4.3	8	↑	8	2.40 ± 1.36	4.38 ± 2.35	5
Payette et al. 2009 – Women (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	41	0M/41F	43.7 ± 9.4	26.5 ± 5.7	8	↑	8	2.77 ± 1.81	5.83 ± 3.49	5
Peluso et al. 2012 (37)	Fried potatoes, eggs, cheese, bread	cannula	P	N	55	1344	14	12M/2W	45.1 ± 8.6	26.8 ± 2.2	8	↑	8	0.39 ± 0.27	1.09 ± 0.20	2.5
Phillips et al. 2013 – Lean (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	43.4 ± 11.3	22.8 ± 1.5	6	↑	6	0.9 ± 0.3	NS	6
Phillips et al. 2013 – Obese (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	40.9 ± 9.8	38.2 ± 6.7	6	↑	6	2.0 ± 1.3	NS	6
Poppitt et al. 2008 (39)	Blueberry muffin	cannula	S	Y	71	748	18	18M/0F	23 ± 4	22.9 ± 2.0	6	↑	6	29.3 ± 16.8	33.4 ± 16.4	6.5
Rankin et al. 2008 (40)	Eggs, sausage, biscuit, pancake, jelly candy	venipuncture	S	Y	53	900	17	8M/9F	26.5 ± 7.6	33.5 ± 6.7	4	↔	-	1.5 ± 1.1	-	5.5
Sanders et al. 2011 (41)	Muffin, milkshake	cannula	P	N	53	846	50	25M/25W	M: 25.4 ± 4.2 W: 24.2 ± 6.3	M: 23.3 ± 2.1 W: 23.7 ± 3.4	8	↑	8	0.4 ± 0.1	0.9 ± 0.2	5
Schmid et al. 2015 (42)	Bread, salami, palm fat, boiled eggs	cannula	P	Y	61	1005	21	21M/0W	41.8 ± 9.0	27.1 ± 8.2	6	↑	6	3.0 ± 1.1	5.1 ± 1.9	4.5
Schwander et al. 2014 – NW (43)	Bread, salami, palm fat, boiled eggs	cannula	S	Y	61	1000	19	19M/0W	40.6 ± 9.2	23.6 ± 1.4	6	↔	-	20.1 ± 1.7	-	4.5
Schwander et al. 2014 – obese (43)	Bread, salami, palm fat, boiled eggs	cannula	S	Y	61	1000	17	17M/0W	44.1 ± 8.0	38.8 ± 4.9	6	↔	-	17.9 ± 1.7	-	4.5
Schwander et al. 2014 – NW (43)	Bread, salami, palm fat, boiled eggs	cannula	S	Y	61	1500	19	19M/0W	40.6 ± 9.2	23.6 ± 1.4	6	↔	-	19.8 ± 1.9	-	4.5
Schwander et al. 2014 – obese (43)	Bread, salami, palm fat, boiled eggs	cannula	S	Y	61	1500	17	17M/0W	44.1 ± 8.0	38.8 ± 4.9	6	↑	4	16.7 ± 1.5	21.7 ± 1.9	4.5
Strohacker et al. 2012 (44)	Sausage, egg, cheese, biscuit, hash browns	cannula	P	N	59	1070	8	4M/4F	21 ± 3	23.1 ± 3.9	3	↔	-	1.7 ± 0.9	-	5
Teng et al. 2011 (45)	Mashed potatoes, baked beans, milk, orange juice, lard	NS	S	N	60	683	10	10M/0W	21.9 ± 0.7	21.0 ± 1.6	4	↔	-	14.5 ± 1.0	-	5.5
Tholstrup et al. 2011 (46)	Mashed potatoes with fat powder	NS	S	Y	76	620	10	0M/10F	38.2 ± 10.7	20.9 ± 1.3	6	↓	4	0.81 ± 0.57	0.6 ± 0.3	5
Twickler et al. 2003 (47)	Liquid cream meal	NS	P	Y	40	NS	10	6M/4F	48.6 ± 7.7	25.4 ± 1.6	24	↑	10	0.9 ± 0.7	3.4 ± 2.3	4
Volek et al. 2008 (48)	Whipping cream, pudding, macadamia nuts	cannula	P	Y	84	908	30	16M/14W	30 ± 8	24.1 ± 4.3	6	↑	3	0.85 ± 0.84	1.42 ± 1.36	6
Wood et al. 2011 (49)	Fast food burger, hash browns	venipuncture	P	N	49	919	21	9M/12F	49.6 ± 4.6	24.0 ± 0.7	4	↑	4	0.8 ± 0.2	1.1 ± 4.4	4.5

Table 3-1. Details of studies that assessed pre- and post-HFM IL-6 in healthy participants. Forty-five studies met the inclusion criteria and assessed IL-6 before and after consumption of a HFM. If studies separately assessed different groups, those specific subsets are specified alongside the study. When applicable, data represent Mean ± SD or Mean (Range). Concentration values are in pg/mL. Arrows represent significant increase (↑), significant decrease (↓), or no significant change (↔) detected in response to the HFM.

Abbreviations: BLOOD, method for drawing blood; BMI, body mass index; FASTING, baseline/fasting concentration of the marker; FAT, percentage of energy (%E) in the test meal from fat; M/F, ratio of males to females; MO, whether or not IL-6 was the main outcome being studied; N, sample size; NS, not stated; PEAK, peak or maximal observed concentration of the marker ("-" if not applicable); PPP, length of postprandial period assessment in hours; S/P, serum or plasma; TTP, time to peak or maximal observed concentration if a significant change was detected ("-" if not applicable)

Figures

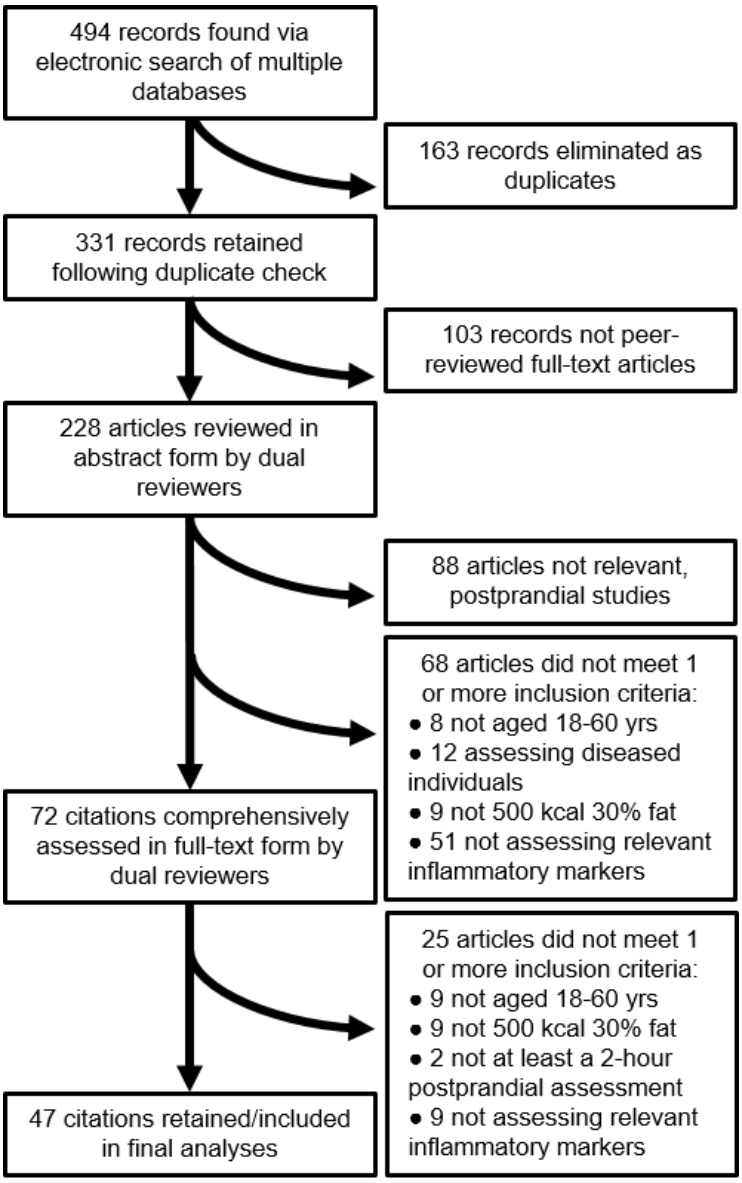

Figure 3-1. Flowchart of article search and selection process.

Ten relevant databases were searched, yielding 494 total citations. The final number of citations included in the present study was 47. Please see *Methods* section for more details.

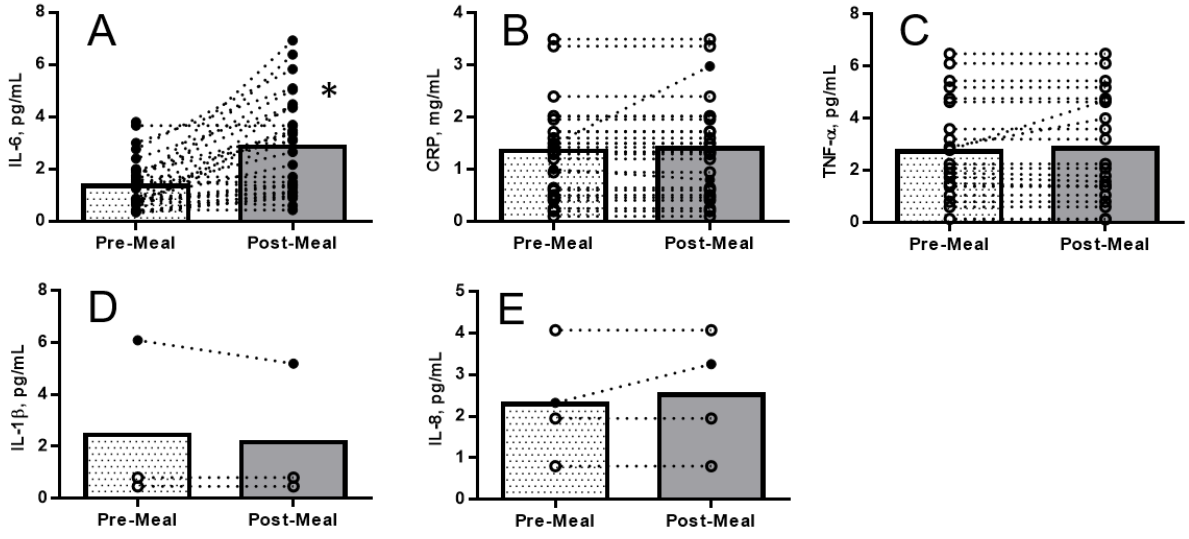

Figure 3-2. Post-HFM responses for the five assessed cytokines in healthy participants.
These figures represent the change from the fasting or baseline concentrations to the peak or maximally observed concentrations for IL-6 (Panel A), CRP (Panel B), TNF-α (Panel C), IL-1β (Panel D), and IL-8 (Panel E). Circles represent individual studies and vertical bars represent mean values. For markers other than IL-6, filled circles represent studies that found a significant change from pre- to post-meal, and open circles represent studies that found no significant postprandial change (difficult to differentiate with IL-6). For IL-6, CRP, and TNF-α, data presented is following removal of formal outliers.

* Significant increase in IL-6 from fasting to peak value ($p < 0.05$)

Abbreviations: IL, interleukin; CRP, C-reactive protein; TNF, tumor necrosis factor.

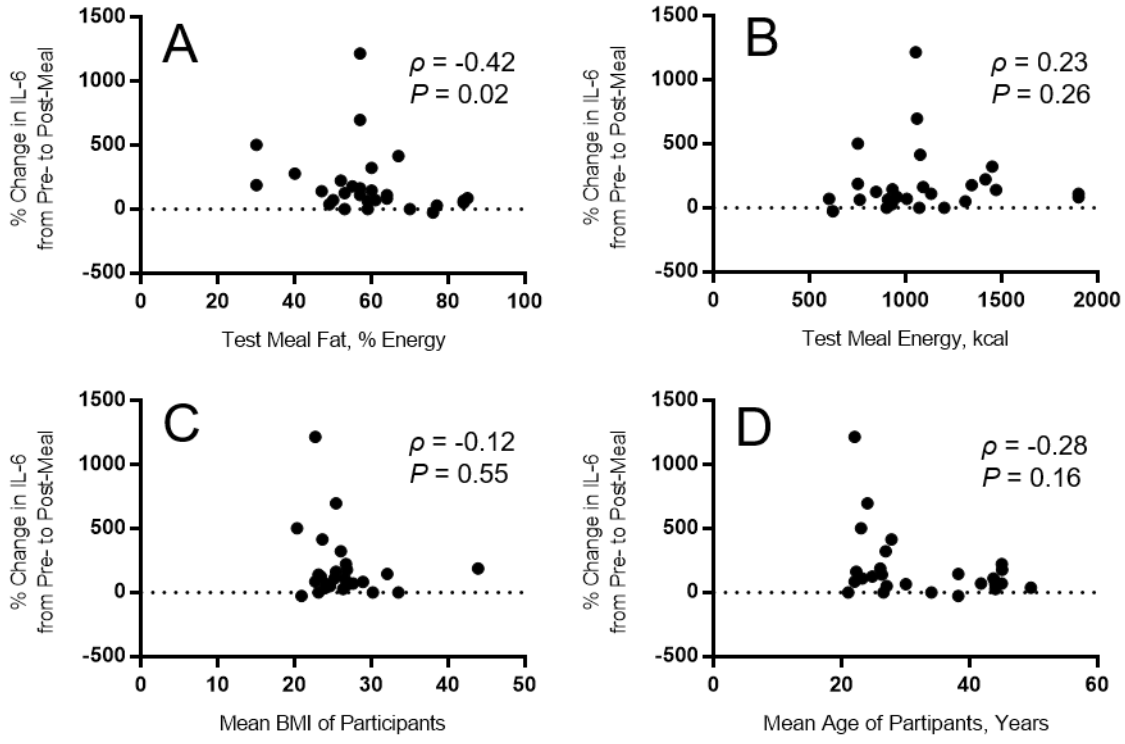

Figure 3-3. Correlations of several study variables with percent change in IL-6 from pre- to post-HFM in healthy participants.

The percent change in IL-6 from baseline to the peak or maximal observed response concentration was assessed for potential correlations with the percent of energy from fat in the test meal (Panel A), the energy content (in kcal) of the test meal (Panel B), the mean BMI of the study participants, and the mean age of the study participants. The only variable that was found to have a significant correlation with percent change in IL-6 was percentage fat in the test meal, which exhibited a moderate negative correlation.

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Chapter 4 - Realistic Test-Meal Protocols Lead to Blunted Postprandial Lipemia but Similar Inflammatory Responses Compared to a Standard High-fat Meal

Abstract

INTRODUCTION: A substantial rise in triglycerides following a meal is associated with increased risk for cardiovascular disease. Most studies investigating the effects of a meal on triglycerides have not utilized meals that reflect typical consumption. The objective of this study was to compare the triglyceride and inflammatory responses of true-to-life meals, containing moderate fat and energy content, with a high-fat, high-energy, low-carbohydrate meal (HFM) typically used to test triglyceride responses. METHODS: Nine healthy, insufficiently active men (age: 25.1 ± 6.7 years; BMI: 25.8 ± 7.0 kg/m2; <150 min/week moderate- to vigorous-intensity physical activity) completed three meal trials in random order: a HFM (17 kcal/kg, 60% fat); a moderate-fat meal (MFM; 8.5 kcal/kg, 30% fat); and a biphasic meal (BPM), in which participants consumed the full MFM at baseline and three hours post-meal. Blood samples were collected via indwelling catheter at baseline and hourly for six hours. RESULTS: Peak blood triglycerides were significantly greater (p = 0.003) following the HFM (285.2 ± 169.7 mg/dL) compared to the MFM (156.0 ± 98.7 mg/dL), but the BPM (198.3 ± 182.8 mg/dL) was not significantly different from the HFM (p = 0.06) or the MFM (p = 0.99). Total area under the curve (AUC) for triglycerides was greater following the HFM (1348.8 ± 783.7 mg/dL x 6 hrs) compared to the MFM (765.8 ± 486.8 mg/dL x 6 hrs; p = 0.0005) and the BPM (951.8 ± 787.7 mg/dL x 6 hrs; p = 0.03), although the MFM and BPM were not significantly different (p = 0.72). There was a significant time-by-meal interaction for interferon-γ, but not for interleukins-

6, 8, or 10. CONCLUSIONS: These findings in insufficiently active healthy young men suggest that the large triglyceride response following HFMs in previous studies may not reflect the metabolic state of many individuals in daily life.

Introduction

Cardiovascular disease (CVD) represents a serious risk and burden for many adults in western society (1). Previous research has linked increased CVD risk to both low levels of physical activity (2) and poor dietary habits (3). Specifically, there is evidence implicating consumption of single high-fat meals (HFMs) in elevating CVD risk. An exaggerated postprandial triglyceride (TG) response, termed postprandial lipemia, has been linked to a high risk for CVD (4). Mechanisms connecting HFM consumption to CVD risk are numerous, and include subendothelial penetration of lipoproteins, increased oxidative stress, and impaired endothelial function (5). In particular, there is evidence that inflammation may increase acutely following a HFM, suggesting the potential activation of an inflammatory cascade after intake of only one meal (6). As inflammation is an important hallmark of CVD (7), inflammation provides a potentially important connection between HFM intake and CVD risk.

However, there are several important methodological considerations when interpreting the results of previous postprandial studies. First, many studies utilize test meals that are quite large, calorie-dense, and rich in fat, such as ~1300 kcal and 60% fat (8,9,10,11,12,13,14,15). Additionally, the standard practice in postprandial studies is to have participants come into the lab fasted, eat the large meal, and then not eat again for 6-8 hours while their post-meal response is monitored. These design features are understandable from a scientific stand-point, but do not necessarily lend themselves well to understanding the actual post-meal challenges that many

individuals face on a daily basis, as they do not represent typical eating patterns (16). More research into the postprandial response under true-to-life scenarios is warranted.

The purpose of this study was to compare metabolic and inflammatory responses to three different meal conditions, in order to better understand the metabolic and inflammatory challenges faced by the body on a daily basis. We utilized a high-fat meal, representative of meals used in previous postprandial studies, as well as a moderate-fat meal, and a third condition in which participants consumed the full moderate-fat meal twice, three hours apart (biphasic meal). We hypothesized that: 1) the high-fat meal would elicit a significantly greater TG response compared to the moderate-fat meal and biphasic meal; and 2) there would be a greater inflammatory response following the high-fat meal compared to the other two meals.

Methods

Participants

Nine young men (age 18-35) were recruited to participate in the present study. Participants were not regularly engaging in exercise (<30 minutes/week) and were not meeting physical activity guidelines (<150 minutes/week of moderate- to vigorous-intensity physical activity; 17), according to self-report via International Physical Activity Questionnaire. Participants were free of any ongoing chronic disease, as confirmed via medical history questionnaire. This study was approved by the Institutional Review Board at Kansas State University.

Overall Study Design

The present study was a randomized crossover design. Participants reported to the laboratory on four separate occasions. The first session was for an initial assessment, in which participants completed paperwork and anthropometric tests were conducted. Participants completed an informed consent, medical history questionnaire, and the physical activity questionnaire. Blood

pressure was assessed at rest using an automated blood pressure cuff (Omron International, Shiokoji Horikawa, Japan). Height was measured using a portable stadiometer (Invictus Plastics, Leicaster, England) and weight was assessed via digital scale (Pelsar LLC, Alsip, IL, USA). Body composition was then measured via dual-energy X-ray absorptiometry (DEXA) scan (GE Lunar Prodigy, Madison, WI, USA). For the three main assessments, participants consumed one of three test meals in randomized order: a standard high-fat meal (HFM), a moderate-fat meal (MFM), and a biphasic meal (BPM), in which participants consumed the MFM twice, separated by a period of three hours. Blood draws were made at baseline and serially (each hour) for six hours to assess the postprandial metabolic and inflammatory responses. Each meal trial was separated by at least one week and no more than three weeks. A wash-out period of at least one week was chosen to eliminate the possibility of a carry-over effect. As previous studies suggest that postprandial TG will return to baseline levels within 8-10 hours post-HFM (18,19), our seven-day wash-out period ensures the observance of independent meal effects.

Test Meals

Three test meals were used in the present study: 1) a HFM (17 kcal/kg body mass; 64% fat (21% saturated fat), 16% CHO, 20% protein; 3 g fiber/serving; energy density: 2.0 kcal/g) that consisted of potatoes, eggs, sausage, and cheddar cheese; 2) a MFM (8.5 kcal/kg body mass; 30% fat (13% saturated fat), 55% CHO, 15% protein; 2 g fiber/serving; energy density: 2.1 kcal/g) comprised of sausage, egg, cheese, and whole grain crust; and 3) a BPM in which the full MFM was consumed twice, three hours apart. The HFM trial was designed to be representative of typical meals used in previous postprandial lipemia studies (8,9,10,11,12,13,14,15). The MFM was designed to be half the kcal of the HFM, while presenting primarily the same contents (i.e. sausage, egg, cheese). In the BPM trial, we wanted to assess the potential compounding effects

of smaller, more moderate meals, as it is reasonable for a person to eat twice in a six-hour period. In total, the BPM was equal in kcal to the HFM trial. When accounting for participant body mass, the HFM contained 1319 ± 338 kcal and the MFM contained 660 ± 169 kcal.

Meal Test Protocol

Prior to the first meal trial (HFM, MFM, or BPM), which was determined via randomization for each participant, the participants were instructed to record their dietary habits for three full days. For the remaining two trials, participants were given a photocopy of their diet record from the first meal trial, which they were instructed to repeat. Participants were instructed to refrain from planned exercise for two full days prior to each meal trial. Participants also abstained from alcohol and caffeine for twelve hours prior to each assessment. For each session, participants were given a hard copy reminder sheet of these instructions, and no participants reported an impactful deviation from these instructions at any point throughout the study.

On each meal trial day, participants reported to the lab following a 10-hour overnight fast. An indwelling safelet catheter was inserted into a forearm vein via 24-gauge needle (Exelint International, Redondo Beach, CA, USA). The IV catheter was kept patent with a steady infusion of 0.9% NaCl solution (~1 drip/second) and fixed in place via placement of tegaderm film (3M Healthcare, Neuss, Germany). When the IV was in place, a baseline blood draw was performed. For each blood draw, a 3 mL syringe (BD, Franklin Lakes, NJ, USA) was used to clear the line of saline, after which a 5 mL syringe (BD, Franklin Lakes, NJ, USA) was used for the actual blood sample. Blood draws were used to assess whole blood TG, glucose, total cholesterol (Total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and markers of inflammation (see Analytical Procedures section). After the baseline/fasting blood draw, participants would eat the respective meal in 20 minutes or less.

Water was available to participants ad libitum with each meal and throughout the post-meal period. Participants remained in the laboratory for six hours after the completion of each meal; the time started following the last bite of the meal. Blood draws were performed hourly for six hours after the meal. Metabolic markers were assessed every hour, while inflammatory markers were assessed at baseline and three and six hours post-meal. Markers of inflammation were assessed less frequently than metabolic markers primarily due to funding limitations. Inflammatory assessments were evenly spaced (baseline, three and six hours post-meal) to best characterize the postprandial inflammatory response given available resources.

Analytical Procedures

Whole blood metabolic measures (TG, glucose, Total-C, HDL-C, and LDL-C) were determined via Cholestech LDX analyzer (Alere Cholestech, San Diego, CA, USA). For each sample, several drops of whole blood were drawn into a capillary tube and plunged into a Cholestech LDX Lipid+Glu cassette (Alere Cholestech, San Diego, CA, USA). The cassette was then inserted into the Cholestech LDX analyzer for processing. The remaining blood sample was then centrifuged for 12 minutes and the plasma was pipetted into 0.6 mL snap-cap containers (Fisher, Hanover Park, IL, USA). Plasma was stored at -60 degrees Celsius until study completion. At the conclusion of the study data collection, plasma samples were analyzed in duplicate via custom high-sensitivity T-cell bead-based 4-plex assay (Eve Technologies, Calgary, Canada). The multiplex assay was conducted at Eve Technologies via the Bio-Plex 200 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and a Milliplex high-sensitivity custom human cytokine kit (Millipore, St. Charles, MO, USA) according to protocol specifications. The four markers of inflammation assessed in the present study were interleukin (IL)-6, IL-8, IL-10, and interferon (IFN)-γ. Coefficients of variation were less than 10% for all inflammatory marker analyses.

Statistical Analyses

An *a priori* sample size calculation ($\alpha = 0.05$; power = 0.80) elucidated that six participants would be needed to detect differences between meals in the postprandial TG total area under the curve (AUC-tot) response. However, nine participants were recruited to increase power to detect differences in other outcomes, such as markers of inflammation (although an *a priori* sample size calculation was not conducted for inflammatory markers). AUC-tot, incremental area under the curve (AUC-inc), peak value, and time to peak value were determined for each of the metabolic and inflammatory markers in each meal trial. The data were checked for normality using the Shapiro-Wilk formal normality test. If the data were normal, a one-way analysis of variance (ANOVA) was conducted to test for differences between meals. If the data were non-normal, a non-parametric Friedman test was utilized to test for meal differences. Time-course changes in metabolic and inflammatory markers in response to each meal were determined via two-way (meal x time) repeated measures ANOVA with a Tukey's adjustment for post hoc pairwise comparisons. A type 1 error rate of 0.05 was allowed for all analyses in determining significant differences.

Results

Participant characteristics are presented in Table 1. Three participants reported with systolic blood pressure >120 mmHg. No participants were found to have high diastolic blood pressure. With regard to BMI, five participants were normal-weight (18.5-24.9 kg/m2), two participants were over-weight (25.0-29.9 kg/m2), and two participants were obese (≥30 kg/m2; 20). Two participants presented with fasting TG greater than 150 mg/dL. All fasting glucose values were below 110 mg/dL. No participants were found to have high fasting Total-C (>200 mg/dL). There were no differences between meals with regard to fasting metabolic markers (p's > 0.05).

Postprandial metabolic data are presented in Table 2. There were significant meal effects with regard to TG peak, time to peak, AUC-tot, and AUC-inc. A significantly higher TG peak was observed in the HFM compared to the MFM ($p = 0.003$), but there were no differences between the BPM and the HFM ($p = 0.06$) or MFM ($p = 0.99$). The time to peak TG response was significantly longer in the HFM ($p = 0.01$) and BPM ($p = 0.01$) trials compared to the MFM trial, with no difference ($p = 0.29$) between HFM and BPM. The AUC-tot response for TG was significantly larger in the HFM compared to the BPM ($p = 0.03$) and MFM ($p = 0.0005$), but there was no difference in the BPM compared to the MFM ($p = 0.72$). Similarly, AUC-inc was greater in the HFM versus the BPM ($p = 0.01$) and MFM ($p = 0.001$), while there was no difference in the BPM compared to the MFM ($p = 0.99$). Figure 1A displays TG time-course for responses during each trial.

There were no significant meal effects with regard to glucose (Table 2). Time-course responses for glucose are displayed in Figure 1B. Overall, there were no substantial differences between the three meal trials in terms of the glucose response.

Metabolic Load Index (MLI) is determined by adding circulating glucose and TG in order to better characterize the metabolic challenge faced by the body, in comparison to looking at glucose and TG separately (5). There was a significant meal effect for MLI across trials in terms of peak, time to peak, AUC-tot, and AUC-inc (Table 2). Within pairwise comparisons, the HFM elicited a greater peak compared to the MFM ($p = 0.007$) and BPM ($p = 0.03$) trials, with no difference between MFM and BPM ($p = 0.99$). Time to peak MLI response was significantly longer in the BPM condition compared to the MFM condition ($p = 0.048$), but the HFM was not significantly different from the MFM ($p = 0.06$) or BPM ($p = 0.09$). With regard to AUC-tot, the HFM elicited a greater MLI response compared to the MFM ($p = 0.003$), but not the BPM ($p =$

0.06); no difference was detected in AUC-tot in the MFM versus BPM ($p = 0.99$). The HFM produced a greater AUC-inc MLI response compared to the MFM ($p = 0.003$) and BPM ($p = 0.02$), and no difference existed between the MFM and BPM ($p = 0.52$). Figure 1C displays time-course changes for MLI in the postprandial period in response to the three meals.

There were no significant meal effects with regard to Total-C. However, significant meal effects were determined for LDL-C peak and AUC-tot responses. The peak LDL-C response was significantly greater in the MFM condition compared to the HFM ($p = 0.007$) and BPM ($p = 0.007$) conditions, although there was no difference between HFM and BPM ($p = 0.77$). With regard to AUC-tot, the LDL-C response was significantly larger in the MFM trial versus the HFM ($p = 0.0009$) and BPM ($p = 0.004$) trials, with no difference between the HFM and BPM conditions ($p = 0.46$). A significant meal effect was detected with regard to the HDL-C AUC-tot response. The MFM elicited a significantly greater HDL-C AUC-tot response when compared to the HFM ($p = 0.02$) and the BPM ($p = 0.047$), but there was no difference between the HFM and BPM ($p = 0.86$). The postprandial time-course for responses generally revealed a steady increase in HDL-C following the MFM, while HDL-C tended to decrease after the HFM (Figure 1D).

Table 3 displays fasting and postprandial values for four markers of inflammation assessed at baseline and three and six hours post-meal: IL-6, IL-8, IL-10, and IFN-γ. A significant meal effect was detected for IFN-γ AUC-inc. However, within this meal effect, there were no significant post-hoc pairwise comparisons. No other meal effects were detected for other inflammatory markers with regard to fasting, peak, time to peak, AUC-tot, or AUC-inc values. There was no significant time x treatment interaction for IL-6 ($p = 0.19$), IL-8 ($p = 0.06$), or IL-10 ($p = 0.16$). However, there was a significant time x treatment interaction for IFN-γ ($p = 0.01$).

In post-hoc pairwise comparisons, IFN-γ was significantly higher (p = 0.02) in the BPM compared to the HFM three hours post-meal.

Discussion

Main Findings

The purpose of the present study was to compare metabolic and inflammatory responses to three different meal conditions, with the intention of better assessing the metabolic and inflammatory challenges faced by the body under real life circumstances. The main finding of the present study was that overall the HFM elicited a substantially greater TG response compared to the other two meal trials. This finding suggests that the single large bolus of fatty food, representative of meals used in previous postprandial studies, may induce a very different TG response compared to more reasonable meals (i.e., fewer kcal, less fat, divided into smaller meals over time) consumed in daily living. We also hypothesized that the HFM would elicit a greater inflammatory response compared to the MFM and BPM. However, this prediction was only partially supported. With the exception of a significant time x meal interaction for IFN-γ, there were no real appreciable differences between meal trials with regard to markers of inflammation.

Postprandial TG Response

We found that, relative to the standard HFM, a meal more moderate in terms of kcal and percent fat (i.e. the MFM) induced a blunted postprandial TG response. However, it should be noted that the MFM contained 660 ± 169 kcal – a relatively substantial meal. The MFM also contained generally the same ingredients (sausage, egg, cheese) as the HFM. Thus, the MFM, arguably representative of a reasonable meal consumed in daily living induced a blunted change in TG over the six-hour time-course, while the HFM produced a large, sustained TG response. It has been reported that elevated postprandial TG can independently increase myocardial infarction

risk by 40% per 100 mg/dL increase in TG (21,22). Since, on average, the HFM elicited a peak TG response >100 mg/dL greater than the MFM, it is very likely that the differences seen between meals with regard to postprandial lipemia have clinical significance.

For the first three hours post-meal, the TG responses of the BPM mimicked the MFM; however, after the consumption of the second meal at hour three, a compounding effect was observed, and the TG values for the BPM were significantly greater than the MFM at four, five and six hours post-meal. These findings support the notion that postprandial lipemic responses can be summative, impacted by both the lingering TG levels of prior meals and the TG flux of a more recent meal. This conclusion is important to consider, as many postprandial studies only feature one meal followed by a prolonged period of assessment in which no additional snacks or meals are consumed. Thus, future research should further assess multiple meals in attempting to characterize the TG flux under normal dietary consumption and timing.

To our knowledge, only one previous study has investigated postprandial lipemia in the context of multiple sequential meals. Pfeiffer et al. (23) assessed postprandial lipemia in response to two meals that each provided ~1/3 of each participant's daily energy needs and were 33% fat. However, the purpose of the study was to determine the minimum amount of walking required to blunt postprandial lipemia to the sequential meals. Thus, no comparison was made between the moderate, sequential meals to a standard HFM, as was done in the present study. Our results support the concept of summative TG responses to sequential meals, but we also found that the HFM elicited a significantly greater postprandial lipemia AUC response compared to the BPM, even though the BPM contained the same amount of kcals. This finding could point to one or several considerations: 1) the 6-hour postprandial duration may not have been sufficient to witness the entire BPM lipemic response; 2) the HFM may overwhelm the metabolic clearing

capacity of the body to a greater degree than the BPM; and 3) the difference between the HFM and BPM may simply be due to the different proportional fat contents. More research is needed to elucidate the potential response differences between two small meals versus one very large meal.

Postprandial HDL-C Response

An important finding in the present study was the divergent postprandial responses with regard to HDL-C. There were no differences in HDL-C between meals at baseline, but over the course of the 6-hour post-meal period, HDL-C tended to decrease in the HFM trial but remain steady in the MFM trial. There were also main meal effects with regard to HDL-C peak and AUC-tot. Clearly, these different meal trials produced different effects on HDL-C. Prior evidence has shown that the response of HDL-C is inversely related to the magnitude of postprandial lipemia (13) and it has been speculated that the lowering of HDL-C in response to HFM consumption can be an avenue by which HFMs induce deleterious cardiovascular effects (24).

Markers of Inflammation

To our knowledge, the present study is the first to investigate inflammatory responses to a HFM as compared to more moderate meals, including sequential moderate meals. Investigating the inflammatory responses to these moderate meals is important, as inflammation has been mechanistically linked to the development and progression of CVD. In our cohort of young, insufficiently active men, with the exception of IFN-γ AUC-inc, there were no main postprandial effects seen across meals among the markers of inflammation. Further, only IFN-γ exhibited a significant time x meal interaction in the postprandial period.

IFN-γ is an important cytokine released from activated macrophages and is an influential player in the inflammatory cascade that promotes atherosclerosis (25). IFN-γ has been minimally

assessed in the post-meal period, but has been shown to increase in one study utilizing a very large HFM (26). In our study employing meals of varying fat and energy content, despite a significant time x meal interaction, there were no clear and consistent postprandial differences between the three meal trials, although BPM was significantly greater than HFM three hours post-meal.

IL-6 is an intriguing inflammatory marker, as it is both a cytokine and a myokine, and there is ongoing debate as to whether it is pro- or anti-inflammatory in nature. Across studies, there is fairly consistent evidence that IL-6 increases in response to HFM intake (27,28). Whether or not this rise in IL-6 is beneficial or deleterious remains to be determined. Interestingly, there was not a significant meal x time interaction for IL-6 in the postprandial period. The reason for the lack of change in IL-6 in the present study, particularly after the HFM, is unclear.

Strengths and Experimental Considerations

In our view, this study has several points of strength. First, this investigation was designed to be true to life and provide valuable data regarding the postprandial metabolic and inflammatory challenges experienced by individuals on a daily basis. The HFM was very large, energy-dense, and rich in fat, similar to previous studies (8,9,10,11,12,13,14,15), while the BPM and MFM were moderate and reflective of more typical dietary behavior (16). Thus, the results of the present study are likely to reflect the postprandial circumstances for many individuals in daily life. Next, this study utilized a randomized cross-over design, eliminating the possibility of systematic participant differences confounding the effects detected from the three meal trials. Lastly, within our cohort, variability existed with regard to body composition, fasting metabolic levels, and age. Nevertheless, we detected visible differences between the meals, thus increasing the generalizability of the present study.

However, several experimental considerations do need to be made when interpreting our findings. Our findings cannot necessarily be extrapolated to women, older adults, physically active individuals, and diseased populations, as each of these groups has been demonstrated to display different postprandial lipemic responses relative to young healthy men. Also, it would have been potentially useful to have included a fourth trial in which the HFM was divided and delivered biphasically, similar to the BPM. From a study design perspective, that point is logical. However, the present study was intentionally designed to compare a HFM representative of those used in the literature to two other meal trials that were more realistic in nature with regard to size and timing. Delivering the HFM biphasically would have nonetheless resulted in participants eating two meals that were >60% fat, and therefore shed light on whether or not TG differences between meal conditions in the present study were simply due to differences in fat content. Next, while participants were continually reminded of the necessary lifestyle controls leading up to each meal trial (avoidance of exercise and caffeine, replication of diet), adherence to these instructions was not formally documented, representing a potential limitation of the present study. Finally, due to cost, markers of inflammation were assessed three and six hours post-meal, as opposed to hourly in the case of metabolic markers. Consequently, our data are not equipped to characterize the postprandial response curve of these inflammatory markers with high precision.

Conclusion

The purpose of this investigation was to compare metabolic and inflammatory responses to three meal conditions, in order to better define and understand the metabolic and inflammatory challenges that individuals experience in daily life. The main finding was that the HFM induced a considerably greater TG response compared to the other realistic meal protocols, signifying

that a single large intake of energy-dense high-fat food, representative of meals used in previous postprandial studies, may result in a markedly different lipemic response compared to smaller, moderate-fat meals consumed in daily living. However, we found few clear and noteworthy differences between meal trials with regard to markers of inflammation. We encourage future research to assess the true-to-life postprandial metabolic and inflammatory responses in other relevant populations that are much less represented in the current literature: women, older adults, and diseased populations. In doing so, we can gain a better understanding of the metabolic and inflammatory challenges faced by individuals on a daily basis.

Tables

	Mean ± SD
Age (years)	25.1 ± 6.8
Height (cm)	173.5 ± 6.1
Mass (kg)	77.6 ± 19.9
Body mass index (kg/m^2)	25.8 ± 7.0
Body fat (%)	20.5 ± 11.9
Trunk fat (%)	26.5 ± 14.0
Fasting TG (mg/dL)	109.9 ± 65.0
Fasting glucose (mg/dL)	88.0 ± 8.0
Fasting total cholesterol (mg/dL)	146.0 ± 20.8
Fasting LDL-C (mg/dL)	90.5 ± 20.3
Fasting HDL-C (mg/dL)	35.7 ± 7.3

Table 4-1. Participant characteristics.

Data are Mean ± SD.

	HFM	MFM	BPM	*p*-value
Triglycerides				
Peak (mg/dL)	214.0 (154.5-415.5)[a]	113.0 (85.0-221.0)[b]	129.0 (103.5-228.5)[ab]	0.0013
Time to peak (hours)	3.8 ± 1.3[a]	2.4 ± 1.4[b]	4.4 ± 1.0[a]	0.0017
AUC-tot (mg/dL x 6 hr)	1087.0 (769.5-1957.5)[a]	546.0 (411.3-1150.3)[b]	645.0 (514.0-1213.3)[b]	<0.0001
AUC-inc (mg/dL x 6 hr)	439.0 (360.8-939.0)[a]	167.0 (66.1-313.8)[b]	153.0 (111.7-381.5)[b]	0.0002
Glucose				
Peak (mg/dL)	98.0 (94.5-105.0)	102.0 (93.0-136.5)	110.0 (91.5-120.0)	0.99
Time to peak (hours)	1.0 (0.5-3.5)	1.0 (1.0-2.0)	1.0 (1.0-5.0)	0.30
AUC-tot (mg/dL x 6 hr)	531.2 ± 51.3	528.2 ± 55.8	543.0 ± 72.3	0.72
AUC-inc (mg/dL x 6 hr)	-4.0 ± 44.2	12.2 ± 70.5	12.2 ± 60.1	0.70
Metabolic Load Index				
Peak (mg/dL)	306.0 (240.5-510.5)[a]	223.0 (188.5-314.5)[b]	214.0 (194.0-331.5)[b]	0.0030
Time to peak (hours)	3.4 ± 1.1[ab]	2.2 ± 1.6[a]	4.3 ± 1.6[b]	0.01
AUC-tot (mg/dL x 6 hr)	1607.0 (1299.0-2558.0)[a]	1046.0 (919.8-1714.5)[b]	1130.0 (1028.3-1798.0)[ab]	0.0013
AUC-inc (mg/dL x 6 hr)	648.7 ± 394.5[a]	257.2 ± 198.2[b]	316.4 ± 327.0[b]	0.001
Total Cholesterol				
Peak (mg/dL)	152.1 ± 21.8	165.3 ± 20.0	148.9 ± 22.4	0.07
Time to peak (hours)	2.9 ± 2.1	4.1 ± 2.2	1.6 ± 1.8	0.06
AUC-tot (mg/dL x 6 hr)	856.2 ± 125.6	914.7 ± 99.4	822.6 ± 121.4	0.08
AUC-inc (mg/dL x 6 hr)	-3.0 (-25.6-34.4)	-38.0 (-69.0-27.7)	-53.1 (-100.8-31.3)	0.40
LDL-Cholesterol				
Peak (mg/dL)	86.3 ± 21.4[a]	104.0 ± 19.9[b]	85.1 ± 23.9[a]	0.0004
Time to peak (hours)	0.0 (0.0-1.0)	3.0 (0.0-5.5)	1.0 (0.0-2.0)	0.19
AUC-tot (mg/dL x 6 hr)	360.8 ± 198.2[a]	539.8 ± 130.5[b]	396.1 ± 162.0[a]	0.0013
AUC-inc (mg/dL x 6 hr)	-68.3 ± 41.7	-28.2 ± 92.9	-47.4 ± 45.0	0.39
HDL-Cholesterol				
Peak (mg/dL)	36.8 ± 8.5	43.3 ± 6.3	37.3 ± 7.6	0.02
Time to peak (hours)	1.0 (0.0-3.0)	5.0 (0.0-6.0)	0.0 (0.0-1.5)	0.12
AUC-tot (mg/dL x 6 hr)	191.7 ± 53.4[a]	224.8 ± 44.1[b]	194.3 ± 53.4[a]	0.03
AUC-inc (mg/dL x 6 hr)	-15.7 ± 18.6	-0.4 ± 25.4	-10.8 ± 20.8	0.35

Table 4-2. Postprandial metabolic data for the three meal trials (HFM, MFM, BPM) in insufficiently active healthy young men.

All metabolic markers were measured in whole blood. Normally distributed data are presented as Mean ± SD and non-normally distributed data are presented as Median (Interquartile Range), n = 9. The *p*-value column represents main effects between meals. Labeled means in a row without a common letter differ, $p < 0.05$. See Results section for pairwise comparison *p*-values.

HFM, high-fat meal; MFM, moderate-fat meal; BPM, biphasic meal; AUC-tot, total area under the curve; AUC-inc, incremental area under the curve; LDL, low-density lipoprotein; HDL, high-density lipoprotein

	HFM	MFM	BPM	*p*-value
IL-6				
Fasting (pg/mL)	1.41 ± 0.65	1.64 ± 1.13	1.66 ± 0.86	0.53
Peak (pg/mL)	1.75 ± 0.75	2.18 ± 1.04	1.83 ± 0.82	0.26
Time to peak (hours)	6.0 (1.5-6.0)	6.0 (1.5-6.0)	1.5 (0.0-5.3)	0.12
AUC-tot (pg/mL x 6 hr)	3.08 ± 1.09	3.98 ± 2.47	3.78 ± 2.00	0.79
AUC-inc (pg/mL x 6 hr)	-0.10 (-0.48-0.63)	-0.10 (-0.75-0.45)	-0.18 (-0.41-0.10)	0.97
IL-8				
Fasting (pg/mL)	6.29 ± 1.09	6.38 ± 1.12	6.13 ± 0.93	0.13
Peak (pg/mL)	6.73 ± 1.04	6.43 ± 1.12	6.33 ± 1.02	0.12
Time to peak (hours)	2.6 ± 3.0	0.8 ± 1.4	2.6 ± 2.5	0.18
AUC-tot (pg/mL x 6 hr)	12.31 ± 2.14	12.20 ± 2.47	11.96 ± 1.96	0.24
AUC-inc (pg/mL x 6 hr)	-0.27 ± 0.79	-0.56 ± 0.59	-0.30 ± 0.63	0.47
IL-10				
Fasting (pg/mL)	4.28 ± 2.16	4.01 ± 1.91	4.27 ± 1.79	0.42
Peak (pg/mL)	4.87 ± 2.54	4.20 ± 1.83	4.42 ± 1.85	0.18
Time to peak (hours)	4.5 (0.8-6.0)	0.0 (0.0-3.0)	1.5 (0.0-5.3)	0.27
AUC-tot (pg/mL x 6 hr)	26.48 ± 14.70	22.98 ± 10.48	23.86 ± 10.93	0.18
AUC-inc (pg/mL x 6 hr)	1.17 ± 3.28	-0.73 ± 2.01	-1.37 ± 2.32	0.12
IFN-γ				
Fasting (pg/mL)	15.73 ± 4.71	14.75 ± 4.11	14.59 ± 5.42	0.26
Peak (pg/mL)	15.75 ± 4.64	15.32 ± 4.05	15.07 ± 4.91	0.45
Time to peak (hours)	0.0 (0.0-0.0)	0.0 (0.0-3.0)	1.5 (0.0-3.0)	0.56
AUC-tot (pg/mL x 6 hr)	81.08 ± 25.62	83.64 ± 24.49	84.04 ± 27.33	0.56
AUC-inc (pg/mL x 6 hr)	-13.28 ± 5.99	-4.88 ± 8.66	-3.50 ± 7.74	0.046

Table 4-3. Postprandial inflammatory data for the three meal trials (HFM, MFM, BPM) in insufficiently active healthy young men.

All inflammatory markers were measured in plasma. Normally distributed data are presented as Mean ± SD and non-normally distributed data are presented as Median (Interquartile Range), n = 9. The *p*-value column represents main effects between meals.

HFM, high-fat meal; MFM, moderate-fat meal; BPM, biphasic meal; AUC-tot, total area under the curve; AUC-inc, incremental area under the curve; IL, interleukin; IFN, interferon.

Figures

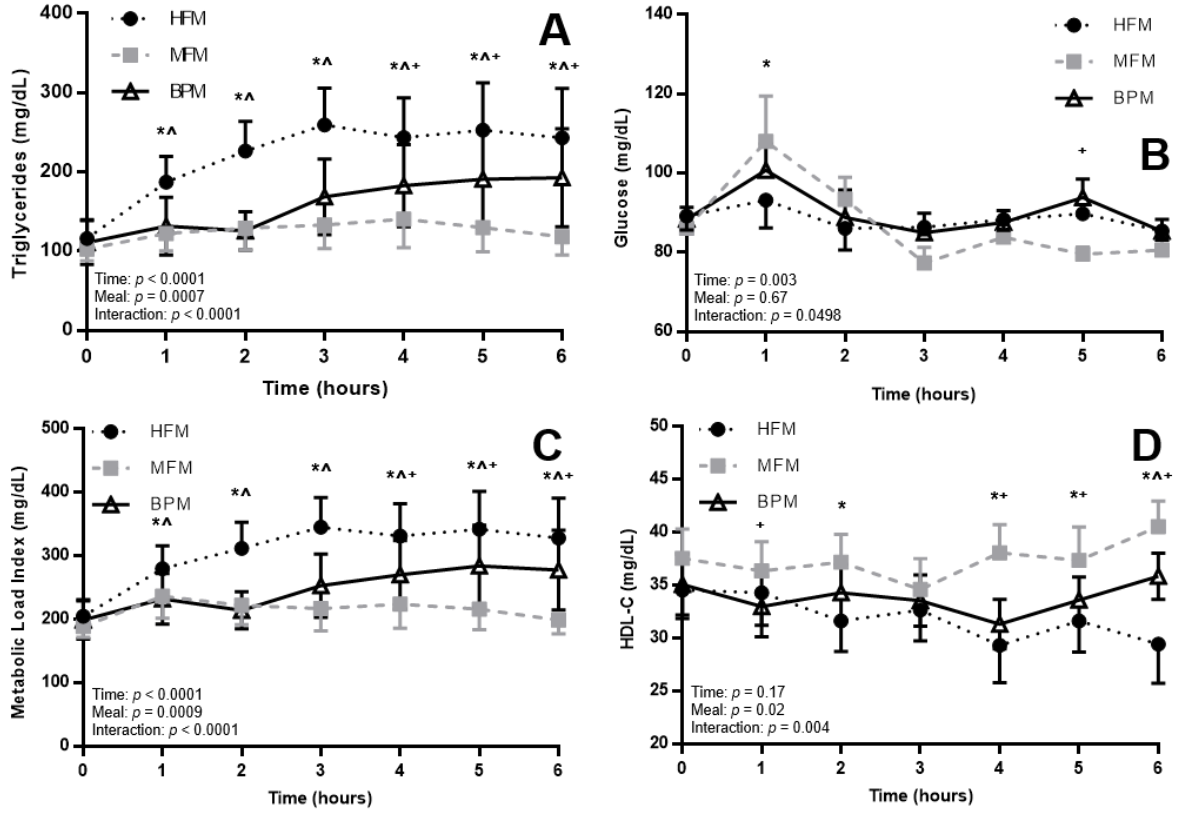

Figure 4-1. Postprandial metabolic responses following the three meal trials (HFM, MFM, BPM) in insufficiently active healthy young men.

Metabolic markers in whole blood, including TG (Panel A), glucose (Panel B), MLI (Panel C), and HDL-C (Panel D) were assessed at baseline (time 0) and serially for six hours after each meal trial (HFM, MFM, and BPM). Data are Mean ± SEM, n = 9.

* HFM vs MFM; ^ HFM vs BPM; + MFM vs BPM

HFM, high-fat meal; MFM, moderate-fat meal; BPM, biphasic meal; HDL-C, high-density lipoprotein cholesterol

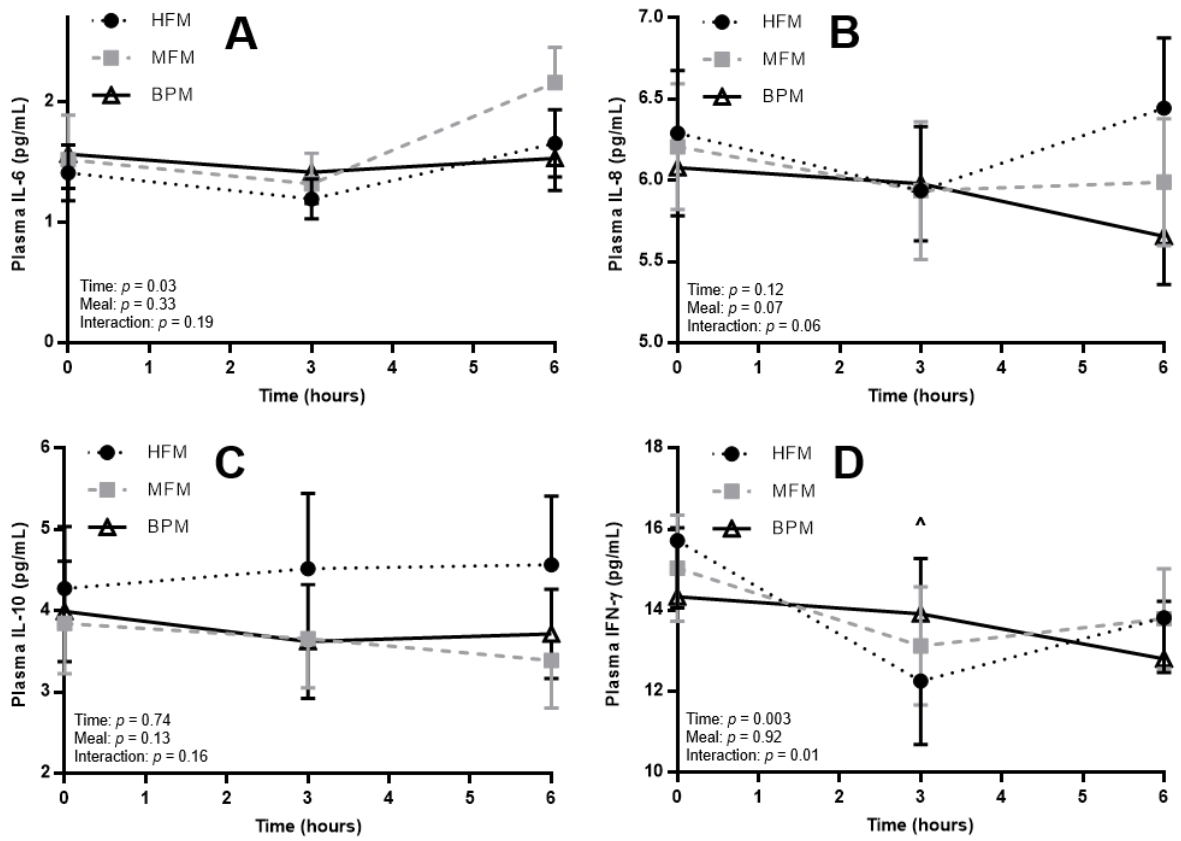

Figure 4-2. Postprandial inflammatory responses following the three meal trials (HFM, MFM, BPM) in insufficiently active healthy young men.

Plasma inflammatory markers, including IL-6 (Panel A), IL-8 (Panel B), IL-10 (Panel C), and IFN-γ (Panel D) were assessed at baseline (time 0) and three and six hours after each meal trial (HFM, MFM, and BPM). Data are Mean ± SEM, n = 9.

^ HFM vs BPM

HFM, high-fat meal; MFM, moderate-fat meal; BPM, biphasic meal; IL, interleukin; IFN, interferon

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Chapter 5 - Postprandial Metabolic Responses Differ by Age Group and Physical Activity Level

Abstract

Objectives: To compare the postprandial metabolic responses to a high-fat meal in healthy adults who differ by age and physical activity level.

Design: Cross-sectional, quasi-experimental design

Setting: Physical Activity and Nutrition Clinical Research Consortium (PAN-CRC) at Kansas State University (Manhattan, KS, USA)

Participants: Twenty-two healthy adults: 8 younger active (YA) adults (4M/4W; 25 ± 5 yr), 8 older active (OA) adults (4M/4W; 67 ± 5 yr), and 6 older inactive (OI) adults (3M/3W; 68 ± 7 yr).

Intervention: Following an overnight (10-hour) fast and having abstained from exercise for 2 days, participants consumed a high-fat meal (63% fat, 34% CHO; 12 kcal/kg body mass; 927 ± 154 kcal). To assess the metabolic response, blood draws were performed at baseline and each hour following the meal for 6 hours.

Measurements: Fasting and postprandial triglycerides (TG), glucose, Total-C, and HDL-C were measured. Metabolic load index (MLI) and LDL-C were calculated.

Results: There were significant group x time interactions for TG ($p < 0.0001$) and MLI ($p = 0.004$). The TG total area-under-the-curve (tAUC) response was significantly lower in YA (407.9 ± 115.1 mg/dL x 6 hr) compared to OA (625.6 ± 169.0 mg/dL x 6 hr; $p = 0.02$) and OI (961.2 ± 363.6 mg/dL x 6 hr; $p = 0.0002$), while the OA group TG tAUC was lower than the OI group ($p = 0.02$). The TG peak was significantly lower in YA (90.5 ± 27.0 mg/dL) than OA (144.0 ± 42.2 mg/dL; $p = 0.03$) and OI (228.2 ± 96.1 mg/dL; $p = 0.0003$), and was lower in the

OA group compared to the OI group (p = 0.03). Glucose was significantly lower 1 hour after the meal in YA (89.4 ± 10.1 mg/dL; p = 0.01) and OA (87.3 ± 22.3 mg/dL; p = 0.005) versus OI (110.7 ± 26.9 mg/dL). MLI tAUC was significantly lower in YA (936.8 ± 137.7 mg/dL x 6 hr; p = 0.0007) and OA (1133.0 ± 207.4 mg/dL x 6 hr; p = 0.01) versus OI (1553.8 ± 394.3 mg/dL x 6 hr), with no difference (p = 0.14) between YA and OA groups. Total-C and LDL-C were generally lower in younger compared to older participants at baseline and throughout the postprandial period, while no group or time effects were evident in HDL-C.

Conclusion: Both physical activity status and aging appear to affect the postprandial metabolic, namely TG, response to a high-fat meal. These findings point to an inherently diminished metabolic capacity with aging, but suggest that physical activity may help minimize this decrement.

Introduction

Cardiovascular disease (CVD) represents a serious health risk for many individuals in Western society. There are several lifestyle factors that are broadly accepted as modifiers of a person's CVD risk, but a particular lifestyle feature that has received consistent attention over the last several decades is dietary intake (1). Specifically, there is evidence that consumption of a single high-fat meal (HFM) can increase a person's CVD risk through a variety of mechanisms (2,3,4). There is a strong association between the postprandial triglyceride (TG) response and CVD risk, with higher non-fasting TG being related to increased CVD risk (5,6). The impact of the postprandial TG response ("postprandial lipemia") is important, as individuals spend the majority of their day in a postprandial state (7). Other deleterious postprandial phenomena include evidence of a strong relationship between postprandial hyperglycemia with type 2

diabetes mellitus and CVD development (8,9,10), as well as a decrease in high-density lipoprotein cholesterol (HDL-C) following consumption of a HFM (11).

There is a large body of evidence that suggests that exercise can be an effective means for reducing postprandial lipemia (12,13). Acute exercise performed 10-12 hours before, immediately before, or immediately after consumption of a HFM have all been found to lessen the postprandial TG response (14,15). However, there are indications that the capacity of physical activity to reduce postprandial lipemia is primarily transitory in nature (16), as active individuals who avoid exercise for several days prior to a HFM may exhibit a post-meal TG response similar to matched inactive individuals (17,18).

Interestingly, nearly all postprandial lipemia studies, including those investigating the effects of exercise, involve young or middle-aged individuals. While the evidence is scarce, it appears that older individuals tend to exhibit a greater postprandial TG response compared to younger individuals (19). Nevertheless, the physiological explanations for this difference have not been fully elucidated, but could partially be due to decreasing physical activity with increasing age (20). To date, no study of which we are aware has investigated both physical activity and aging as potential factors in altering the postprandial HFM response.

Therefore, the purpose of this investigation was to study the independent effects of aging and physical activity status on the postprandial metabolic response. We assessed postprandial responses to a HFM in three groups: younger active (YA), older active (OA), and older inactive (OI) adults. To our knowledge, no previous study has assessed the postprandial metabolic response in clearly defined cohorts of men and women that differ by both age and physical activity level. We predicted that both age and physical activity level would independently alter the postprandial metabolic response. Specifically, we hypothesized that: 1) Participants in the

YA group would exhibit a smaller postprandial TG response as compared to both OA and OI groups, but the OA group would show a smaller response as compared to the OI group; 2) Given the macronutrient composition of the test meal, there would be no differences over time or between groups with regard to postprandial glucose; and 3) Metabolic Load Index (MLI), representative of the total metabolic challenge and calculated by summing TG and glucose (21), in the postprandial period would be smaller in YA compared to the OA and OI groups, but OA would exhibit a lesser postprandial MLI response than the OI group.

The hypotheses of the present study were based on TG and glucose (and subsequently MLI), as these are substrates widely recognized to change acutely following meal consumption (22). Total-C, LDL-C, and HDL-C were secondary metabolic markers in the current study – informative of overall metabolic status, but not viewed as primary outcomes in the postprandial period.

Methods

Participants and Physical Activity Level

Twenty-two participants participated in the present study: eight YA adults (age 18-35 years; 4M/4W), eight OA adults (age 60+ years; 4M/4W), and six OI adults (3M/3W). Active participants were regularly meeting physical activity guidelines (≥150 minutes/week of moderate- to vigorous-intensity physical activity; MVPA) (23). Inactive participants were not regularly engaging in planned exercise (<30 minutes/week) and reported engaging in a generally inactive lifestyle (i.e. not meeting physical activity guidelines). Participants had not changed physical activity habits dramatically in the past five years. OA and OI adults reported to having been generally active or insufficiently active, respectfully, for most of their lives. Because there is not a validated questionnaire to assess lifetime physical activity, whether participants fit the

physical activity inclusion criteria was determined via extensive interviewing with an investigator, as has been done previously (24). Current physical activity status was objectively measured using accelerometry (Actical; Respironics; Bend, OR, USA). Accelerometers were worn on the non-dominant wrist for 5-7 continuous days, including at least 1 weekend day, and were initialized to record data in 30-second epochs. Participants were free of any ongoing chronic disease, as confirmed via medical history questionnaire. No participants were taking lipid-lowering medications. This study was approved by the Institutional Review Board at Kansas State University.

Initial Assessment

Participants reported to the laboratory on two occasions: an initial assessment and a meal assessment. The initial assessment entailed paperwork and anthropometric testing. Height was measured via portable stadiometer (Invictus Plastics, Leicaster, England) and weight was assessed using a digital scale (Pelsar LLC, Alsip, IL, USA). Height and weight were each measured twice, and a third measurement was performed if the values differed by more than 0.5 cm or 0.5 kg, respectively. The values were then averaged together. Body composition was assessed via a dual-energy X-ray absorptiometry (DEXA) scan (GE Lunar Prodigy, Madison, WI, USA).

Meal Test Protocol

The HFM used in the present study was chocolate pie (Marie Callender's Chocolate Satin Pie; Conagra Brands; Omaha, NE, USA). The primary ingredients of the pie were sugar, water, eggs, enriched wheat flour, soybean oil, palm oil, milk, butter, margarine, high fructose corn syrup, cocoa powder, and milk chocolate. The macronutrient distribution was 63% fat, 34% carbohydrate, and 3% protein. The amount of test meal that each participant consumed was

relative to their body mass (12 kcal/kg body mass; 0.84 g/kg fat, 1.02 g/kg carbohydrate, 0.09 g/kg protein). When accounting for participant body mass, the HFM contained 927 ± 154 kcal across all of the participants. Table 1 displays kcal consumed in the test meal by group. The amount of pie consumed was generally similar to a typical serving at a restaurant or social gathering (1-2 servings).

Participants were instructed to avoid planned exercise for two full days before their main assessment. Participants were given a 270-kcal snack (Little Debbie Swiss Cake Roll; McKee Foods; Collegedale, TN, USA) that they were instructed to consume in the evening, ten hours prior to their appointment.

On the morning of the meal assessment, participants arrived to the laboratory after a 10-hour overnight fast. An indwelling safelet catheter was inserted into a forearm vein via 24-gauge needle (Exelint International, Redondo Beach, CA, USA). The catheter was kept clear with a consistent infusion of 0.9% NaCl solution (~1 drip/second) and maintained stationary via placement of tegaderm film (3M Healthcare, Neuss, Germany). When the catheter was set in place, a fasting blood draw was conducted. For each blood draw, a 3 mL syringe (BD, Franklin Lakes, NJ, USA) was used to remove saline from the line, after which the actual blood sample was drawn into a 5 mL syringe (BD, Franklin Lakes, NJ, USA). The 5 mL syringe was emptied into a 6 mL Vacutainer test tube (BD, Franklin Lakes, NJ, USA) coated with EDTA (anticoagulant) and inverted three times to ensure adequate mixing with the EDTA. Whole blood from the blood draws was utilized to measure TG, glucose, total cholesterol (Total-C), and high-density lipoprotein cholesterol (HDL-C) using a Cholestech LDX analyzer (Alere Cholestech, San Diego, CA, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated by the LDX analyzer using the Friedewald equation (25). For each blood sample, a few drops of whole blood

were drawn into a capillary tube and plunged into a Cholestech LDX Lipid+Glu cassette (Alere Cholestech, San Diego, CA, USA). The cassette was then placed in the Cholestech LDX analyzer for measurement. After the baseline blood draw, participants consumed the test meal within 20 minutes. Water was available ad libitum with the meal and during the post-meal period. Participants stayed in the laboratory for six hours after consumption of the test meal (the six-hour time period began after the last bite of the test meal). Blood draws were conducted serially each hour for six hours post-HFM.

Statistical Analyses

An *a priori* sample size calculation based on the findings of previous studies (26,27) revealed that 3-5 participants would need to be recruited to each group to detect statistically significant differences in the postprandial TG response (power = 0.80; $\alpha = 0.05$). We aimed to recruit 8 participants to each group (24 participants total) in order to increase power to detect differences in other metabolic markers. However, due to the considerable challenge of recruiting inactive individuals over age 60 who were not taking lipid-lowering medications, only 6 participants recruited to the OA group met our *a priori* inclusion criteria.

MLI is calculated by adding TG and glucose values. As postprandial TG and glucose responses have been shown to be interrelated (28,29), MLI is intended to represent the cumulative metabolic challenge faced by the body, either fasting or following a meal (21).

Total area under the curve (tAUC), incremental area under the curve (iAUC), peak value, and time to peak value were determined for each of the metabolic markers. tAUC and iAUC were calculated using the trapezoid method. All data were assessed for normality via Shapiro-Wilk formal normality test and analysis of frequency distribution. If data were not normally distributed, a square root transformation was performed. Differences between groups with regard

to participant characteristics and postprandial values were tested via one-way Analysis of Variance (ANOVA) with Holm-Sidak adjustment for multiple comparisons. However, since iAUC analyses can potentially produce negative values, a square root transformation was not performed. Instead, a non-parametric Friedman test was utilized to test for group differences if iAUC data were not normally distributed.

Time-course changes in metabolic markers in the postprandial period were determined via two-way (group x time) repeated measures ANOVA with a Tukey's adjustment for multiple comparisons. A type 1 error rate of 0.05 was used in all analyses for the determination of statistically significant differences. Statistical analyses were conducted using GraphPad Prism statistical software (Version 6.05; GraphPad Software, Inc; La Jolla, CA).

Results

Participant Characteristics

Participant characteristics are displayed in Table 1. The YA group was younger than the OA ($p < 0.0001$) and the OI ($p < 0.0001$) groups. The OA and OI groups did not differ in age ($p = 0.60$). With regard to anthropometric variables, there were no differences between groups ($p > 0.05$) with respect to height, body mass, body mass index (BMI), or percentage body fat. However, the OI group had significantly more trunk fat compared to the YA group ($p = 0.02$), although there were no differences between YA and OA ($p = 0.09$) or OA and OI ($p = 0.33$).

Physical Activity Level

There was no difference in steps/day in the YA group versus the OA group ($p = 0.56$) or the OA group compared to the OI group ($p = 0.06$). However, the YA group did obtain significantly more steps than the OI group ($p = 0.03$). With regard to minutes per day spent in MVPA, the YA

and OA groups both engaged in more MVPA compared to the OI group ($p = 0.01$; $p = 0.003$, respectively), while YA and OA did not differ ($p = 0.44$).

Fasting Values

There was no difference in fasting TG in the YA group compared to the OA group ($p = 0.55$) or the OA group compared to the OI group ($p = 0.07$). Fasting TG was significantly lower in the YA group versus the OI group ($p = 0.03$). No participants presented with fasting TG >150 mg/dL and only one participant (belonging to the OI group) had fasting TG >100 mg/dL (136 mg/dL). Thus, all participants presented with optimal fasting TG. There were no group differences ($p > 0.05$) with regard to fasting glucose. However, fasting MLI was higher in the OI adults compared to both the YA ($p = 0.01$) and OA adults ($p = 0.03$), with no difference between the YA and OA ($p = 0.62$). Fasting Total-C was not different between groups ($p > 0.05$). Only one participant (an OI adult) presented with fasting total cholesterol >200 mg/dL (209 mg/dL). Fasting Total-C was in the optimal range for all other participants. There were group differences with regard to fasting LDL-C, as OA had higher LDL-C levels compared to YA ($p = 0.04$). However, the OI group was not different from the YA group ($p = 0.06$) or the OA group ($p = 0.80$). No participants presented with fasting LDL-C greater than 129 mg/dL, the upper limit for optimal fasting LDL-C. There were no group differences with regard to fasting HDL-C ($p > 0.05$). Three participants (1 YA, 2 OI) had below-optimal HDL-C (40-60 mg/dL).

Postprandial Metabolic Responses

Postprandial metabolic values and time-course responses are presented in Table 3 and Figures 1 and 2, respectively. In a two-way repeated measures ANOVA, a significant group x time interaction was detected in the postprandial TG response ($p < 0.0001$). TG peaked at a significantly lower level in the YA group compared to the OA ($p = 0.03$) and OI ($p = 0.0003$)

groups, while the OA group peaked at a significantly lower level than the OI group ($p = 0.03$). TG tAUC (representative of the total magnitude and duration of the postprandial response for the 6-hour period) was lower in YA adults versus OA adults ($p = 0.02$) and OI adults ($p = 0.0002$), whereas TG tAUC was lower in OA adults compared to OI adults ($p = 0.02$). With regard to TG iAUC (representative of the magnitude and duration of the postprandial response above fasting/baseline), YA had a lower response than OA ($p = 0.006$) and OI ($p = 0.0002$), with no difference ($p = 0.06$) in OA and OI groups.

For the postprandial glucose response, there was no significant group x time interaction following the HFM ($p = 0.24$), although there was a significant time effect ($p = 0.002$). There was a significant group effect ($p = 0.04$) for peak glucose values. However, no group pairwise comparisons were statistically significant ($p > 0.05$). With the exception of a few time-point specific differences (Figure 1), there were no other significant differences between groups in the postprandial glucose response.

The repeated measures two-way ANOVA revealed a significant group x time interaction in the MLI response to the HFM ($p = 0.004$). The OI group exhibited a greater MLI peak compared to the YA ($p = 0.0007$) and OA ($p = 0.02$) groups, while there was no difference between YA and OA ($p = 0.09$). With regard to tAUC, there was no difference between the YA and OA groups (Mean diff: -196.2 mg/dL x 6 hr; 95% CI: (-516.9, 124.5); $p = 0.14$), but the OI group exhibited a greater MLI response compared to YA (Mean diff: 617.0 mg/dL x 6 hr; 95% CI: (270.6, 963.4); $p = 0.0007$) and OA (Mean diff: 420.8 mg/dL x 6 hr; 95% CI: (74.4, 767.2); $p = 0.01$). The YA group also displayed a significantly lower (Mean diff: -375.9 mg/dL x 6 hr; 95% CI: (-614.7, -137.2); $p = 0.002$) MLI iAUC response compared to the OI group, with no difference

between YA and OA (Mean diff: -160.9 mg/dL x 6 hr; 95% CI: (-381.9, 60.2); $p = 0.08$) or OA and OI (Mean diff: -215.0 mg/dL x 6 hr; 95% CI: (-453.8, 23.7); $p = 0.07$).

Postprandial Total-C levels in each group are presented in Figure 2. No significant group x time interaction was observed in Total-C ($p = 0.49$), although there was a significant group effect ($p = 0.004$). Peak Total-C levels were lower in the YA group compared to the OA (Mean diff: -36.8 mg/dL; 95% CI: (-64.2, -9.3); $p = 0.009$) and OI (Mean diff: -32.7 mg/dL; 95% CI: (-62.4, -3.1); $p = 0.02$) groups, with no difference between OA and OI (Mean diff: 4.04 mg/dL; 95% CI: (-25.6, 33.7); $p = 0.73$). Total-C tAUC was significantly lower in YA compared to OA (Mean diff: -219.8 mg/dL x 6 hr; 95% CI: (-370.9, -68.6); $p = 0.005$) and OI (Mean diff: -189.9 mg/dL x 6 hr; 95% CI: (-353.2, -26.7); $p = 0.02$), but there was no difference between OA and OI (Mean diff: 29.8 mg/dL x 6 hr; 95% CI: (-133.4, 193.1); $p = 0.65$).

With regard to LDL-C, in a two-way repeated measures ANOVA, although there was no significant group x time interaction ($p = 0.46$), significant time ($p < 0.0001$) and group ($p = 0.03$) effects were detected. YA adults exhibited a significantly lower LDL-C peak compared to OA ($p = 0.02$) and OI ($p = 0.04$) adults, with no difference between OA and OI adults ($p = 0.68$). The LDL-C tAUC response was lower (Mean diff: -151.5 mg/dL x 6 hr; 95% CI: (-285.3, -17.8); $p = 0.03$) in the YA group versus the OA group, but there was no difference in YA versus OI (Mean diff: -95.9 mg/dL x 6 hr; 95% CI: (-238.9, 47.1); $p = 0.20$) or OA versus OI (Mean diff: 55.6 mg/dL x 6 hr; 95% CI: (-78.1, 189.4); $p = 0.30$).

There was no significant group x time interaction ($p = 0.47$) with regard to HDL-C. There were also no statistical group differences ($p > 0.05$) in peak, time to peak, tAUC, or iAUC.

Discussion

Main Findings

The main finding of the present study was the distinct difference in the postprandial TG response between the three groups. Supporting our first hypothesis, the YA group exhibited an attenuated postprandial lipemic response relative to OA and OI groups, and OA displayed a tempered response compared to OI. These findings suggest that both age and physical activity status independently impact the postprandial TG response. With regard to our second hypothesis, we predicted no group- or time-based differences in the postprandial glycemic response. Interestingly, we found that glucose was significantly higher in the OI group compared to the YA and OA groups one hour after the HFM, although there was not an overall group x time interaction. Finally, in partial agreement with our third hypothesis, the YA and OA groups displayed smaller postprandial MLI responses compared to the OI group, with no differences between YA and OA groups.

Postprandial TG Responses

While there is a well-established connection between postprandial lipemia and CVD risk (30), most studies investigating postprandial lipemia have utilized young and middle-aged individuals, as opposed to older adults (31,32). This omission is problematic, considering that: 1) older individuals are at a higher risk for CVD (33), and 2) older adults, as a segment of the population, are becoming more numerous in Western society (34). The current study found an age-related increase in postprandial lipemia: YA adults showed a comprehensively lower postprandial TG response compared to OA and OI adults. The finding of an increase in the postprandial lipemic response with age is in agreement with previous studies (26, 27, 35-38). However, the reason(s) for the greater postprandial TG response in older individuals remains unclear, with several

potential mechanisms in consideration (19). First, there is evidence that lipoprotein lipase (LPL) activity decreases with age (39). As LPL is the rate-limiting step for the clearance of TG in circulation (19), the increase in postprandial lipemia with age could be partly due to a decrease in LPL activity. Next, age-related changes in liver physiology result in impairment of uptake and metabolism of chylomicron remnants, including the TG portion (40). Related to this, liver fat content is typically higher in older individuals (41), and there is evidence that increased liver fat content is accompanied by increased circulating lipid concentrations, including TG (42). Therefore, there are several potential physiological explanations for the greater postprandial TG response displayed by older adults.

To our knowledge, only one previous study has assessed postprandial lipemia in older adults, while also considering chronic physical activity level (27). Miyashita and colleagues (27) tested the postprandial TG response in 26 older adults (mean age: ~70 years), divided into active and inactive groups based on whether they obtained less or more than 150 minutes per week of MVPA. The authors found a significantly lower postprandial TG response in the active older adults, despite requiring that all participants avoid physical activity for 48 hours prior to their assessment. There are several noteworthy differences in study design between the present investigation and that of Miyashita et al. (27), including that the present study matched groups by sex, obtained metabolic values every hour post-meal (instead of every other hour), and utilized a fattier test meal (63% vs 35% kcal from fat). Despite these differences, both investigations found a significantly lower postprandial lipemic response in OA than OI groups. These data disagree with the previously accepted notion that the lipid-lowering effects of physical activity were limited to the acute time-frame, as OA adults appear to experience a lesser post-meal TG response than their inactive counterparts, even when required to abstain from exercise for two

days prior to the meal. Therefore, there are potentially chronic physical activity effects on the postprandial TG response in older adults that may not be present in younger individuals. Finally, the most notable difference between the present study and that of Miyashita and colleagues (27) is that our study included a YA group. In doing so, the present study was designed to assess whether both aging and physical activity impacted the postprandial TG response. Results from the current investigation indicated that there was a lower lipemic response in OA compared to OI, while YA exhibited the lowest lipemic response of the three groups. As there were no differences between the YA and OA groups with regard to physical activity level, it appears that both physical activity status and aging may have independent effects on the postprandial lipemic response.

Other Metabolic Markers

For glucose, there were no group differences with regard to the primary postprandial indices. This is not surprising, given the quicker postprandial response of glucose relative to TG, so that group differences would be difficult to detect over a six-hour period. Notably, we did find that glucose was significantly higher in the OI group compared to the YA and OA groups one hour after the meal. This finding was unexpected, since the HFM only included 34% of kcals as carbohydrate. While the present study was not designed or intended to robustly assess glucose tolerance, our findings point to potentially greater capacity for glucose clearance in OA adults compared to OI adults.

In accordance with this, we also utilized the MLI response to the HFM in assessing postprandial metabolism in the three groups. Noteworthy group differences were exhibited with regard to both TG and glucose, but these differences generally occurred at different phases of the postprandial period. Specifically, there were group differences in glucose (but not TG) early in the

postprandial period, while group differences were evident in TG (but not glucose) later in the postprandial period. However, consideration of the postprandial MLI response reveals significant group differences throughout the postprandial period. Thus, the metabolic differences between active and inactive adults in the present study were not merely an issue of lipid clearance or glucose uptake, but an overall difference in metabolic capacity to clear the mixed meal, as represented by the group differences in MLI throughout the postprandial period. (However, it should be noted that the mixed meal in the present study was 63% fat, 34% carbohydrate. Thus, the MLI response primarily represents the postprandial lipemic response.) As both elevated postprandial glycemia and lipemia have been independently linked to CVD risk (21,22), this finding of an overall diminished metabolic capacity in OI adults is noteworthy.

Previous research suggests that Total-C is elevated in older individuals compared to younger individuals (43), and that it is not very responsive in the acute postprandial period (44). These notions were supported by the present findings, as Total-C was lower at baseline and throughout the postprandial period in the younger adults compared to the older adults, and there was no main effect of time for Total-C. Additionally, despite previous evidence suggesting lower Total-C in regularly active individuals (45), the lack of difference between the OA and OI adults is not surprising, since we excluded individuals who were taking lipid-lowering medication.

In the present study, although it was generally lower in the younger adults, LDL-C did not dramatically change in the postprandial period in any of the groups. On the other hand, it has previously been demonstrated that HDL-C has a tendency to decrease following consumption of a HFM, another potentially deleterious feature of the postprandial metabolic response (11). Accordingly, we hypothesized that we would see a decrease in HDL-C following the HFM, with the OI adults displaying the greatest decrease and that YA adults showing the most negligible

decrease. Contrary to expectation, we found no changes in the postprandial period, or differences among groups, with regard to HDL-C. The explanation for this null finding remains unclear.

Strengths and Experimental Considerations

There are several strengths of the present investigation that deserve to be highlighted. First, a strong point of the present study is its realistic test meal. Prior postprandial metabolic studies have tended to utilize test meals that are not very "true-to-life" (e.g. 1500 kcal) (46). In the present investigation, the average kcal value for the test meal was ~930 kcal. Thus, while the meal was not small, it was nevertheless considerably smaller than many previous studies (46), and yet, in our view, still representative of an unhealthy Western meal. Finally, an asset of this study was the blood sample frequency and the duration of postprandial period. It is ideal to collect frequent blood samples during the postprandial period to optimally characterize the response curve. With regard to duration of assessment, measuring the postprandial TG response for four hours is typically sufficient (47). However, as older adults tend to have a delayed TG response relative to younger adults, it is imperative that postprandial metabolic tests in older adults extend beyond four hours (19). In utilizing a six-hour postprandial period, with hourly blood samples, we view the present study to be well-suited to characterize the postprandial metabolic response.

However, the present study is not without limitations, and considerations need to be made in interpreting the findings. First, the present study did not include a younger inactive group. The primary research purpose was to determine whether there were independent effects of aging and chronic physical activity on the postprandial response. We did not view including a younger inactive group as pertinent to accomplishing this purpose. Specifically, we were most interested in comparing YA adults to OA adults, and OA adults to OI adults. Further, the comparison

between younger active and inactive individuals has been tested numerous times in the past (16). Consequently, we directed our resources toward obtaining sufficient data to adequately answer our research question with the essential YA, OA, and OI groups. However, relevant to our research question, had we included a younger inactive group, we could have compared the effects of physical activity in younger participants to the activity effects in older participants. Thus, we acknowledge not including a younger inactive group as a limitation. Another consideration is the limited degree to which we can connect the postprandial responses in the present study to CVD risk. While there is a fasting TG recommendation of <150 mg/dL (48), and on average the OI group exceeded this cut point for most of the postprandial period, there is no established post-meal TG cut point for increased CVD risk. However, an expert panel statement has suggested non-fasting TG ≥180 mg/dL is "undesirable" (49). More research is needed to better determine TG reference ranges that represent increased risk in the acute postprandial period. Similarly, MLI is a novel, recently proposed marker of metabolic status. As such, the strength of MLI as an independent CVD risk factor is yet to be determined, and no cut points exist for fasting and postprandial MLI. Therefore, at this point, the higher MLI response in OI participants cannot be directly linked to increased CVD risk. Next, it was crucial that individuals on lipid-lowering medications be excluded from the present study. However, not only does this requirement make recruiting more difficult, but it potentially limits external applicability for the OI group. There were simply not very many OI individuals who were not on lipid-lowering medications in our target population. However, it could be argued that OI individuals on lipid-lowering medications could, theoretically, display a similar response as the OI adults in the present study who were not on lipid-lowering medications. This is an interesting issue, and

should be investigated further. In addition, given the important roles of insulin in regulating substrate utilization, it would have been valuable to assess fasting and postprandial insulin.

Conclusion and Future Directions

We found that both aging and physical activity level likely impact the postprandial TG response, as YA exhibited a lesser response than OA and OI groups, but OA adults showed a reduced response compared to OI. OI adults also displayed higher glucose in the 1-2 hours post-meal, pointing to an overall diminished capacity for metabolic clearance, as supported by the MLI findings. Overall, these findings point to the possibility of an age-related decline in metabolic clearance capacity, but this deterioration can be partially alleviated with chronic physical activity – an important public health message. Future research should continue to study the relationship between physical activity, postprandial metabolism, and CVD risk in aging individuals, as older adults represent a growing segment of the population that is at increased risk for CVD development.

Tables

	Younger Active n=8	Older Active n=8	Older Inactive n=6	*P*-value
Age (years)	25.1 ± 4.8[a]	66.5 ± 5.2[b]	68.2 ± 7.4[b]	<0.0001
Height (cm)	174.2 ± 10.8	175.0 ± 8.8	168.3 ± 8.5	0.40
Body mass (kg)	71.9 ± 10.4	81.6 ± 15.4	78.6 ± 11.3	0.32
Body mass index (kg/m^2)	23.6 ± 2.0	26.6 ± 4.1	27.9 ± 4.7	0.11
Body fat (%)	21.5 ± 8.9	30.0 ± 11.7	33.4 ± 8.9	0.09
Trunk fat (%)	22.5 ± 9.2[a]	32.6 ± 11.5[ab]	37.7 ± 5.9[b]	0.02
Steps (x10^3)/day	16.6 ± 6.5[a]	14.9 ± 5.5[ab]	8.8 ± 2.8[b]	0.03
MVPA (minutes/day)	159.4 ± 48.6[a]	182.4 ± 77.1[a]	62.3 ± 14.1[b]	0.003
Test meal energy (kcal)	863 ± 125	980 ± 185	943 ± 136	0.32

Table 5-1. Participant characteristics.

Data are Mean ± SD. The *P*-value column denotes main effects between groups assessed via one-way ANOVA. Within main effects (by row), column values with shared superscript letters are not significantly differrent, determined by *post hoc* pairwise comparisons. Rows with no superscript letters present contain no significant differences. See Results section for post hoc pairwise comparison *p*-values.

n, number of participants; MVPA, moderate- to vigorous-intensity physical activity

Fasting Values (mg/dL)	Optimal Values (mg/dL)	Younger Active n=8	Older Active n=8	Older Inactive n=6	*P*-value
TG	< 150	47.4 ± 4.6[a]	52.3 ± 9.0[ab]	75.8 ± 35.3[b]	0.03
Glucose	< 100	89.6 ± 11.2	90.6 ± 8.3	101.3 ± 9.7	0.08
MLI	N/A	137.0 ± 11.5[a]	142.9 ± 15.0[a]	177.2 ± 39.5[b]	0.01
Total-C	< 200	137.5 ± 24.5	165.4 ± 18.1	165.8 ± 32.1	0.06
LDL-C	100-129	76.3 ± 18.9[a]	103.9 ± 17.1[b]	101.3 ± 19.9[ab]	0.03
HDL-C	40-60	52.0 ± 10.9	50.8 ± 6.0	48.3 ± 17.4	0.84

Table 5-2. Metabolic values measured in fasting participants.

Data are Mean ± SD. The *P*-value column denotes main effects between groups assessed via one-way ANOVA. Within main effects (by row), column values with shared superscript letters are not significantly different, determined by *post hoc* pairwise comparisons. Rows with no superscript letters present contain no significant differences. Optimal values are based on references 48 and 50.

n, number of participants; TG, triglycerides; MLI, metabolic load index; Total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

	Younger Active n=8	Older Active n=8	Older Inactive n=6	*P*-value
Triglycerides				
Peak (mg/dL)	82.0 (75.5-97.5)[a]	133.0 (120.3-160.0)[b]	182.0 (155.3-341.5)[c]	0.0004
Time to peak (hours)	3.5 (2.0-4.0)	4.0 (3.0-4.8)	4.5 (3.0-5.0)	0.18
tAUC (mg/dL 6 hr)	372.8 (334.0-436.1)[a]	570.0 (486.5-712.3)[b]	794.8 (677.6-1367.0)[c]	0.0003
iAUC (mg/dL 6 hr)	78.3 (59.3-154.6)[a]	288.0 (216.5-358.3)[b]	453.0 (307.3-687.8)[b]	0.0002
Glucose				
Peak (mg/dL)	99.9 ± 7.8	99.0 ± 16.4	118.2 ± 17.2	0.04
Time to peak (hours)	1.5 (0.0-3.5)	1.0 (0.0-3.8)	1.0 (0.8-1.3)	0.96
tAUC (mg/dL 6 hr)	528.9 ± 43.2	507.3 ± 82.2	592.5 ± 59.9	0.07
iAUC (mg/dL 6 hr)	-8.9 ± 58.5	-36.4 ± 49.5	-15.5 ± 44.9	0.56
Metabolic Load Index				
Peak (mg/dL)	174.0 (165.3-193.5)[a]	222.0 (207.0-245.8)[a]	275.0 (256.3-445.3)[b]	0.0009
Time to peak (hours)	3.4 ± 1.1	3.6 ± 0.9	4.2 ± 1.0	0.35
tAUC (mg/dL 6 hr)	936.8 ± 137.7[a]	1133.0 ± 207.4[a]	1553.8 ± 394.3[b]	0.0009
iAUC (mg/dL 6 hr)	114.8 ± 131.2[a]	275.6 ± 135.1[ab]	490.7 ± 255.7[b]	0.003
Total Cholesterol				
Peak (mg/dL)	144.1 ± 24.2[a]	180.9 ± 13.8[b]	176.8 ± 26.2[b]	0.006
Time to peak (hours)	2.1 ± 2.0	3.1 ± 1.4	2.8 ± 2.3	0.56
tAUC (mg/dL 6 hr)	803.8 ± 123.8[a]	1023.5 ± 84.0[b]	993.7 ± 149.9[b]	0.003
iAUC (mg/dL 6 hr)	-21.3 ± 57.6	31.1 ± 53.9	-1.4 ± 54.1	0.19
LDL-Cholesterol				
Peak (mg/dL)	77.5 (65.5-90.5)[a]	104.5 (95.8-123.5)[b]	106.0 (87.0-122.0)[b]	0.01
Time to peak (hours)	1.0 (0.0-6.0)	1.0 (0.0-3.5)	0.0 (0.0-1.5)	0.52
tAUC (mg/dL 6 hr)	427.9 ± 88.4[a]	579.4 ± 80.2[b]	523.8 ± 121.9[ab]	0.03
iAUC (mg/dL 6 hr)	-30.1 ± 39.5	-43.8 ± 41.1	-84.2 ± 64.7	0.16
HDL-Cholesterol				
Peak (mg/dL)	57.5 ± 11.9	56.4 ± 9.0	51.0 ± 17.7	0.62
Time to peak (hours)	3.4 ± 2.1	2.6 ± 2.3	1.8 ± 2.3	0.45
tAUC (mg/dL 6 hr)	305.8 ± 67.4	317.2 ± 47.1	276.9 ± 106.1	0.60
iAUC (mg/dL 6 hr)	-6.2 ± 17.4	12.7 ± 27.0	-13.1 ± 19.9	0.10

Table 5-3. Postprandial metabolic outcomes.

Normally distributed data are Mean ± SD, and non-normally distributed data are Median (Interquartile Range). Non-normally distributed data were transformed before analysis. A one-way ANOVA was conducted to test for differences between groups. However, due to negative numbers, a non-parametric Friedman test was conducted for TG iAUC. The *P*-value column denotes main effects between groups assessed via one-way ANOVA. Within main effects (by

row), column values with shared superscript letters are not significantly different, determined by post hoc pairwise comparisons. Rows with no superscript letters present contain no significant differences. See Results section for *post hoc* pairwise comparison *p*-values.

tAUC, total area under the curve; iAUC, incremental area under the curve; LDL, low-density lipoprotein, HDL, high-density lipoprotein; ANOVA, analysis of variance

Figures

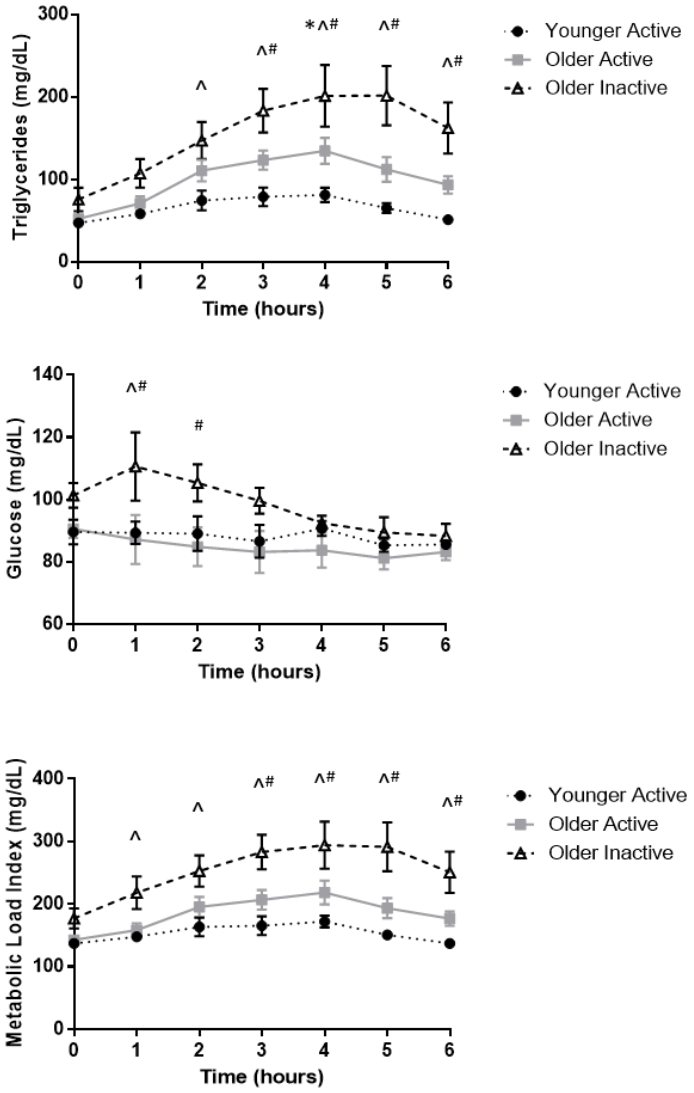

Figure 5-1. Postprandial responses for triglycerides, glucose, and metabolic load index. Triglycerides (top panel), glucose (middle panel), and metabolic load index (bottom panel) were assessed at baseline/fasting (time 0) and serially for six hours after the high-fat meal. Significant differences ($p < 0.05$) at specific time-points reflect the results of a two-way (group x time) repeated measures ANOVA. Error bars reflect standard error.

* YA vs OA; ^ YA vs OI; # OA vs OI

YA, younger active adults; OA, older active adults; OI, older inactive adults

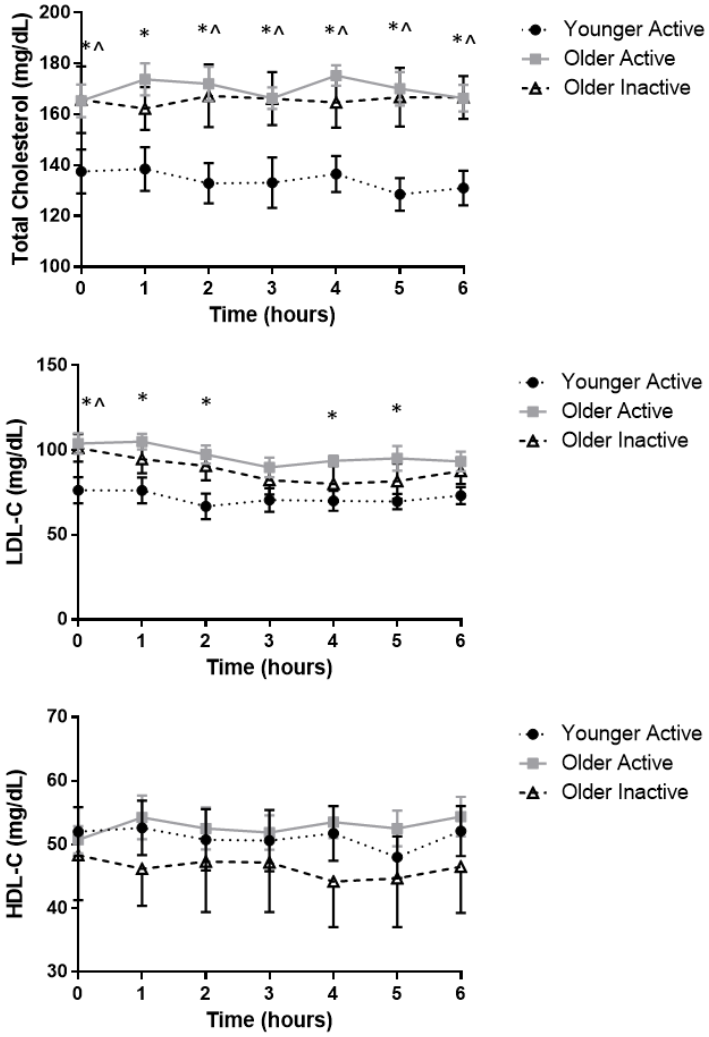

Figure 5-2. Postprandial cholesterol responses.

Total cholesterol (top panel), LDL-C (middle panel), and HDL-C (bottom panel) were assessed at baseline/fasting (time 0) and serially for six hours after the high-fat meal. Significant differences ($p < 0.05$) at specific time-points reflect the results of a two-way (group x time) repeated measures ANOVA. Error bars reflect standard error.

* YA vs OA; ^ YA vs OI; # OA vs OI

YA, younger active adults; OA, older active adults; OI, older inactive adults; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

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Chapter 6 - Conclusion

Large postprandial metabolic fluxes and inflammation have been shown to be linked to CVD risk through a variety of mechanisms. Given this link, the present dissertation sought to better understand the relationship between single-meal intake and postprandial metabolic and inflammatory responses. Collectively, this series of papers 1) proposed a novel index of metabolic challenge (MLI) that considers both glucose and TG; 2) characterized the magnitude and timing of the postprandial response for IL-6, the only marker systematically shown to increase in circulation following a HFM; 3) revealed the substantially different postprandial metabolic responses induced by a HFM, representative of meals used in previous postprandial studies, compared to more "true-to-life" meals; and 4) showed that aging and chronic physical activity level independently influence the postprandial metabolic response to a HFM.

In the Chapter 2, we reviewed the evidence connecting both postprandial glycemia and lipemia to chronic disease risk. Since large glucose and TG fluctuations appear to alter CVD risk through similar mechanisms, we suggested the utility of a single metabolic index intended to represent the total metabolic challenge experienced by the body. The novel MLI, simply the sum of glucose and TG in circulation, was formally proposed (then used as a metabolic outcome in Chapters 4 and 5). We are optimistic that MLI will be increasingly used in research and clinical environments.

In the Chapter 3, the focus shifted from postprandial metabolism to postprandial inflammation. We conducted the first systematic review attempting to characterize the magnitude and timing of the postprandial inflammatory response for five commonly assessed markers of inflammation. Interleukin-6 was the only assessed inflammatory marker found to consistently increase in response to a HFM, starting at a baseline of ~1.4 pg/mL and peaking at ~2.9 pg/mL

approximately six hours after the meal. On the other hand, CRP, TNF-α, IL-1β, and IL-8 infrequently changed in response to a HFM. Therefore, we propose that future postprandial inflammatory research should focus on IL-6, as well as other novel inflammatory markers.

Chapter 4 was a randomized cross-over trial comparing the postprandial metabolic and inflammatory responses to three meal conditions: HFM (17 kcal/kg; 63% fat), MFM (8.5 kcal/kg; 30% fat), and a BPM, in which participants consumed the MFM twice, separated by three hours. We found a significantly greater TG tAUC response following the HFM compared to both the MFM and BPM, with no difference between the MFM and BPM. Additionally, the TG peak was significantly greater following HFM compared to MFM, although there was no difference between HFM and BPM. These findings suggest that the large TG responses seen in previous postprandial studies may not be reflective of the metabolic challenge experienced by many individuals in daily life.

Finally, Chapter 5 investigated the postprandial metabolic responses to a single HFM (12 kcal/kg; 63% fat) in three groups of healthy adults: YA, OA, and OI adults. Assessed via several indices, the largest postprandial TG response was exhibited by the OI adults, followed by the OA adults. Despite similar levels of physical activity, YA displayed a significantly lower postprandial TG response relative to OA adults. Further, glucose was significantly greater in OI adults compared to OA and YA adults one hour post-meal. Overall, these findings point to a diminished metabolic capacity that accompanies aging, in agreement with the MLI results. However, this age-related metabolic decline can be partially ameliorated by higher levels of chronic physical activity.

In conclusion, this series of papers enhances our understanding of the relationship between diet and CVD risk by focusing on the acute metabolic and inflammatory responses to single-meal

intake. Aging, chronic physical activity level, meal size, meal macronutrient distribution, and meal frequency are all factors that appear to modify the postprandial response. Considering the connection between the postprandial response and CVD risk, and that many individuals spend most of their day in the postprandial state, our findings have clear real-life relevance, as well as the potential to enhance clinical understanding. Additionally, our results point to intriguing future research endeavors, such as testing the strength of MLI (fasting or postprandial) to predict disease risk compared to TG and glucose alone, further investigating the role of the postprandial increase in IL-6 in CVD development, studying other meal consumption factors that can minimize postprandial fluxes, and further investigating the physiological mechanisms behind aging and chronic physical activity with regard to their modification of the postprandial response.

Appendix A - Supplemental Tables

ARTICLE (REF)	MEAL	BLOOD	S/P	MO	FAT	ENERGY	N	M/F	AGE	BMI	PPP	↑↓↔	TTP	FASTING	PEAK	QA
					%E	*kcal*			*y*	*kg/m²*	*hrs*		*hrs*	*CRP, mg/mL*	*CRP, mg/mL*	
Bell et al. 2010 [1]	Heavy whipping cream, water	venipuncture	P	N	68	1200	10	0M/10W	29 ± 11	22 ± 3	6	↔	-	2.03 ± 1.43	-	6.5
Bidwell et al. 2014 (15)	Eggs, muffin, butter, sugary drink	cannula	P	N	40	600	22	11M/11F	M: 20.8 ± 0.7 F: 21.5 ± 0.9	M: 23.9 ± 0.9 F: 21.1 ± 0.5	6	↑	NS	NS	NS	6
Blum et al. 2006 - Med meal (77)	Vegetables, olive oil, orange juice, cheese, bread, yogurt, fish	NS	P	N	43	982	10	10M/0F	26.6 ± 1.1	24.86 ± 0.80	7	↓	2	1.5 (0.35 - 3.15)	NS	2.5
Blum et al. 2006 - Wes meal (77)	White bread, egg, sour cream, cheese	NS	P	N	46	972	10	10M/0F	26.6 ± 1.1	24.86 ± 0.80	7	↔	-	1.5 (0.35 - 3.15)	-	2.5
Caixas et al. 2008 – lean (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	23.0 (21.0 - 26.0)	20.3 (18.9 - 25.1)	6	↔	-	0.65 (0.36 - 4.85)	-	4
Caixas et al. 2008 – obese (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	26.0 (23.0 - 27.0)	43.9 (32.8 - 48.0)	6	↔	-	3.37 (2.79 - 9.48)	-	4
Campbell et al. 2006 (19)	Apple muffins, milk shake	venipuncture	S	N	41	976	15	15M/0F	28 ± 9	NS	6	↔	-	0.1 ± 0.04	-	6.5
Coutinho et al. 2008 [2]	Milk cream, egg yolk	NS	S	Y	74	683	28	13M/15F	39.96 ± 6.29	23.38 ± 2.41	5	↔	-	1.34 ± 1.40	-	2.5
Dandona et al. 2015 [3]	Egg, sausage, muffin, hash browns	NS	P	N	42	910	10	6M/4F	33 ± 4	22.8 ± 0.7	5	↔	-	1.33 ± 0.3	-	2.5
Denniss et al. 2008 [4]	Egg, sausage, muffin, hash browns, milk	venipuncture	S	N	49	1107	18	18M/0F	23 ± 2	20 - 30	3	↔	-	0.41 ± 0.35	-	4
Esser et al. 2013 (23)	Milk shake (cream, sugar, water)	cannula	P	Y	85	954	20	20M/0F	22 ± 2	22.7 ± 2.4	6	↔	-	0.46 ± 0.62	-	5
Ghanim et al. 2009 (78)	Egg, sausage, muffin, hash browns	NS	P	N	42	910	10	5M/5F	32.4 ± 1.3	23.1 ± 0.6	3	↔	-	1.43 ± 0.2	-	3
Johnson et al. 2016 (28)	Ice cream, whipping cream	cannula	P	N	45	1360 - 2160	12	12M/0F	23.0 ± 3.2	24.5 ± 2.7	4	↔	-	0.25 ± 0.32	-	4
Kackov et al. 2013 [5]	French bread, salami, cheese, margarine	venipuncture	S	N	55	823	102	102M/0F	58 (52 - 68)	28.3 ± 3.8	3	↔	-	1.72 (0.88 - 3.04)	-	4
Neri et al. 2010 [6]	Homogenized milkshake	NS	S	N	NS	1480	40	20M/20W	41 ± 2	23 ± 1	2	↑	2	1.48 ± 0.60	2.98 ± 0.70	4
Payette et al. 2009 - Men (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	39	39M/0F	44.0 ± 9.1	28.9 ± 4.3	8	↔	-	2.02 ± 2.30	-	5
Payette et al. 2009 - Women (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	41	0M/41F	43.7 ± 9.4	26.5 ± 5.7	8	↔	-	1.96 ± 2.03	-	5
Phillips et al. 2013 – Lean (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	43.4 ± 11.3	22.8 ± 1.5	6	↑	6	0.2 ± 0.1	NS	6
Phillips et al. 2013 – Obese (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	40.9 ± 9.8	38.2 ± 6.7	6	↑	6	1.3 ± 0.4	NS	6
Poppitt et al. 2008 (39)	Blueberry muffin	cannula	S	Y	71	748	18	18M/0F	23 ± 4	22.9 ± 2.0	6	↔	-	0.51 ± 0.1	-	6.5
Rankin et al. 2008 (40)	Eggs, sausage, biscuit, pancake, jelly candy	venipuncture	S	Y	53	900	17	8M/9F	26.5 ± 7.6	33.5 ± 6.7	4	↔	-	5.5 ± 5.8	-	5.5
Rosenkranz et al. 2010 [7]	Ice cream, whipping cream	venipuncture	P	N	45	982 - 2590	20	10M/10W	21.9 ± 1.9	24.4 ± 4.5	2	↔	-	1.73 ± 1.67	-	5.5
Schmid et al. 2015 (42)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1005	21	21M/0W	41.8 ± 9.0	27.1 ± 8.2	6	↔	-	1.2 ± 1.2	-	4.5
Schwander et al. 2014 – NW (43)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1000	19	19M/0W	40.6 ± 9.2	23.6 ± 1.4	6	↔	-	0.6 ± 0.2	-	4.5
Schwander et al. 2014 – obese (43)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1000	17	17M/0W	44.1 ± 8.0	38.8 ± 4.9	6	↔	-	2.4 ± 0.4	-	4.5
Schwander et al. 2014 – NW (43)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1500	19	19M/0W	40.6 ± 9.2	23.6 ± 1.4	6	↓	6	1.0 ± 0.3	0.8 ± 0.2	4.5
Schwander et al. 2014 – obese (43)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1500	17	17M/0W	44.1 ± 8.0	38.8 ± 4.9	6	↔	-	3.5 ± 1.0	-	4.5
Tholstrup et al. 2011 (46)	Mashed potatoes with fat powder	NS	S	Y	76	620	10	0M/10F	38.2 ± 10.7	20.9 ± 1.3	6	↔	-	0.95 ± 0.24	-	5
Wood et al. 2011 (49)	Fast food burger, hash browns	venipuncture	P	N	49	919	21	9M/12F	49.6 ± 4.6	24.0 ± 0.7	4	↔	-	1.6 ± 1.0	-	4.5

Supplemental Table 1. Details of studies that assessed pre- and post-HFM CRP in healthy participants.

Twenty-nine studies met the inclusion criteria and assessed CRP before and after consumption of a HFM. If studies separately assessed different groups or utilized different test meals (each had to meet established participant or meal criteria), those specific subsets are specified alongside the study. When applicable, data represent Mean ± SD or Mean (Range). Concentration values are in mg/mL. Arrows represent significant increase (↑), significant decrease (↓), or no significant change (↔) detected in response to the HFM. References in parentheses are originally cited in the main printed paper, while references in brackets are located only in the Supplemental References section.

Abbreviations: BLOOD, method for drawing blood; BMI, body mass index; FASTING, baseline/fasting concentration of the marker; FAT, percentage of energy (%E) in the test meal from fat; M/F, ratio of males to females; MO, whether or not CRP was the main outcome being studied; N, sample size; NS, not stated; PEAK, peak or maximal observed concentration of the marker ("-" if not applicable); PPP, length of postprandial period assessment in hours; S/P, serum or plasma; TTP, time to peak or maximal observed concentration if a significant change was detected ("-" if not applicable).

ARTICLE (REF)	MEAL	BLOOD	S/P	MO	FAT	ENERGY	N	M/F	AGE	BMI	PPP	↑↓↔	TTP	FASTING	PEAK	QA
					%E	*kcal*			*y*	*kg/m²*	*hrs*		*hrs*	*TNF, pg/mL*	*TNF, pg/mL*	
Bidwell et al. 2014 (15)	Eggs, muffin, butter, sugary drink	cannula	P	N	40	600 kcal	22	11M/11F	M: 20.8 ± 0.7 F: 21.5 ± 0.9	M: 23.9 ± 0.9 F: 21.1 ± 0.5	6	↑	NS	NS	NS	6
Brandauer et al. 2013 (16)	Sugar, heavy cream, chocolate syrup, powdered milk	cannula	P	Y	84	1310 ± 34.1	10	10M/0F	27 ± 1	24.6 ± 0.7	4	↔	-	5.2 ± 1.2	-	4.5
Caixas et al. 2008 – lean (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	23.0 (21.0 - 26.0)	20.3 (18.9 - 25.1)	6	↔	-	0.14 (0.05 - 0.19)	-	4
Caixas et al. 2008 – obese (18)	Liquid test meal	cannula	P	Y	30	750	7	6M/1F	26.0 (23.0 - 27.0)	43.9 (32.8 - 48.0)	6	↔	-	0.13 (0.13 - 0.16)	-	4
Drew et al. 2014 (21)	Turkey burger, white bread	cannula	P	N	50	600	16	16M/0F	45 ± 11	27.6 ± 5.3	6	↔	-	2.26 ± 0.64	-	3.5
Esser et al. 2013 (23)	Milk shake (cream, sugar, water)	cannula	P	Y	85	954	20	20M/0F	22 ± 2	22.7 ± 2.4	6	↔	-	6.12 ± 1.70	-	5
Ghanim et al. 2009 (78)	Egg, sausage, muffin, hash browns	NS	P	N	42	910	10	5M/5F	32.4 ± 1.3	23.1 ± 0.6	3	↔	-	2.09 ± 0.32	-	3
Gill et al. 2003 (24)	Whipping cream, fruit, cereal, nuts, chocolate	cannula	P	N	67	1075	8	8M/0F	27.8 ± 12.1	23.6 ± 1.0	6	↔	-	1.40 ± 0.52	-	5
Jimenez-Gomez et al. 2009 (27)	Butter, wholemeal bread, hard-boiled egg, whole milk	venipuncture	P	Y	60	NS	20	20M/0F	NS	NS	9	↔	-		-	6
Johnson et al. 2016 (28)	Ice cream, whipping cream	cannula	P	N	45	1360 - 2160	12	12M/0F	23.0 ± 3.2	24.5 ± 2.7	4	↔	-	6.48 ± 2.13	-	4
Kiecolt-Glaser et al. 2015 (29)	Eggs, turkey sausage, biscuits, gravy	cannula	S	N	60	930	86	43M/43W	38.22 ± 8.18	32.07 ± 5.83	7	↔	-	4.77 ± 1.12	-	4.5
Kracmerova et al. 2014 (30)	Pork meat, egg, French fries, hazelnut spread, croissant	cannula	P	Y	47	1470	10	10M/0W	26.3 ± 1.04	23.11 ± 0.59	4	↔	-	4.632 ± 0.410	-	3.5
Mariotti et al. 2015 (33)	Milk cream, sucrose, whey protein	cannula	P	N	70	1200	10	10M/0F	34 ± 9	30.2 ± 1.5	6	↔	-	5.44 ± 1.04	-	3.5
Miglio et al. 2013 (34)	Fried potatoes, eggs, cheese, bread rolls	cannula	P	Y	52	1416	15	13M/2F	45 ± 8	26.7 ± 1.9	8	↑	8	29.1 ± 29.0	50.5 ± 32.3	4
Nappo et al. 2002 (35)	Sausage, bread, egg, butter, olive oil	venipuncture	P	N	59	760	20	10M/10F	44 ± 5	26.8 ± 1.2	4	↑	2	2.9 ± 0.8	4.0 ± 0.8	5
Payette et al. 2009 - Men (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	39	39M/0F	44.0 ± 9.1	28.9 ± 4.3	8	↓	4	1.74 ± 0.48	1.57 ± 0.50	5
Payette et al. 2009 - Women (36)	Cheese, eggs, toast, butter, cream, milk, peanut butter	cannula	P	Y	64	1600 - 2200	41	0M/41F	43.7 ± 9.4	26.5 ± 5.7	8	↓	4	1.91 ± 1.35	1.80 ± 1.29	5
Peluso et al. 2012 (37)	Fried potatoes, eggs, cheese, bread	cannula	P	N	55	1344	14	12M/2W	45.1 ± 8.6	26.8 ± 2.2	8	↑	8	31.3 ± 30.2	53.5 ± 16.9	2.5
Phillips et al. 2013 – Lean (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	43.4 ± 11.3	22.8 ± 1.5	6	↔	-	0.6 ± 0.3	-	6
Phillips et al. 2013 – Obese (38)	Bacon, egg, muffin, hash browns, milk	cannula	P	N	52	989	10	10M/0W	40.9 ± 9.8	38.2 ± 6.7	6	↔	-	0.8 ± 0.4	-	6
Poppitt et al. 2008 (39)	Blueberry muffin	cannula	S	Y	71	748	18	18M/0F	23 ± 4	22.9 ± 2.0	6	↓	1	736.1 ± 156.8	698.4 ± 164.5	6.5
Sanders et al. 2011 (41)	Muffin, milkshake	cannula	P	N	53	846	50	25M/25W	M: 25.4 ± 4.2 W: 24.2 ± 6.3	M: 23.3 ± 2.1 W: 23.7 ± 3.4	8	↓	4	1.5 ± 0.05	1.4 ± 0.05	5
Schmid et al. 2015 (42)	Bread, salami, palm fat, boiled eggs	cannula	P	N	61	1005	21	21M/0W	41.8 ± 9.0	27.1 ± 8.2	6	↔	-	3.6 ± 1.3	-	4.5
Strohacker et al. 2012 (44)	Sausage, egg, cheese, biscuit, hash browns	cannula	P	N	59	1070	8	4M/4F	21 ± 3	23.1 ± 3.9	3	↔	-	3.2 ± 1.4	-	5
Teng et al. 2011 (45)	Mashed potatoes, baked beans, milk, orange juice, lard	NS	S	N	60	683	10	10M/0W	21.9 ± 0.7	21.0 ± 1.6	4	↔	-	14.5 ± 2.0	-	5.5
Twickler et al. 2003 (47)	Liquid cream meal	NS	P	Y	40	NS	10	6M/4F	48.6 ± 7.7	25.4 ± 1.6	24	↑	4	2.8 ± 0.8	4.7 ± 3.4	4
Volek et al. 2008 (48)	Whipping cream, pudding, macadamia nuts	cannula	P	Y	84	908	30	16M/14W	30 ± 8	24.1 ± 4.3	6	↔	-	1.06 ± 0.49	-	6
Wood et al. 2011 (49)	Fast food burger, hash browns	venipuncture	P	N	49	919	21	9M/12F	49.6 ± 4.6	24.0 ± 0.7	4	↔	-	1.4 ± 0.3	-	4.5

Supplemental Table 2. Details of studies that assessed pre- and post-HFM TNF-α in healthy participants.

Twenty-eight studies met the inclusion criteria and assessed TNF-α before and after consumption of a HFM. If studies separately assessed different groups, those specific subsets are specified alongside the study. When applicable, data represent Mean ± SD or Mean (Range). Concentration values are in pg/mL. Arrows represent significant increase (↑), significant decrease (↓), or no significant change (↔) detected in response to the HFM.

Abbreviations: BLOOD, method for drawing blood; BMI, body mass index; FASTING, baseline/fasting concentration of the marker; FAT, percentage of energy (%E) in the test meal from fat; M/F, ratio of males to females; MO, whether or not TNF-α was the main outcome being studied; N, sample size; NS, not stated; PEAK, peak or maximal observed concentration of the marker ("-" if not applicable); PPP, length of postprandial period assessment in hours; S/P, serum or plasma; TTP, time to peak or maximal observed concentration if a significant change was detected ("-" if not applicable).

ARTICLE (REF)	MEAL	BLOOD	S/P	MO	FAT	ENERGY	N	M:F	AGE	BMI	PPP	↑↓↔	TTP	FASTING	PEAK	QA
					%E	*kcal*			*y*	*kg/m²*	*hrs*		*hrs*	*IL-8, pg/mL*	*IL-8, pg/mL*	
Brandauer et al. 2013 (16)	Sugar, heavy cream, chocolate syrup, powdered milk	cannula	P	Y	84	1310 ± 34.1	10	10M/0F	27 ± 1	24.6 ± 0.7	4	↑	4	2.33 ± 1.16	3.26 ± 2.56	4.5
Esser et al. 2013 (23)	Milk shake (cream, sugar, water)	cannula	P	Y	85	954	20	20M/0F	22 ± 2	22.7 ± 2.4	6	↔	-	4.08 ± 0.95	-	5
Kracmerova et al. 2014 (30)	Pork meat, egg, French fries, hazelnut spread, croissant	cannula	P	Y	47	1470	10	10M/0W	26.3 ± 1.04	23.11 ± 0.59	4	↔	-	1.95 ± 0.418	-	3.5
Sanders et al. 2011 (41)	Muffin, milkshake	cannula	P	N	53	846	50	25M/25W	M: 25.4 ± 4.2 W: 24.2 ± 6.3	M: 23.3 ± 2.1 W: 23.7 ± 3.4	8	↔	-	0.8 ± 0.1	-	5

Supplemental Table 3. Details of studies that assessed pre- and post-HFM IL-8 in healthy participants.

Four studies met the inclusion criteria and assessed IL-8 before and after consumption of a HFM. When applicable, data represent Mean ± SD. Concentration values are in pg/mL. Arrows represent significant increase (↑), significant decrease (↓), or no significant change (↔) detected in response to the HFM.

Abbreviations: BLOOD, method for drawing blood; BMI, body mass index; FASTING, baseline/fasting concentration of the marker; FAT, percentage of energy (%E) in the test meal from fat; M/F, ratio of males to females; MO, whether or not IL-8 was the main outcome being studied; N, sample size; NS, not stated; PEAK, peak or maximal observed concentration of the marker ("-" if not applicable); PPP, length of postprandial period assessment in hours; S/P, serum or plasma; TTP, time to peak or maximal observed concentration if a significant change was detected ("-" if not applicable).

ARTICLE (REF)	MEAL	BLOOD	S/P	MO	FAT	ENERGY	N	M:F	AGE	BMI	PPP	↑↓↔	TTP	FASTING	PEAK	QA
					%E	*kcal*			*y*	*kg/m²*	*hrs*		*hrs*	*IL-1, pg/mL*	*IL-1, pg/mL*	
Cheng et al. 2010 [8]	Heavy whipping cream, milk powder, syrup	cannula	S	Y	78	782/ sq m	838	456M/382W	44 ± 14	26.6 ± 4.4	6	↔	-	0.8 (0.8 - 0.8)	-	6
Esser et al. 2013 (23)	Milk shake (cream, sugar, water)	cannula	P	Y	85	954	20	20M/0F	22 ± 2	22.7 ± 2.4	6	↔	-	0.47 ± 0.31	-	5
Teng et al. 2011 (45)	Mashed potatoes, baked beans, milk, orange juice, lard	NS	S	N	60	683	10	10M/0W	21.9 ± 0.7	21.0 ± 1.6	4	↓	4	6.1 ± 1.0	5.2 ± 1.0	5.5

Supplemental Table 4. Details of studies that assessed pre- and post-HFM IL-1β in healthy participants.

Three studies met the inclusion criteria and assessed IL-1β before and after consumption of a HFM. When applicable, data represent Mean ± SD. Concentration values are in pg/mL. Arrows represent significant increase (↑), significant decrease (↓), or no significant change (↔) detected in response to the HFM. References in parentheses are originally cited in the main printed paper, while references in brackets are located only in the Supplemental References section.

Abbreviations: BLOOD, method for drawing blood; BMI, body mass index; FASTING, baseline/fasting concentration of the marker; FAT, percentage of energy (%E) in the test meal from fat; M/F, ratio of males to females; MO, whether or not IL-1β was the main outcome being studied; N, sample size; NS, not stated; PEAK, peak or maximal observed concentration of the marker ("-" if not applicable); PPP, length of postprandial period assessment in hours; S/P, serum or plasma; TTP, time to peak or maximal observed concentration if a significant change was detected ("-" if not applicable).

Appendix B - Supplemental References

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