ABSTRACT

Title of Thesis: EFFECTS OF A HIGH-FAT MEAL ON THE INFLAMMATORY PHENOTYPE AND FUNCTION OF MONOCYTES

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Cardiovascular disease (CVD) remains the leading cause of mortality throughout the world. Atherosclerosis, the buildup of plaque within the arteries, is the main cause of CVD. An early and essential step in the pathogenesis of atherosclerosis is the formation of foam cells, derived from lipid-laden macrophages that become trapped within the tunica intima. Macrophages, through the scavenger receptor CD36, take up a modified form of cholesterol, oxidized low-density lipoprotein (oxLDL), at a rapid rate, which causes them to become lipid-laden and trapped. Chronic inflammation causes endothelial damage and dysfunction, increasing the permeability to circulating LDL, which becomes oxidized within the arteries. Due to the difficulty of studying the macrophages within atherosclerotic plaque, recent research has shifted to the study of their biological precursor: monocytes. A high-fat meal (HFM), an experimental model used to assess postprandial inflammation, was used to assess the role of this HFM-induced inflammation on the likelihood of monocytes to eventually become foam cells. We also included an additional oxLDL *ex vivo* treatment to gain further insight into the potential "priming" effect of a single HFM. While there was a significant increase in

the inflammatory cytokine tumor necrosis factor alpha (TNF-α) in response to the HFM, there were no significant changes in monocyte oxLDL uptake or cell surface marker expression. Future studies may want to examine the inflammatory role that higher concentrations of oxLDL may have or examine other postprandial markers of inflammation in an older or at-risk population.

EFFECTS OF A HIGH-FAT MEAL ON THE INFLAMMATORY PHENOTYPE AND FUNCTION OF MONOCYTES

by

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List of Abbreviations

acLDL	Acetylated low-density lipoprotein
ANOVA	Analysis of Variance
apoE	Apolipoprotein E
BMI	Body Mass Index
CD	Cluster of Differentiation
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FACS	Fluorescent-activated Cell Sorting
FBS	Fetal Bovine Serum
FFA	Free Fatty Acids
HFM	High Fat Meal
HDL	High-density Lipoprotein
ICAM-1	Intercellular Adhesion Molecule 1
IL	Interleukin
IMTG	Intramuscular Triglycerides
kg	Kilogram
LDL	Low-Density Lipoprotein
LPL	Lipoprotein Lipase
LPS	Lipopolysaccharide
MCP-1	Monocyte Chemoattractant Protein 1
MFI	Mean Fluorescent Intensity
mL	Milliliter
NF-kB	Nuclear Factor Kappa B
oxLDL	Oxidized Low-Density Lipoprotein
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate-buffered Saline
PG	Picogram
PPAR	Peroxisome Proliferator-Activated Receptor
SR	Scavenger Receptor
TG	Triglycerides
TGRL	Triglyceride-rich lipoprotein
TLR	Toll-like Receptor
TNF-α	Tumor Necrosis Factor alpha
VCAM-1	Vascular Cell Adhesion Molecule 1

Chapter 1: Introduction

For the last 100 years, cardiovascular disease (CVD) has been the leading cause of mortality in the United States (Sawyer & Flagg, 2000). Though often only associated with more highly developed nations, CVD has rapidly become a global issue and is the leading cause of death worldwide (Libby, 2021). Atherosclerosis, the buildup of plaque within the arteries, is widely considered to be the largest contributor to the development of CVD (Libby, 2021)

While there are many factors that influence the development and progression of atherosclerosis (genetics, diet, activity, environment, etc.), recent attention has been devoted to the role of innate immunity (Frostegård, 2013; Maguire et al., 2019). The link between atherosclerotic plaque and foam cells (the prototypical atherosclerotic cell created by immune cells) has been thoroughly examined (Maguire et al., 2019; Y. M. Park, 2014; Yu et al., 2013), but less is known about the acute, inflammatory processes that ultimately lead to this chronic disease.

A high-fat meal (HFM) experimental model is often used in the study of cardiovascular disease and its surrounding risk factors (Emerson et al., 2017; Herieka & Erridge, 2014). This model is helpful because of the role of nutrition in the development of atherosclerosis and CVD (Torres et al., 2015). Additionally, many humans spend the majority of their time in a postprandial state, making this state a valuable window to understand the cellular responses that occur multiple times a day and develop into lifestyle-related diseases. Thus, a single HFM is a practical way to measure lifestyle choices that can influence the progression of CVD.

Cholesterol is often studied when measuring the postprandial inflammatory response. A modified form of cholesterol, oxidized low-density lipoprotein (oxLDL), is considered one of the most important forms of cholesterol in the development of atherosclerosis (Frostegård, 2013; Zingg et al., 2021). In humans, chronically elevated, circulating levels of oxLDL have been linked to cardiovascular disease risk (Gao & Liu, 2017). Animal models reveal high-fat, Western diets increase circulating oxLDL (Jia et al., 2019). Even a single HFM can significantly raise the circulating levels of oxLDL (Burton-Freeman et al., 2010; Jenkins et al., 2011), though not all studies support this (E. Park et al., 2018). This elevated level of oxLDL is linked to the development of atherosclerosis through several pathways—the most important being the development of macrophage-derived foam cells (Yu et al., 2013).

While there are many animal models examining foam cell formation, the invasive nature of the procedure to collect these cells drastically reduces feasibility of this in human studies. Thus, recent work has examined monocytes as they are an important precursor to macrophage-derived foam cells (Henning et al., 2018). For example, monocytes have the ability to take up oxLDL (Henning et al., 2018), express a proinflammatory phenotype (Khan et al., 2016; McFarlin et al., 2017), alter their surface marker expression (Gower et al., 2011; Henning et al., 2018), and produce cytokines (Oh et al., 2020). However, few studies have examined the connection between a HFM model and an additional *ex vivo* oxLDL treatment in monocytes. Understanding this connection could serve to bridge the gap between acute, postprandial changes and chronic, dietary choices. Therefore, the aim of this

review is to examine the current evidence on the relationship between a HFM, oxLDL uptake in monocytes, and the pathogenesis of atherosclerosis.

Chapter 2: Literature Review

Atherosclerosis and Foam Cell Formation

Atherosclerosis is essentially the build-up of plaque (composed of mainly lipid-laden foam cells) in the arteries, leading to increased risk of high blood pressure, thrombotic events, and other CVDs (Insull, 2009). The role of foam cells in the development and progression of atherosclerosis has previously been well established (Chistiakov et al., 2017; Maguire et al., 2019; Marchio et al., 2019; Yu et al., 2013). The decades-long progression of atherosclerosis can be examined under several different lenses but underlying each of these would include chronic pro-inflammatory state.

Most of the pro-inflammatory events that relate to atherosclerosis occur within the tunica intima—the innermost layer of the arteries. A disturbed, atherogenic vascular wall has increased permeability to LDL (Marchio et al., 2019). As LDL begins to accumulate within the arteries, it is oxidized via myeloperoxidase, hydrogen peroxide, and/or nitric oxide (Guy et al., 2007). The oxLDL is now recognized as a damage-associated molecular pattern and can bind to pattern recognition receptors, such as toll-like receptor 4 (TLR4) (Marchio et al., 2019), which is preferentially upregulated on macrophages and endothelial cells at the site of atherosclerotic plaque (Edfeldt et al., 2002). This binding creates a pro-inflammatory cascade during which chemokines and adhesion molecules (MCP-1, ICAM-1, and others) are upregulated, attracting monocytes that mature into pro-inflammatory macrophages (M1 macrophages) (Marchio et al., 2019).

In a healthy state, macrophages assist in maintaining a cholesterol balance via cholesterol ester formation and the cleaving of cholesterol esters into free fatty acids that can be used for other cellular purposes (Marchio et al., 2019). In an atherosclerotic state, however, there is an upregulation of oxLDL that gets taken up into macrophages via scavenger receptors such as CD36 (Marchio et al., 2019). Once internalized, oxLDL serves as a ligand for peroxisome proliferator-activated receptor (PPAR-γ), a nuclear hormone receptor, which upregulates CD36 expression (Y. M. Park, 2014). The increase in both oxLDL and CD36 leads to an overaccumulation of lipids within the macrophages and a production of pro-inflammatory cytokines and chemokines, as the macrophages can produce cytokines, and oxLDL continues to serve as a ligand for TLR2 and TLR4 (Chávez-Sánchez et al., 2014; Y. M. Park, 2014). These cytokines and chemokines recruit monocytes and other immune cells to the developing atherosclerotic lesion, worsening the problem over time (Y. M. Park, 2014). These macrophages are now considered to be lipid-laden foam cells, and the development of foam cells is considered an early and critical step of atherosclerosis development (Y. M. Park, 2014).

Although there are other oxLDL receptors, CD36 is of particular importance in the uptake of oxLDL and resultant formation of atherosclerotic lesions. A member of the scavenger receptor (SR) class B family, CD36, and another receptor, SR-A, account for 75-90% of oxLDL uptake in macrophages (Yu et al., 2013). CD36 is expressed on monocytes and macrophages, along with other cell types, such as adipocytes and platelets (Y. M. Park, 2014). In animal studies, CD36 has been found to play a significant role in atherosclerotic lesion development in apoE-/- mice fed a

Western diet for 35 weeks (Guy et al., 2007). Additionally, uptake of oxLDL was significantly decreased in macrophages taken from CD36/apoE double-null mice compared with apoE null mice, while uptake of native LDL was not different between the two (Febbraio et al., 2000).

In human studies, monocytes and monocyte-derived macrophages isolated from CD36-deficient individuals had a significantly reduced capacity to bind to oxLDL compared with healthy controls (Nozaki et al., 1995). However, CD36-deficient patients tend to exhibit many of the metabolic disorders associated with metabolic syndrome, including hypertension, dyslipidemia, and hyperglycemia (Masuda et al., 2009). Additional studies have shown that CD36-deficient individuals exhibit elevated triglyceride (TG) and free fatty acids (FFA) at baseline as well as a heightened postprandial response (Masuda et al., 2009).

Higher levels of plasma soluble CD36 are associated with atherosclerosis, markers of insulin resistance, and increased likelihood of fatty liver in a non-diseased population (Handberg et al., 2012). Additionally, in patients with acute coronary syndrome, monocytes express higher levels of CD36 on the cell surface, and an increased density of CD36 expression is correlated with higher circulating levels of oxLDL (Piechota et al., 2012). While the data on the role of CD36 in human health cover a broad spectrum of pathologies, the current data clearly point to a high relevance of CD36 in the development of atherosclerosis. More information is needed to understand the effects of a HFM on CD36 in order to understand potential acute changes that form long-term problems.

Cytokine Responses to a High-Fat Meal

The HFM experimental model is commonly used to assess the postprandial state in which most people spend the majority of their time. However, there is a large amount of variability when considering both the magnitude and timing of the inflammatory, postprandial response (Emerson et al., 2017). This variation can be attributed to a number of different factors including study population, meal content or calories, the inclusion of pre- or post-meal exercise, supplementation, duration of the postprandial assessment, and timing of blood draws, among others (Emerson et al., 2017). In order to present the current HFM literature in a manner relevant to the proposed study, this review will mainly focus on HFM models in a healthy, adult population—unless a study uses a different population with particular significance. All meal types will also be included, though it will be noted if a particular food or nutrient is included that may have impacted the study outcome. Additionally, there are many elements of the postprandial milieu that contribute to an inflammatory state, and the current literature presents a wide range of postprandial data. However, this review will mainly focus on acute postprandial changes in cytokines, oxLDL, and peripheral blood mononuclear cells (PBMCs) that may reflect the eventual development of atherosclerosis.

A 2014 review thoroughly examined the HFM literature to assess correlations among commonly reported variables in the postprandial, inflammatory response (Herieka & Erridge, 2014). That review assigned an inflammatory score to all of the studies calculated by dividing the inflammatory outcome variables that increased by the inflammatory outcomes that were measured (Herieka & Erridge, 2014). They

reported no statistical significance between these inflammatory scores, subject characteristics (age, sex, BMI), or meal/energy content (Herieka & Erridge, 2014). While it can be useful to compare HFM studies that use similar study designs, these data suggest that comparing between various populations and meals may not have a negative impact on the accuracy of comparisons.

Additionally, that review (Herieka & Erridge, 2014) and a later review (Emerson et al., 2017) noticed similar trends in which plasma markers of inflammation seemed irregular and inconsistent. To understand this, they examined markers of leukocyte activation, monocyte protein markers, and peripheral blood mononuclear cell (PBMC) markers, in which postprandial inflammatory changes seem to be more consistent and reliable (Herieka & Erridge, 2014). These variables support the notion of a HFM being an inflammatory stimulus eliciting an immune response (Herieka & Erridge, 2014). While there are inconsistencies regarding which variables change in the postprandial state and the ideal way to measure these variables, it is typically agreed that the pro-inflammatory, postprandial state is characterized by increases in some cytokines.

According to a 2017 review examining HFM studies in healthy adult men and women, 71% of studies report an increase in interleukin-6 (IL-6), a cytokine commonly used to measure postprandial inflammation (Emerson et al., 2017). Of note, there was a moderate inverse correlation between the percent change in IL-6 and the percent of energy from fat in the experimental meal (Emerson et al., 2017). This could help explain some of the variation that can be seen between different meal types. Tumor necrosis factor alpha (TNF-α), another commonly studied and highly

inflammatory cytokine, did not change in 68% of studies (Emerson et al., 2017). IL-1β, an important and often unreported cytokine, was only measured in 3 of the reviewed studies—two of which reported no significant change, while one reported a significant decrease (Emerson et al., 2017). IL-8, a chemokine involved in the recruitment of immune cells, increased in only 1 of 4 studies in which it was measured, while the remaining 3 studies reported no change from baseline (Emerson et al., 2017).

While pro-inflammatory cytokines and chemokines are some of the most common postprandial measurements, anti-inflammatory cytokines are not reported as often. IL-10, a potent anti-inflammatory cytokine, has been measured in several papers with varying results. For example, some studies report no increase from baseline (Altman, 2014; Emerson et al., 2016; Gower et al., 2011; Strohacker et al., 2012) while others report a significant increase (Fogarty et al., 2015; Rocha et al., 2017) or decrease (Teeman et al., 2016a). However, IL-10 remains an interesting cytokine to study to understand the anti-inflammatory, postprandial changes that may work to combat the robust pro-inflammatory changes.

This postprandial change in cytokines is noteworthy as certain cytokines (specifically, IL-6, TNF-α, and IL-1β) released into circulation trigger the release of C-reactive protein (CRP) and other acute phase proteins (Herieka & Erridge, 2014). Chronically elevated CRP is a well-established risk factor for CVD, type 2 diabetes, obesity, and other chronic diseases (Herieka & Erridge, 2014). These pro-inflammatory cytokines also play an important role in the progression of atherosclerosis, and there is abundant pro-inflammatory cytokine production at the

site of atherosclerotic plaque (Poznyak, Bharadwaj, et al., 2021). These cytokines play a role in increasing chemokines and adhesion molecule expression on the endothelium (Ait-Oufella et al., 2011) which creates a positive feedback loop of inflammation at the atherosclerotic lesion.

Triglycerides, Lipopolysaccharide, and the Inflammatory Responses to a High-Fat Meal

It is important to describe the mechanisms by which this postprandial inflammation leads to this increase in cytokines after a HFM in order to understand exactly how a single meal contributes to an elevated disease risk profile. In the context of a HFM, it is thought that TLRs can be activated via triglycerides (TG) and/or lipopolysaccharide (LPS). TLR binding of LPS and/or TG leads to an activation of nuclear-factor kappa B (NF-kB) and the resulting production of cytokines (Mo et al., 2020). Many studies report that consuming a HFM leads to an increase in circulating TG (Emerson et al., 2016; Kackov et al., 2013; Kennedy et al., 2015; Lassenius et al., 2014a; Lyte et al., 2016; Meher et al., 2014; Rosenkranz et al., 2010; Teeman et al., 2016a), which could bind with TLRs and increase circulating cytokine levels. The postprandial changes in LPS, however, are more controversial with some studies reporting an increase (Erridge et al., 2007; Henning et al., 2018; Lassenius et al., 2014b; Lyte et al., 2016) and others reporting no change (Ahn et al., 2017; Bakker et al., 2019; Fuller et al., 2017; Gower et al., 2011; Milan et al., 2017; Mo et al., 2020).

In an effort to clarify the differences in TLR activation, one study (Mo et al., 2020) compared the cytokine production in heparinized whole blood samples after a

HFM with and without LPS inhibitor polymyxin B. They concluded that even if a HFM increases LPS absorption, it may be metabolized too quickly to have a significant effect on postprandial cytokine production as the addition of polymyxin B did not significantly decrease postprandial cytokines (Mo et al., 2020). Additionally, they tested the production of cytokines in an LPS-independent manner and concluded that TGs may be the cause of the cytokine production as non-esterified fatty acids and saturated fat can activate TLR4 (Mo et al., 2020).

However, other research demonstrates that the fat content (i.e., monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, etc.) rather than the amount of fat in a given meal may have an impact on LPS concentration, after a test meal high in saturated fatty acid increased postprandial LPS significantly more than a separate test meal containing higher amounts of polyunsaturated fatty acid (Lyte et al., 2016). Of note, the HFM meals used in this study were drastically lower in percent calories from fat than many other studies (Emerson et al., 2017), and the inclusion of orange juice in the test meal could have influenced the postprandial inflammatory profile (Rocha et al., 2017).

While this study (Mo et al., 2020) and others (Fogarty et al., 2015) may suggest LPS has less to do with the postprandial response than originally thought, there is still a connection between dietary fat consumption and LPS—evidenced by high-fat diets that increase circulating LPS (Pendyala et al., 2012). Additionally, current research reveals a connection between metabolic disorders and LPS (Cani & Delzenne, 2009), as well as a correlation between increased LPS and risk for atherosclerosis (Wiedermann et al., 1999).

The activation of TLRs via LPS and/or TGs is important to understand as this activation is integral to the postprandial cytokine response. While there is clearly an important connection between diet, LPS, and risk factors for CVD, the conflicting evidence surrounding LPS and high-fat meals will not be the focus of the remainder of this literature review. It is discussed to simply mention other variables that play a role in the postprandial cytokine response, though the increase in circulating TGs seems to be a more reliable source of postprandial TLR activation. This review will now focus on the role of dietary fat and its modified forms, particularly the role of oxLDL and the pathogenesis of atherosclerosis.

Cholesterol Responses to a High-Fat Meal

High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) are the two most common forms of cholesterol reported in HFM studies. Elevated levels of LDL-C are associated with increased risk of atherosclerosis and CVD (Luo et al., 2020). HDL-C is colloquially considered the "good" form of cholesterol and is responsible for transporting circulating cholesterol back to the liver in order to maintain an optimal cholesterol balance (Luo et al., 2020). LDL-C is the form in which most circulating cholesterol is found and can be taken up by peripheral cells (Luo et al., 2020).

In the current literature, some studies report total cholesterol changes in response to a HFM while others report changes in HDL-C and/or LDL-C. The literature also does not provide consistent results when it comes to postprandial cholesterol responses. For example, some report an increase in total cholesterol (Bakker et al., 2019; Benson et al., 2018; Demmer et al., 2016; Rosenkranz et al.,

2010), a decrease in total cholesterol, LDL-C, and HDL-C (Gower et al., 2011; Meher et al., 2014; Teeman et al., 2016a), a decrease in just LDL-C and/or HDL-C (Bakker et al., 2019; Demmer et al., 2016; Nestel et al., 2012) no change in HDL (Kennedy et al., 2015; Lassenius et al., 2014b), or no change in any form of cholesterol (Ahn et al., 2017; Strohacker et al., 2012). Likely, these variations are due to the meal compositions, postprandial time window, and inevitable participant variability. However, it is important to mention these changes in cholesterol, particularly in LDL-C, as they impact the availability of LDL to be oxidized, contributing to foam cell formation.

OxLDL is a modified form of cholesterol and is often not reported in studies of responses to a HFM. However, it is important to study oxLDL due to its integral role in the inflammatory process via activation of monocytes and macrophages and eventual formation of foam cells. The oxidation of LDL, the uptake of the oxidized form via scavenger receptors, and the storage of cholesterol within macrophages is intimately connected with the development and progression of atherosclerosis (Poznyak, Nikiforov, et al., 2021) and can be controlled via diet (Fitó et al., 2007; Wang et al., 2020).

There are a few human studies that report circulating oxLDL changes after a single HFM. Some studies report an increase in postprandial oxLDL (Burton-Freeman et al., 2010; Jenkins et al., 2011), though the former study was conducted in hyperlipidemic and overweight men and women and the latter conducted in active men. Another study (Phillips et al., 2013) reported no change in oxLDL post-HFM in lean, obese, or diabetic men. Clearly, there is a current gap in the literature regarding

the effects of a single meal (not to mention the varying effects of different meal compositions) on fluctuations in circulating oxLDL.

Despite the variations in the literature regarding postprandial inflammation, it is generally agreed that a HFM is an experimental model that allows researchers to gain greater insight into the acute, inflammatory effects a single meal can have. Given the frequency with which HFMs are consumed in the typical, Western diet, this postprandial window gives us great insight into the acute changes that create chronic health challenges.

Monocytes as a Precursor to Macrophages/Foam Cells

While macrophages are the cell type responsible for foam cell formation *in vivo*, it is not feasible to obtain tissue-resident macrophages from human atherosclerotic lesions in most scenarios. Thus, some studies have transitioned to using circulating monocytes as they are a precursor to foam cells (Henning et al., 2015, 2018; Henning & McFarlin, 2017).

Specifically, these studies examine changes in monocyte phenotype and function after a HFM in an attempt to understand this connection between HFMs and foam cell formation. After consumption of a HFM, there is an increase in adhesion molecule expression (CD11a+CD18+) in both classical (CD14+CD16-) and nonclassical (CD14+CD16+) monocytes (Henning et al., 2018). Furthermore, this same study examined acetylated LDL (acLDL) uptake and CD36 expression after a HFM and 1hr acLDL treatment and reported that both classical and nonclassical monocytes that were positive for acLDL internalization expressed more CD36 on their surface than monocytes without acLDL internalization (Henning et al., 2018).

While this study does not examine any postprandial changes that may have occurred without acLDL treatment or measure CD36 expression over time, their results demonstrate that a HFM does influence monocyte adhesion markers and acLDL uptake – perhaps making them more likely to adhere to the endothelium and transform into foam cells (Henning et al., 2018).

Further evidence demonstrates that postprandial monocytes increase expression of CD11b and CD11c – which can recognize vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, aiding in monocyte binding (Gower et al., 2011). Also, this research found a correlation between elevated postprandial TGs and monocyte adhesion using a microfluidic flow channel (Gower et al., 2011). Additional studies have noted postprandial increases in CD11a/b/c and CD18 after one HFM (Kračmerová et al., 2014) or two consecutive HFMs (McFarlin et al., 2017).

Previous animal research also demonstrates the important role of monocytes in the development of atherosclerosis. One study fed apoE-/- mice a high-fat Western diet and characterized the development of foamy monocytes – monocytes with confirmed intracellular stores of lipids (Xu et al., 2015). After just 3 days of a Western diet, slightly more than 10% of monocytes were foamy, with this number increasing to 20% after 1 week, and 30% after 3 weeks (Xu et al., 2015). In addition to this shift, the majority of these foamy monocytes were CD11c+CD36+, suggesting that a chronic Western diet in mice may lead to phenotypic changes in monocytes that predispose them to becoming macrophages and ultimately foam cells (Xu et al., 2015). Additionally, animal models using hypercholesteremic mice fed a high-fat diet

have shown the progression of atherosclerosis that correlates with increase in numbers and changes to the phenotype of monocytes (Swirski et al., 2007).

Since this study examined postprandial monocytes and their response to an oxLDL treatment, CD11c+CD36+ cells were quantified to identify monocytes that have an enhanced ability to uptake oxLDL, adhere to the endothelium, and become foam cells. Additionally, non-classical (CD14+CD16+) monocytes were examined to compare differences in postprandial inflammation and oxLDL uptake, as non-classical monocytes are considered to be pro-inflammatory (Mukherjee et al., 2015) and can be impacted by a HFM (Henning et al., 2018).

Interaction between Oxidized LDL and Monocytes

Previous studies have examined the interaction between oxLDL and monocytes *in vitro*. Currently, the literature is inconsistent regarding *ex vivo* oxLDL-induced cytokine production. For example, it has been reported that treatment with oxLDL results in cytokine production from monocytes and macrophages, particularly TNF-α (Chen & Khismatullin, 2015; Jovinge et al., 1996), but other studies note that an overnight or 24hr incubation with oxLDL did not induce IL-6, TNF-α, or IL-1B production (Bekkering et al., 2014; Sieg et al., 2020). However, the experiment conducted by Bekkering et al. in 2014, restimulated the monocytes with TLR ligands 6 days later and reported an increase in IL-6, TNF-α, IL-8, and MCP-1 production, relative to monocytes that had not been previously treated with oxLDL (Bekkering et al., 2014). Native LDL did not have the same results (Bekkering et al., 2014). Additionally, this study reported that monocytes that were treated with oxLDL on day 1, cultured into macrophages, and then treated with oxLDL again on day 7 became

foam cells with a greater frequency than control cells (Bekkering et al., 2014). This suggests that the monocytes were potentially primed with a pro-inflammatory stimulus, which elicited a stronger cytokine response to a second stimulus. Furthermore, this same study noted that in addition to changes in cytokine production, oxLDL treatment can change monocyte phenotypes as monocytes treated with oxLDL upregulated CD36 compared to control monocytes (Bekkering et al., 2014).

In an effort to bridge the gap between human experimental models and cell culture models, this study used monocytes obtained before and after a HFM and expose them to oxLDL. The exposure to both the HFM-induced inflammation *in vivo* and the oxLDL treatment *ex vivo* may elicit stronger cytokine responses than either stimulus on its own.

In support of this, a previously mentioned study that combined both a HFM and an acLDL treatment reported both non-classical and classical monocytes obtained after a HFM increased their uptake of acLDL more than those obtained before the HFM (Henning et al., 2018). Furthermore, the pro-inflammatory monocytes that had internalized acLDL had a greater expression of CD36 than the monocytes that had not (Henning et al., 2018). These researchers concluded that a single HFM has the ability to alter monocyte surface marker expression and subsequent acLDL uptake in the postprandial window (Henning et al., 2018).

The literature clearly demonstrates that HFMs and/or high-fat diets can influence markers of atherosclerosis and that monocytes play a particularly important role in this pathogenesis. Since it is not often feasible to study macrophage-derived foam cells, the literature often shifts to studying their precursor: monocytes. The data

demonstrate that monocytes express CD36, an important scavenger receptor for the uptake of oxLDL – a modified lipid that contributes to inflammation and highly influences the development of foam cells and atherosclerosis. Additionally, the current data reveal that a HFM or diet can alter the function of these monocytes and may predispose them to becoming these macrophage-derived foam cells. However, there is a current gap in the literature connecting the transient changes after a single HFM to the long-term, systemic changes that may occur over time. It is important to understand these changes to accurately assess preclinical markers of atherosclerosis and to further understand the postprandial role of monocytes. Additionally, only one study mentioned above (Henning et al., 2018) attempted to understand the relationship between the postprandial window and acLDL treatment.

Therefore, the overarching aim of this study was to assess the impacts of a single HFM on the ability of monocytes to take up oxLDL, alter cell surface expression of adhesion markers and CD36, and produce inflammatory cytokines. The initial exposure to the HFM-induced inflammation may "prime" monocytes and predispose them to take up more oxLDL *ex vivo*. This is a potentially important mechanism when considering the impact of repeated bouts of inflammation that may occur over a day or a lifetime of postprandial inflammation.

Chapter 3: Aims and Hypotheses

Aim 1: To quantify the difference in oxLDL uptake in monocytes after a high-fat meal with or without oxLDL treatment

Hypothesis 1: After a high-fat meal, more monocytes will take up oxLDL than monocytes obtained prior to a high-fat meal.

Aim 2: To quantify differences in cell surface markers indicating inflammation (CD16), oxLDL uptake (CD36), and adhesion (CD11c) on monocytes after a high-fat meal with or without oxLDL treatment

Hypothesis 2a: oxLDL treatment will increase the number of monocytes expressing CD16, CD36, and CD11c.

Aim 3: To quantify differences in pro-inflammatory (TNF-α) and anti-inflammatory cytokine (IL-10) production from monocytes after a high-fat meal with or without oxLDL treatment

Hypothesis 3: Monocytes exposed to oxLDL treatment after a high-fat meal will release greater amounts of TNF-α and IL-10 than monocytes exposed to a high-fat meal or oxLDL treatment alone.

Chapter 4: Methods

Experimental Approach

This research study was designed to test the response of monocytes to two inflammatory stimuli: a HFM and an oxLDL treatment. Obtaining monocytes after a HFM and then exposing these cells to an *ex vivo* oxLDL treatment allowed us to test the hypothesis that the two inflammatory stimuli would result in greater uptake of oxLDL, increased expression of adhesion and pro-inflammatory markers on the cell surface, and increased secretion of cytokines. Study participants underwent blood draws at baseline (BL), 2 hours post-HFM, and 4 hours post-HFM. PBMCs were isolated immediately after obtaining blood samples at each time point and frozen. After cryopreservation, cells were thawed and split into two experimental groups: HFM or HFM + oxLDL. This allowed us to test the above responses in monocytes only exposed to a HFM versus monocytes exposed to both a HFM and an oxLDL treatment.

Subjects and Screening

This study was approved by the Institutional Review Board at the University of Maryland, College Park. All study procedures and potential risks were explained to the participant in detail and all questions were answered prior to obtaining written informed consent. Participants were between the ages of 18-35 years old, had a calculated body mass index (BMI) less than 30 kg/m^2, had a resting blood pressure lower than 140/90 mmHg, had no current or previous heart, lung or metabolic disease or diabetes, were not currently pregnant, had regular menstrual cycles

(females), had not been sick in the past two weeks, had not been users of tobacco or nicotine products, and were not currently taking any prescription medications (other than oral contraceptive pills where participants were tested during their placebo phase). To recruit participants, flyers were placed throughout the University of Maryland campus and brief presentations were given at undergraduate courses. The participants included in this study were chosen because they had full sets of PBMCs from each time point to be used for analysis.

High-fat Meal Preparation and Administration

During the first visit to the lab, participants underwent a VO_2peak exercise test on a cycle ergometer (Parvo Medics, Salt Lake City) to establish cardiovascular fitness level in order to consider the impact of fitness on our results. Following 5-10 minutes of a warm-up, participants began cycling at an intensity of 50 Watts at 75 revolutions per minute (rpm) or more. The intensity was increased by 25 Watts every 2 minutes until the participate could not maintain the cadence. Heart rate was continuously monitored via an exercise strap, and oxygen consumption was monitored with the use of a face mask and metabolic cart. In addition, basic anthropometric measurements (height, weight) were taken. The second visit occurred at least 24 hours later. Participants were instructed to arrive after an overnight fast and to avoid exercise 24 hours before the visit. During this visit, participants underwent a baseline blood draw, a HFM, and subsequent blood draws (at 2 and 4 hours postprandial). After the baseline blood draw, the participants were instructed to consume the HFM within 10 minutes. The meal consisted of heavy whipping cream, chocolate syrup, and non-fat powdered milk normalized to the subject's body surface

area. Calorically, the meal was 85.3% fat, 11.3% carbohydrates, and 3.4% protein, with a 386g serving containing 1362 kcal. The meal size was normalized to the participant's body surface area (386g per $2m^2$ body surface area). This meal has previously been used by studies in our laboratory (Jenkins et al., 2011).

Blood Collection and PBMC Isolation

Blood was collected via venipuncture at each time point (baseline, 2 hours postprandial, and 4 hours postprandial) in a 10 mL ethylenediaminetetraacetic acid (EDTA) tube. EDTA tubes were centrifuged at 1500xg for 15 minutes. Plasma was then aliquoted and stored at -80°C for future analysis. The remaining blood was then diluted 1:1 in 1x phosphate buffer solution (PBS). This mixture was layered onto 3-5mL of Lymphocyte Separation Medium (Corning, Corning), before being spun at 400xg for 20 minutes at 4°C. Then the buffy coat was collected and mixed with 2mL 1x PBS before being spun at 500xg for 5 minutes at 4°C. After aspirating the supernatant, the cells were incubated with 2mL 1x RBC lysis buffer (Biolegend, San Diego) for 10 minutes. After spinning at 500xg for 5 minutes at 4°C, the cells were washed and spun twice more with 2mL 1x PBS. They were counted and stored in 1.5mL aliquots containing 900uL of cell solution that includes PBS with 2% Fetal Bovine System (FBS) (Lifeline Cell Technology, Frederick), and 100uL of DMSO (Fisher Chemical, Pittsburgh) at -80°C for future analysis.

OxLDL Uptake and Cell Surface Marker Expression: Aims 1 and 2

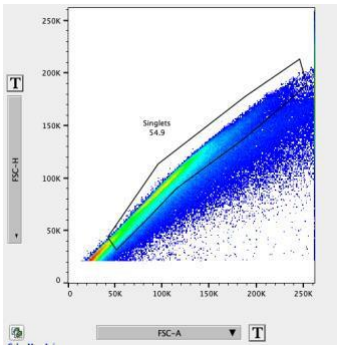
In order to address Aims 1 and 2, PBMCs were thawed and 2.0×10^6 cells were used per time point per assay. After thawing, cell viability was confirmed via

Trypan blue (Thermo Fisher Scientific, Waltham) for all assays. PBMCs from each time point were divided into two conditions: HFM and HFM+oxLDL.

For the HFM condition, each time point (BL, 2 hours post-HFM, 4 hours post-HFM) was stained with the following fluorescent antibodies: anti-CD45 (APC Cy7) (Biolegend, San Diego), anti-CD14 (APC) (Biolegend, San Diego), anti-CD11c (PE Cy7) (Biolegend, San Diego), anti-CD16 (PE Dazzle 594) (Biolegend, San Diego), and anti-CD36 (PerCP eFluor 710) (Biolegend, San Diego). A viability dye (DAPI) (Thermo Fisher Scientific, Waltham) was also included in the antibody cocktail to verify the presence of live or dead cells. The antibody titration and staining protocols have been previously optimized by our lab and others (Henning et al., 2018). Cells were fixed with a solution of PBS and 2% paraformaldehyde (PFA) before analysis in a flow cytometer.

For the HFM+oxLDL condition, each time point (BL, 2 hours post-HFM, 4 hours post-HFM) was treated with 1ug/mL oxLDL, as this was determined to be the optimal dosage to observe differences in oxLDL uptake while minimizing apoptosis during our optimizations. Cells were then incubated at 37°C at 5% CO_2 for 1 hour. This incubation duration was optimized by our lab to allow for sufficient uptake while minimizing apoptosis. After incubation, the excess oxLDL was washed off by spinning the cells at 500xg for 5 minutes and aspirating the supernatant. Then, the cells were stained and fixed exactly as described for the HFM condition. PBMCs were analyzed via a BD FACS II Canto Flow Cytometer at 250,000 events/sample.

A


Singlets
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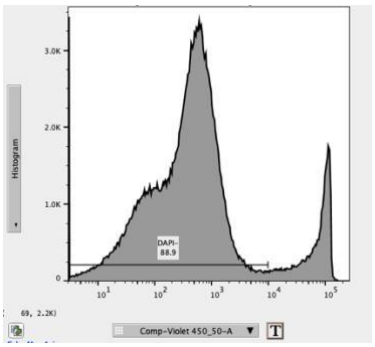
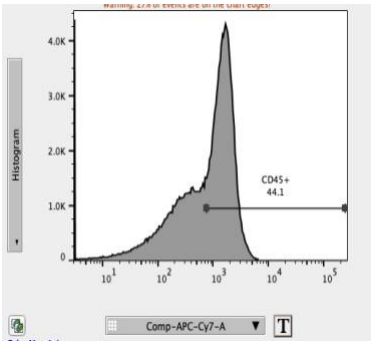
FSC-H
FSC-A

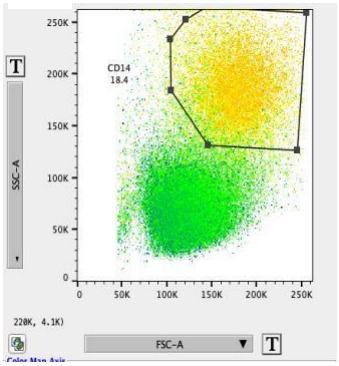
B


Histogram
DAPI-
88.9
Comp-Violet 450_50-A

C


Histogram
CD45+
44.1
Comp-APC-Cy7-A

D


CD14
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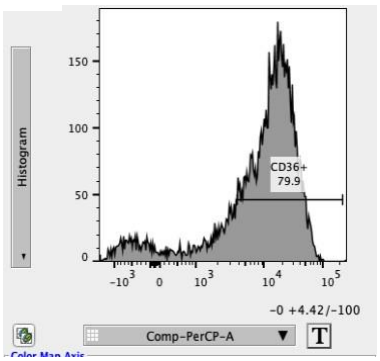
SSC-A
FSC-A

E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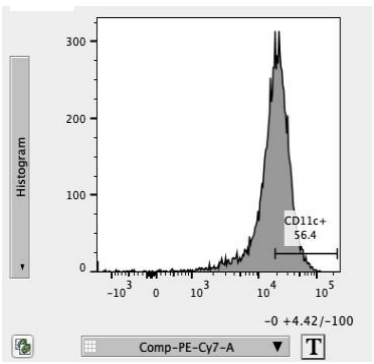
CD36+
79.9
-0 +4.42/-100
Comp-PerCP-A

F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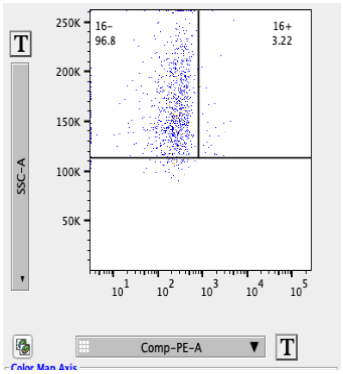
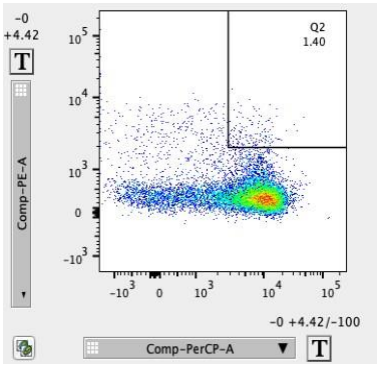
CD11c+
56.4
-0 +4.42/-100
Comp-PE-Cy7-A

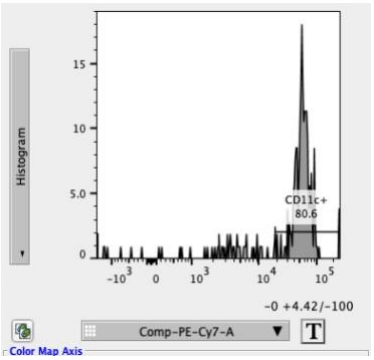
G


16-
96.8
16+
3.22
SSC-A
Comp-PE-A

H


-0
+4.42
Q2
1.40
Comp-PE-A
-0 +4.42/-100
Comp-PerCP-A

I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CD11c+
80.6
-0 +4.42/-100
Comp-PE-Cy7-A
Color Map Axis

J

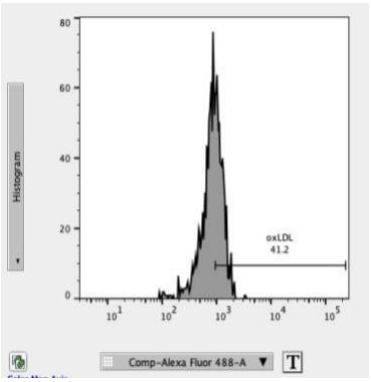
Histogram
oxLDL
41.2
Comp-Alexa Fluor 488-A

Figure 1: Gating strategy used for all flow analysis. PBMCs were analyzed via a BD FACS II Canto Flow Cytometer at 250,000 events/sample. (A) Singlet gate obtained via FSC-A and FSC-H. (B) Cells were then gated as DAPI- cells to ensure a population of only live cells. (C) Histogram gate used to quantify CD45+ cells, a pan-leukocyte marker. (D) CD14+ monocytes were gated based on size and color. (E) Histogram gate was used to quantify CD14+/CD36+ monocytes. (F) Histogram gate used to quantify CD14+/CD11c+ monocytes. (G) CD14+ monocytes were split into CD16- and CD16+ cells. (H) The 16+ monocytes from Figure G were gated based on CD36+ marker. (I) Of the cell population obtained in Figure H, they were further quantified by the CD11c+ histogram. (J) A representative histogram gate for oxLDL+ monocytes. Abbreviations: peripheral blood mononuclear cells (PBMCs), forward side scatter area/height (FSC-A/H), oxidized low-density lipoprotein (oxLDL).

Production of Cytokines: Aim 3

In order to address Aim 3, PBMCs were thawed for each condition: HFM and HFM+oxLDL. For the HFM condition, 600,000 cells/well were plated in duplicate in a 24-well plate with 600uL Roswell Park Memorial Institute (RPMI) 1640 medium (Thermo Fisher Scientific, Waltham) supplemented with 2% FBS. For the HFM+oxLDL condition, cells were plated the same way, but with the addition of 5ug/mL oxLDL for 24 hours. This dosage was optimized by our lab to maximize cell viability after 24 hours. These cells were incubated at 37°C and 5% CO_2 for 24 hours. After 24 hours, conditioned media was collected, centrifuged at 2500xg for 20 minutes, and the supernatant was frozen at -80°C for at least 24 hours before being analyzed via an enzyme-linked immunosorbent assay (ELISA) for TNF-a and IL-10 (Thermo Fisher Scientific, Waltham).

Statistical Analysis

All analyses were performed using GraphPad Prism. A 2x3 repeated measures analysis of variances (ANOVA) was used for each aim with condition (HFM or HFM+oxLDL) and HFM timepoint (BL, 2 hours postprandial, 4 hours postprandial)

as separate factors. A one-way ANOVA was used for the oxLDL uptake outcome in the HFM + oxLDL condition. Post hoc t-tests were conducted when the ANOVA provided statistically significant results with an accepted p-value of $p<0.05$. Essentially, most of the following data are presented as the percent of CD14+ monocytes. All data are presented as means ± SEM unless otherwise specified.

Chapter 5: Results

Subject Characteristics

Participant demographic information is shown in Table 1.

Table 1: Subject Demographics

Age (years)	24.1 ± 3.9
Sex (male/female)	9/5
BMI (kg/m^2)	22.3 ± 2.8
VO_2 peak (mL/kg/min)	44.4 ± 2.6

n = 14
Data are expressed as means ± SD.

CD14+ monocytes and oxLDL uptake

We first assessed the absolute values of CD14+ monocytes in each sample, revealing no significant interaction or main effect of the HFM or oxLDL treatment (Figure 2A, p=0.18-0.84). We next analyzed the percentage of CD14+ cells that co-expressed CD16, CD36, or CD11c in both the HFM condition and the HFM + oxLDL condition, finding no effect of the HFM or oxLDL treatment on the numbers of these cells. There were no interaction or main effects of the HFM or oxLDL treatment on the number of CD14+ cells that co-expressed CD36 (Figure 2B, p=0.13-0.36). The same was true for the number of CD14+ cells that co-expressed CD16 (Figure 2C, p=0.28-0.89) or CD11c (Figure 2D, p=0.28-0.97).

Figure 2: CD14+ monocytes co-expressing CD36, CD16, or CD11 after a HFM, with or without oxLDL treatment. PBMCs from baseline, 2hrs post-HFM, and 4hrs post-HFM were split into two groups (HFM and HFM + oxLDL). The HFM + oxLDL group was incubated with 1ug/mL of fluorescently-tagged oxLDL for 1hr. Both groups were stained with fluorescent antibodies, and the sample were run through a BD FACS II Canto Flow Cytometer and analyzed via FlowJo. Absolute CD14+ cell count is depicted in Figure A, revealing no differences after the HFM or oxLDL treatment. The percentages of CD14+ monocytes that co-express CD36+ (B), CD16 (C), and CD11c (D) follow. A 2x3 ANOVA revealed no significant interactions or main effects of time or condition in any group. Data are presented as means ± SEM. Abbreviations: fluorescence activated cell sorting (FACS), high fat meal (HFM), oxidized low-density lipoprotein (oxLDL).

Then, we assessed the changes in oxLDL uptake in these same populations after a 1-hour incubation (Figure 3). There were no significant differences before or after the HFM (Figure 3A, $p=0.42$) in the number of CD14+ monocytes positive for oxLDL. We then examined changes in the number of oxLDL+ cells in the subsets of CD14+ cell co-expressing CD36, CD16, or CD11c, revealing no significant

differences in CD14+/CD36+/oxLDL+ monocytes (Figure 3B, p=0.38), CD14+/CD16+/oxLDL+ monocytes (Figure 3C, p=0.35), or CD14+/CD11c+/oxLDL+ monocytes (Figure 3D, p=0.50).

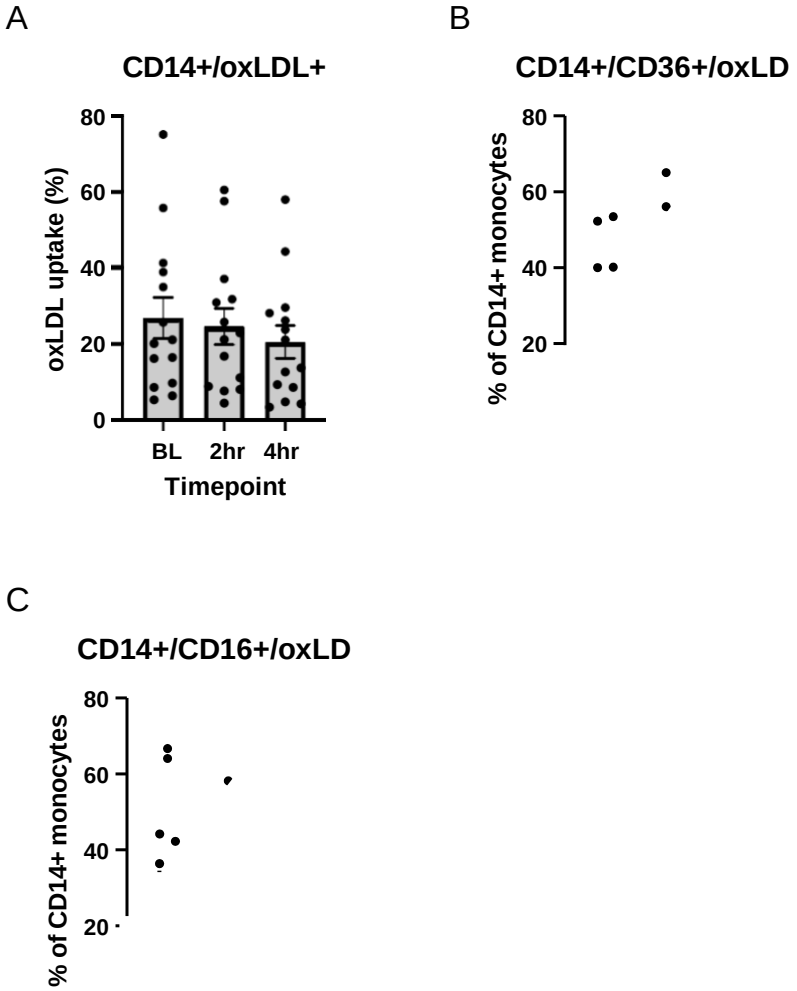

Figure 3: oxLDL uptake in subsets of CD 14+ monocytes after a HFM. Cells in the HFM + oxLDL condition were treated with 1ug/mL of fluorescently-tagged oxLDL for 1 hour. To examine potential changes in oxLDL uptake in various cell types, cells were stained and analyzed accordingly. An ANOVA revealed no significant differences in oxLDL uptake in CD14+ monocytes (A) or CD14+ monocytes that expressed CD36 (B), CD16 (C), or CD11c (D). All data are presented as means ± SEM. Abbreviations: high fat meal (HFM), oxidized low-density lipoprotein (oxLDL).

CD14+/CD16+ monocytes and oxLDL uptake

To analyze potential inflammatory changes in cell phenotypes after a HFM and oxLDL incubation, we further assessed the numbers of CD14+/CD16+ monocytes co-expressing CD36 or CD11c (Figure 4). As there were no differences in the absolute numbers of CD14+/CD16+ cells among conditions (Figure 4A, p=0.31-0.99), we quantified the percentage of CD14+/CD16+ cells that co-expressed CD36 or CD11c. There was no effect of the HFM or the oxLDL treatment on CD14+/CD16+/CD36+ monocytes (Figure 4B, p=0.76-0.95). Then, we quantified CD16+ monocytes that also co-expressed CD11c, but there were no significant differences between the two experimental groups (Figure 4C, p=0.22-0.71). Lastly, we wanted to assess the cells that expressed all three of these markers, starting with the CD14+ monocytes that were previously determined to be CD16+/CD36+ and then isolating the percentage of those cells that were also CD11c+. There were no significant interaction or main effects of the HFM or oxLDL treatment on the population of CD14+/CD16+/CD36+/CD11c+ monocytes (Figure 4D, p=0.37-0.65).

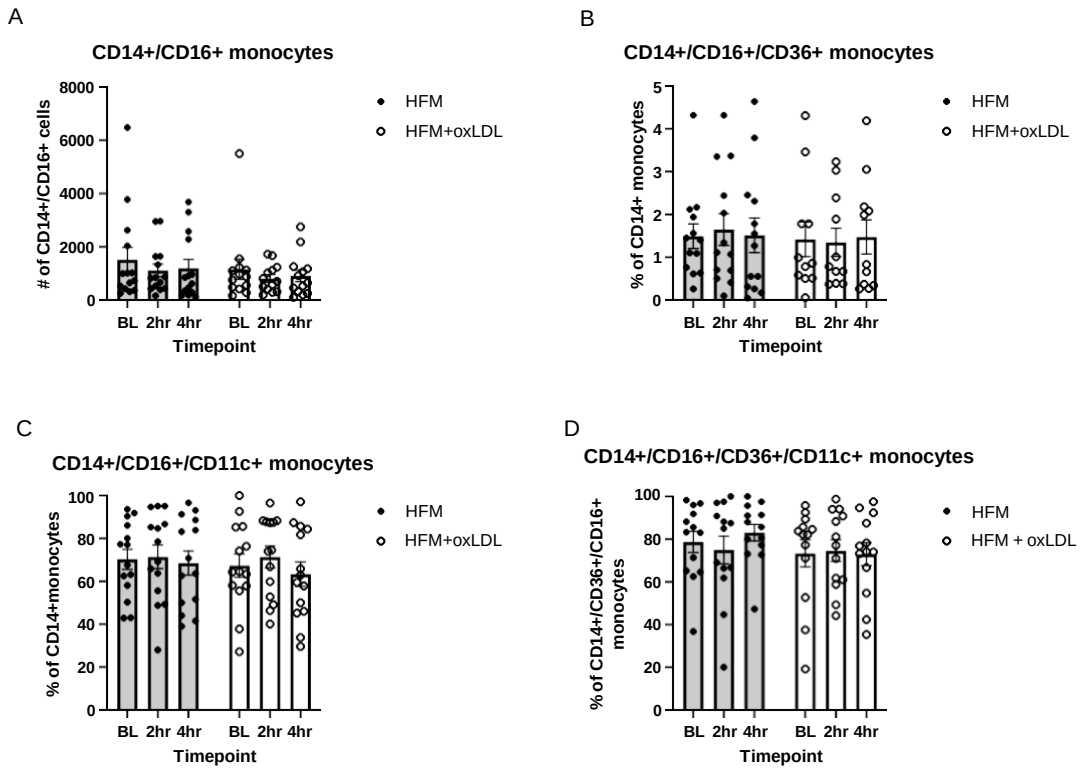

Figure 4: CD14+/CD16+ monocytes co-expressing CD36 and/or CD11 in the HFM and HFM + oxLDL conditions. PBMCs from BL, 2hrs post-HFM, and 4hrs post-HFM were split into two groups (HFM and HFM + oxLDL). The HFM + oxLDL group was incubated with 1ug/mL of fluorescently-tagged oxLDL for 1hr. Both groups were stained with fluorescent antibodies, and the sample were run through a BD FACS II Canto Flow Cytometer and analyzed via FlowJo. These cell populations are all double-positive for CD14+/CD16+, and there was no difference in CD14+/CD16+ monocytes in either condition (A). (B) and (C) represent the percentage of CD14+/CD16+ that co-express CD36 and CD11c, respectively. (D) depicts the CD14+/CD16+/CD36+, that also co-express CD11c. A 2x3 ANOVA revealed no significant interaction effects or main effects of time or condition. All data are presented as means ± SEM. Abbreviations: Abbreviations: fluorescence activated cell sorting (FACS), high fat meal (HFM), oxidized low-density lipoprotein (oxLDL).

We also quantified the oxLDL uptake in these cell phenotypes. As shown above in Figure 3C, there were no significant differences in oxLDL uptake for CD14+/CD16+ monocytes. Additionally, there were no significant differences in oxLDL uptake in CD14+/CD16+/CD36+monocytes, (Figure 5B, p=0.32),

CD14+/CD16+/11c+ monocytes (Figure 5C, p=0.62), or

CD14+/CD16+/CD36+/CD11c+ monocytes (Figure 5D, p=0.34).

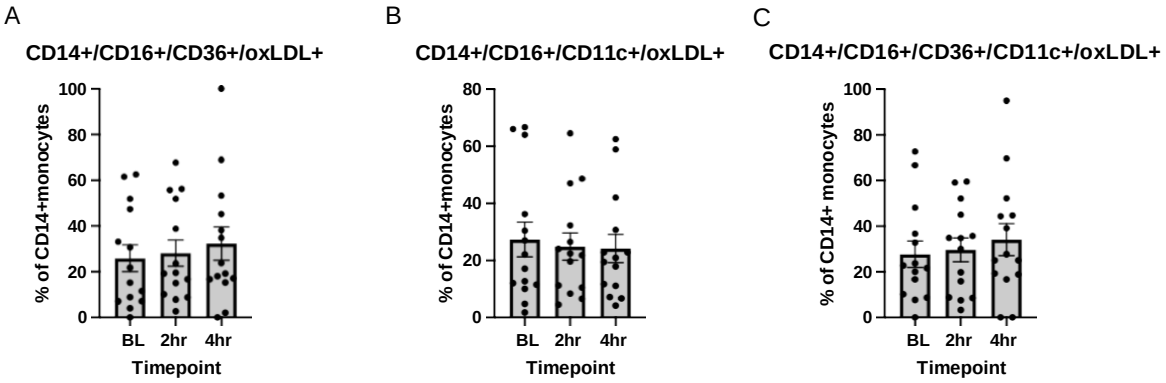

Figure 5: oxLDL uptake in subsets of CD14+/CD16+ monocytes after a HFM. Cells in the HFM + oxLDL condition were treated with 1ug/mL of fluorescently-tagged oxLDL for 1 hour. To examine potential changes in oxLDL uptake in various cell types, cells were stained and analyzed accordingly. A 1x3 ANOVA revealed no significant differences in oxLDL uptake in CD14+/CD16+/CD36+ monocytes (A), CD14+/CD16+/CD11c+ monocytes (B), or CD14+/CD16+/CD36+/CD11c+ monocytes (C). All data are presented as means ± SEM. Abbreviations: high fat meal (HFM), oxidized low-density lipoprotein (oxLDL).

Production of Cytokines

TNF-α

We measured TNF-α production in conditioned media after a HFM and an oxLDL incubation and found that the HFM caused an increase in TNF-α secretion from PBMCs (Figure 6, p=0.02).

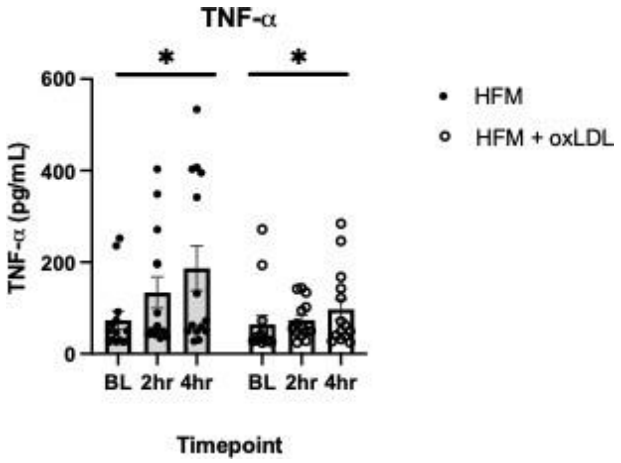

Figure 6: TNF-α production (pg/mL) in 24-hour conditioned media. PBMCs were thawed and plated in a 24-well plate with 600,000 cells in 600uL FBS-supplemented RPMI. The samples in the HFM + oxLDL condition were treated with 5ug/mL of oxLDL. Conditioned media was collected 24 hours later and analyzed via an ELISA. *A 2x3 ANOVA revealed a main effect of the HFM to increase TNF-α secretion (p=0.01). All data are presented as means ± SEM. Abbreviations: enzyme-linked immunosorbent assay (ELISA), fetal bovine serum (FBS), oxidized low-density lipoprotein (oxLDL), peripheral blood mononuclear cells (PBMCs), tumor necrosis factor alpha (TNF-α).

TNF-α and VO_2

In order to examine the potential influence of fitness status on inflammatory responses post-HFM, we assessed the relationship between 4-hour post TNF-α and VO_2 peak values, revealing a moderate, inverse relationship between the two variables ($r = -0.55$, $p=0.04$). Additionally, the change in TNF-α values from the BL timepoint to the 4hr timepoint also significantly correlated with VO_2 max values (Figure 6A: $r = -0.55$, $p=0.04$), indicating that participants with higher fitness levels had a less robust inflammatory response to the HFM. There was no relationship between the change in TNF-α values and BMI (Figure 6B).

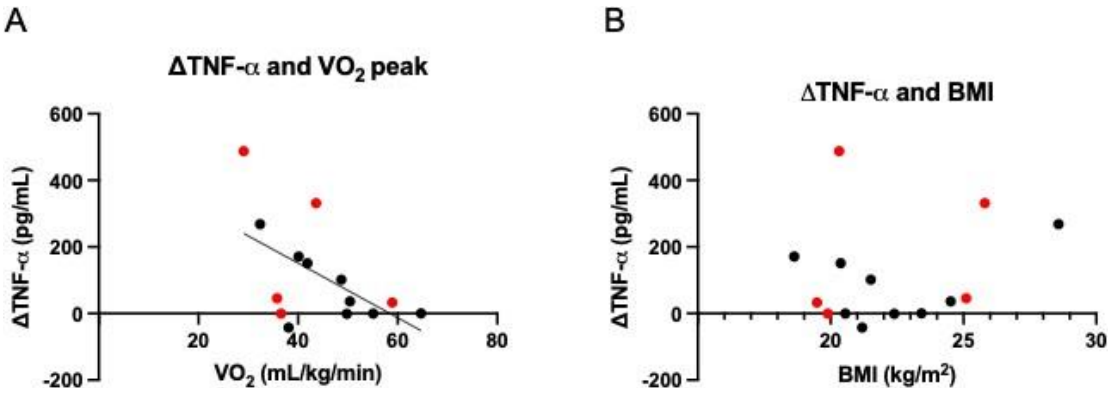

Figure 7: Relationship between TNF-α production (pg/mL) in conditioned media, relative VO_{2peak} (mL/kg/min), and BMI (kg/m^2). Figure A depicts that the overall change in TNF-α from BL to 4 hours post was significantly correlated with relative VO_2 values (r=-0.55, p=0.04) There was no relationship between overall change in TNF-α and BMI (B). Red circles indicate females and black circles indicate males. Abbreviations: minute (min), milliliter (mL), kilogram (kg), picogram (pg), tumor necrosis factor alpha (TNF-α), volume of oxygen (VO_2).

IL-10

We also measured IL-10 concentrations in conditioned media after the HFM and oxLDL incubation, revealing no significant interaction or main effect of the HFM or oxLDL treatment (Figure 8: p=0.08-0.8).

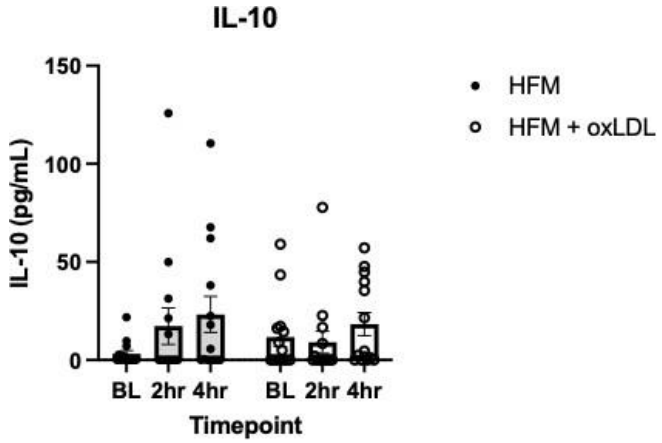

Figure 8: IL-10 production (pg/mL) in 24-hour conditioned media. PBMCs were thawed and plated in a 24-well plate with 600,000 cells in 600uL FBS-supplemented RPMI. The samples in the HFM + oxLDL condition were treated with 5ug/mL of oxLDL. Conditioned media was collected 24 hours later and analyzed via an ELISA. A 2x3 ANOVA revealed no significant interaction or main effect of time or condition. All data are presented as means ± SEM. Abbreviations: enzyme-linked immunosorbent assay (ELISA), fetal bovine serum (FBS), interlekin-10 (IL-10), milliliter (mL), oxidized low-density lipoprotein (oxLDL), peripheral blood mononuclear cells (PBMCs), picogram (pg).

Chapter 6: Discussion

This study assessed the effects of two inflammatory stimuli on the phenotype and function of circulating monocytes. The main findings of this study are: (1) monocytic oxLDL uptake did not change within 4 hours after a HFM; (2) expression of cell surface markers on monocytes indicating enhanced capacity for oxLDL uptake, inflammation, and endothelial adhesion did not change within 4 hours after a HFM or exposure to a oxLDL treatment; (3) PBMC production of TNF-α increased after a HFM with or without oxLDL treatment, while production of IL-10 remained unchanged after both conditions. Thus, contrary to many of our initial hypotheses, the HFM may not be a sufficient inflammatory stimulus to prime monocytes in young, healthy adults, within the assessed time course.

OxLDL uptake in monocytes

Previous literature has demonstrated an enhanced monocyte responsiveness to modified LDL after a HFM (Henning et al., 2018). This study, however, used acLDL instead of oxLDL, which we believe does not accurately reflect the postprandial changes that most directly correlate with foam cell formation. Despite our preliminary experiments that suggested oxLDL uptake would increase in CD14+ monocytes after a HFM, our study data did not confirm these findings.

Furthermore, we used a 1ug/mL dose of oxLDL for the incubation, which is a lower concentration than the doses (5ug/mL-15ug/mL) used in other studies of changes in monocyte responsiveness to oxLDL (Bekkering et al., 2014; Henning et al., 2018). We tested many concentrations during our optimizations, and this

concentration appeared to elicit a range of oxLDL uptake in monocytes from different individuals and minimize apoptosis, as opposed to a higher concentration of oxLDL which may have overloaded the monocytes with oxLDL and caused an inflated response in all monocytes. However, using the 1ug/mL dose may have limited our ability to compare our data with previous studies or observe significant changes in oxLDL uptake. There were no differences in oxLDL uptake among the baseline, 2-hour and 4-hour timepoints in any of the cell types we examined, even when analyzed using mean fluorescence intensity (MFI), which would indicate not only that a cell was positive for oxLDL uptake, but if that cell had taken up more or less oxLDL.

Additionally, previous studies suggest that oxLDL tends to upregulate the expression of CD36 (Bekkering et al., 2014; Y. M. Park, 2014). In our study, there were no significant differences in the number of monocytes expressing CD36 after the oxLDL incubation. There are several possible explanations for this finding. First, the concentration of oxLDL may not have been high enough to induce CD36 expression. However, our results showed the vast majority of the CD14+ monocytes co-expressed CD36+ at baseline, so we find it is more likely that there was a limited capacity to increase (a) the number of CD14+/CD36+ monocytes and/or (b) the number of CD36 receptors on CD14+ monocytes.

The timing of sample collection in this study may also have impacted the ability to observe differences in oxLDL uptake. Previous literature indicates that treating monocytes with even low doses of TNF-α can increase oxLDL uptake and upregulate CD36 and adhesion marker expression on the cell surface (Zhu et al., 2015). Ultimately, that study concluded that TNF-α plays an important role in

enhancing the inflammatory capacity of monocytes and increasing their likelihood to become foam cells (Zhu et al., 2015). This conclusion leads us to believe that if the postprandial measures in our study exceeded the 4-hour timepoint, the significant increase in TNF-α at 4 hours may have enhanced monocytic capacity to take up oxLDL and may have altered the expression of the cell surface markers we examined at a later timepoint.

Cell Surface Makers on Monocytes

For our analysis, we used both a size gate and CD14+ gate to ensure we were analyzing only CD14+ monocytes. The primary goal of Aim 2 was to quantify the percentage of monocytes that appeared to have the greatest propensity to become foam cells, indicated by co-expression of CD16 (a marker of non-classical monocytes), CD36 (the main receptor involved in oxLDL uptake) and CD11c (a marker of endothelial adhesion capacity).

We examined all combinations of these cell surface markers but found no changes in the proportions of the various cell types in response to the HFM or exposure to oxLDL. This is consistent with a previous study that reported no significant changes in the number of CD16+ monocytes post HFM (Henning et al., 2018). However, that same study did report an increase in oxLDL uptake in CD14+/CD16+/CD36+ monocytes compared with monocytes lacking the CD16 marker (Henning et al., 2018). Furthermore, additional studies have noted postprandial changes in CD11c corresponding with the uptake of triglyceride-rich lipoprotein (Gower et al., 2011), but our data did not indicate significant changes in CD11c+ monocytes with the HFM or oxLDL treatment. In general, most of the

monocytes were CD14+/CD16- (classical monocytes), and highly expressed CD36, which is standard for monocyte phenotypes (Boyette et al., 2017; Moniuszko et al., 2006). Given that there were no significant changes in these cell phenotypes after the HFM or oxLDL treatment, it does not appear that either stimulus elicited a robust inflammatory response in this young and healthy population.

We also quantified the percentage of CD14+ monocytes that co-expressed CD16, CD36, and CD11c together, with the notion that these monocytes are the most likely to take up oxLDL, adhere to the endothelium, and transform into foam cells. However, there were no differences in the numbers of CD14+ monocytes co-expressing CD16, CD36, and CD11c after the HFM or oxLDL incubation. Importantly, though the percentage of these cells shown in Figure 4D is very high, this number requires careful interpretation. First, these cells were isolated as CD14+ monocytes that were double-positive for CD16 and CD36 (Figure 4B). These percentages were quite low, with values ranging from <1-5% of all CD14+ monocytes. From there, we quantified the percentage of those double-positive CD14+ monocytes that also co-expressed CD11c. So while the percentage of CD14+/CD16+/CD36+/CD11c+ seems high, it is a percentage of a very small proportion of total CD14+ cells. Given that these samples were obtained from a young, healthy population, it is expected that these values would be low.

The lack of changes to cell surface marker expression could be attributed to a number of factors. As previously mentioned, the 1ug/mL concentration of oxLDL or the 1-hour incubation time may not have been adequate to induce these changes. However, the changes in cell surface markers post-HFM remain inconsistent

throughout the literature, so our results are not completely surprising. Furthermore, it is important to note that we conducted our assays on frozen cells that had been thawed, thus we cannot rule out the possibility that the freezing and thawing of the PBMCs may have impacted the expression of some cell surface markers and potentially reduced their functional capacity (Li et al., 2022). However, prior to data collection, our optimizations involved testing the differences in fresh and frozen cells, and we did not note any significant differences.

Pro- and anti-inflammatory cytokine production

After a HFM, the increase in circulating TGs can lead to an increase in cytokine production via the binding of TGs to TLRs and the subsequent activation of NF-kB (Mo et al., 2020). OxLDL uptake can also lead to cytokine production via NF-kB activation (Chen & Khismatullin, 2015; Janabi et al., 2000). These mechanisms likely explain the increase in TNF-α production in the conditioned media. This increase is consistent with some previous literature in which circulating TNF-α levels increase after a HFM (Demmer et al., 2016; Fogarty et al., 2015; Gower et al., 2011). It is surprising, then, that the oxLDL did not pose as an additional inflammatory stimulus and further increase TNF-α production in our experiments. Once again, this may be related to using a lower oxLDL concentration as previous studies demonstrated a much more robust TNF-α response after incubating PBMCs with >5ug/mL of oxLDL (Bekkering et al., 2014; Jovinge et al., 1996).

We also measured the production of IL-10 in order to understand any potential compensatory, anti-inflammatory effects. There were no differences in IL-10 production after the HFM or after the oxLDL treatment. This is not particularly

surprising, however, as it has previously been noted that the postprandial changes in IL-10 are inconsistent with some studies reporting no change (Gower et al., 2011; Strohacker et al., 2012) and others reporting an increase (Fogarty et al., 2015; Rocha et al., 2017).

Further analysis of the TNF-α data revealed a statistically significant correlation with VO_{2peak} values. Specifically, both the 4-hour post-HFM TNF-α value and the overall change in TNF-α from BL to 4 hours post-HFM revealed a moderate, inverse correlation between the two variables. These data indicate that an individual with a higher VO_2 peak may exhibit a blunted TNF-α response after the HFM.

When comparing the VO_2 values for male and female participants, the average male VO_2 was 46.8 mL/kg/min and the average female VO_2 was 40.2 mL/kg/min. These values are considered "good" for males and "fair-good" for females (ACSM, 2013). The notion that someone who is more fit would be better able to mitigate an inflammatory stimulus is supported by previous literature in which individuals who are classified as "high fit" have a blunted postprandial inflammatory response, likely due to elevated HDL and lipoprotein lipase (LPL) activity, and more efficient replenishment of intramuscular triglycerides (IMTG) (Teeman et al., 2016b). However, previous literature has also demonstrated that this protective effect of exercise training may be negated if more than 60 hours have passed since a subject's last training session (Herd et al., 2000). Participants were instructed to avoid exercise 24 hours prior to the HFM in this study, but we did not control for exercise beyond that time. Though our participants are not considered "high fit," it is likely that their fair-good fitness status impacted their postprandial responses. Furthermore, the young

age range and otherwise healthy status of the participants likely limited postprandial responses as older individuals tend to have a more exaggerated and prolonged postprandial response (Milan et al., 2016).

Limitations

As already mentioned in the discussion, the use of a lower oxLDL concentration may have had a limited inflammatory impact. Future studies in our lab and others could optimize the dosage and perhaps go further to understand how that dose is relevant to physiological levels. Additionally, the majority of the individuals who participated in the study were moderately fit, young males and females. This population will have a more robust ability to counteract inflammation. Furthermore, they are at little risk of developing atherosclerosis, so the practicality of studying these changes in young, healthy individuals is less than studying these changes in older or at-risk populations. Lastly, we did not use a dietary recall or any form of dietary normalization to account for habitual dietary choices that may have impacted some of the outcomes.

Future Directions

Moving forward, conducting this same study in older individuals or individuals exhibiting early signs of atherosclerosis and CVD may prove to be more useful. Additionally, running an oxLDL ELISA on the plasma from these participants would provide useful information on the *in vivo* conditions the monocytes were exposed to. Previous literature has indicated a postprandial increase in circulating oxLDL (Burton-Freeman et al., 2010; Jenkins et al., 2011), which may correspond to

the increase in TNF-α shown in our data and provide insight into the relationship between TNF-α and oxLDL in monocytes. Lastly, the role of a single meal is limited without considering the overall diet. Conducting longer-term studies that examine changes in the monocyte population over time while exposed to sequential meals or various diets could prove to be insightful and more significant given the amount of time it takes to develop atherosclerosis and resultant CVD.

Conclusions

In conclusion, this study demonstrated that in a young, healthy, moderately-fit population, there were no significant changes to monocyte phenotype or function after a single HFM or additional oxLDL treatment. However, we did demonstrate that the HFM was an inflammatory stimulus due to the increase in TNF-α post-HFM. This study is unique in that we have attempted to understand the role of a single HFM and oxLDL treatment in an effort to quantify the preliminary changes that occur in monocytes in response to inflammation; these changes may reflect the phenotype and function of monocyte-derived macrophages and the eventual development of atherosclerosis. Continuing this line of study will provide important information to the field of kinesiology as we seek to understand lifestyle choices that inform disease pathology.

Bibliography

Ahn, C. H., Kim, E. K., Min, S. H., Oh, T. J., & Cho, Y. M. (2017). Effects of gemigliptin, a dipeptidyl peptidase-4 inhibitor, on lipid metabolism and endotoxemia after a high-fat meal in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, *19*(3), 457–462. https://doi.org/10.1111/dom.12831

Ait-Oufella, H., Taleb, S., Mallat, Z., & Tedgui, A. (2011). Recent advances on the role of cytokines in atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(5), 969–979. https://doi.org/10.1161/ATVBAHA.110.207415

Altman, R. (2014). The Postprandial Effects of a Moderately High-Fat Meal on Lipid Profiles and Vascular Inflammation in Alzheimer's Disease Patients: A Pilot Study. *Journal of General Practice*, *02*(06). https://doi.org/10.4172/2329-9126.1000186

Bakker, G. J., Schnitzler, J. G., Bekkering, S., de Clercq, N. C., Koopen, A. M., Hartstra, A. v., Meessen, E. C. E., Scheithauer, T. P., Winkelmeijer, M., Dallinga-Thie, G. M., Cani, P. D., Kemper, E. M., Soeters, M. R., Kroon, J., Groen, A. K., van Raalte, D. H., Herrema, H., & Nieuwdorp, M. (2019). Oral vancomycin treatment does not alter markers of postprandial inflammation in lean and obese subjects. *Physiological Reports*, *7*(16). https://doi.org/10.14814/phy2.14199

Bekkering, S., Quintin, J., Joosten, L. A. B., Van Der Meer, J. W. M., Netea, M. G., & Riksen, N. P. (2014). Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(8), 1731–1738. https://doi.org/10.1161/ATVBAHA.114.303887

Benson, T. W., Weintraub, N. L., Kim, H. W., Seigler, N., Kumar, S., Pye, J., Horimatsu, T., Pellenberg, R., Stepp, D. W., Lucas, R., Bogdanov, V. Y., Litwin, S. E., Brittain, J. E., & Harris, R. A. (2018). A single high-fat meal provokes pathological erythrocyte remodeling and increases myeloperoxidase levels: implications for acute coronary syndrome. *Laboratory Investigation*, *98*(10), 1300–1310. https://doi.org/10.1038/s41374-018-0038-3

Boyette, L. B., MacEdo, C., Hadi, K., Elinoff, B. D., Walters, J. T., Ramaswami, B., Chalasani, G., Taboas, J. M., Lakkis, F. G., & Metes, Di. M. (2017). Phenotype, function, and differentiation potential of human monocyte subsets. *PLoS ONE*, *12*(4). https://doi.org/10.1371/journal.pone.0176460

Burton-Freeman, B., Linares, A., Hyson, D., & Kappagoda, T. (2010). Strawberry modulates ldl oxidation and postprandial lipemia in response to high-fat meal in overweight hyperlipidemic men and women. *Journal of the American College of Nutrition*, *29*(1), 46–54. https://doi.org/10.1080/07315724.2010.10719816

Cani, P. D., & Delzenne, N. M. (2009). Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. In *Current Opinion in Pharmacology* (Vol. 9, Issue 6, pp. 737–743). https://doi.org/10.1016/j.coph.2009.06.016

Chávez-Sánchez, L., Garza-Reyes, M. G., Espinosa-Luna, J. E., Chávez-Rueda, K., Legorreta-Haquet, M. V., & Blanco-Favela, F. (2014). The role of TLR2, TLR4

and CD36 in macrophage activation and foam cell formation in response to oxLDL in humans. *Human Immunology*, *75*(4), 322–329. https://doi.org/10.1016/j.humimm.2014.01.012

Chen, C., & Khismatullin, D. B. (2015). Oxidized low-density lipoprotein contributes to atherogenesis via co-activation of macrophages and mast cells. *PLoS ONE*, *10*(3). https://doi.org/10.1371/journal.pone.0123088

Chistiakov, D. A., Melnichenko, A. A., Myasoedova, V. A., Grechko, A. v., & Orekhov, A. N. (2017). Mechanisms of foam cell formation in atherosclerosis. In *Journal of Molecular Medicine* (Vol. 95, Issue 11, pp. 1153–1165). Springer Verlag. https://doi.org/10.1007/s00109-017-1575-8

Demmer, E., Van Loan, M. D., Rivera, N., Rogers, T. S., Gertz, E. R., Bruce German, J., Zivkovic, A. M., & Smilowitz, J. T. (2016). Consumption of a high-fat meal containing cheese compared with a vegan alternative lowers postprandial C-reactive protein in overweight and obese individuals with metabolic abnormalities: A randomised controlled cross-over study. *Journal of Nutritional Science*, *5*. https://doi.org/10.1017/jns.2015.40

Edfeldt, K., Swedenborg, J., Hansson, G. K., & Yan, Z. (2002). *Expression of Toll-Like Receptors in Human Atherosclerotic Lesions A Possible Pathway for Plaque Activation*. https://doi.org/10.1161/01.CIR.0000012489.17433.31

Emerson, S. R., Kurti, S. P., Harms, C. A., Haub, M. D., Melgarejo, T., Logan, C., & Rosenkranz, S. K. (2017). Magnitude and timing of the postprandial inflammatory response to a high-fat meal in healthy adults: A systematic review. In *Advances in Nutrition* (Vol. 8, Issue 2, pp. 213–225). American Society for Nutrition. https://doi.org/10.3945/an.116.014431

Emerson, S. R., Kurti, S. P., Snyder, B. S., Sitaraman, K., Haub, M. D., & Rosenkranz, S. K. (2016). Effects of thirty and sixty minutes of moderate-intensity aerobic exercise on postprandial lipemia and inflammation in overweight men: A randomized cross-over study. *Journal of the International Society of Sports Nutrition*, *13*(1). https://doi.org/10.1186/s12970-016-0137-8

Erridge, C., Attina, T., Spickett, C. M., & Webb, D. J. (2007). *A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation 1-3*. https://academic.oup.com/ajcn/article/86/5/1286/4651083

Febbraio, M., Podrez, E. A., Smith, J. D., Hajjar, D. P., Hazen, S. L., Hoff, H. F., Sharma, K., & Silverstein, R. L. (2000). Targeted disruption of the class B, scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *Journal of Clinical Investigation*, *105*(8), 1049–1056. https://doi.org/10.1172/JCI9259

Fitó, M., Guxens, M., Corella, D., Sá ez, G., Estruch, R., de la Torre, R., Francés, F., Cabezas, C., del Carmen López-Sabater, M., Marrugat, J., García-Arellano, A., Arós, F., Ruiz-Gutierrez, V., Ros, E., Salas-Salvadó, J., Fiol, M., Solá, R., & Covas, M.-I. (2007). *Effect of a Traditional Mediterranean Diet on Lipoprotein Oxidation A Randomized Controlled Trial*. http://www.predimed.org

Fogarty, C. L., Nieminen, J. K., Peräneva, L., Lassenius, M. I., Ahola, A. J., Taskinen, M. R., Jauhiainen, M., Kirveskari, J., Pussinen, P., Hörkkö, S., Mäkinen, V. P., Gordin, D., Forsblom, C., Groop, P. H., Vaarala, O., & Lehto, M. (2015). High-fat meals induce systemic cytokine release without evidence of

endotoxemia-mediated cytokine production from circulating monocytes or myeloid dendritic cells. *Acta Diabetologica*, *52*(2), 315–322. https://doi.org/10.1007/s00592-014-0641-8

Frostegård, J. (2013). Immunity, atherosclerosis and cardiovascular disease. In *BMC Medicine* (Vol. 11, Issue 1). https://doi.org/10.1186/1741-7015-11-117

Fuller, K. N. Z., Summers, C. M., & Valentine, R. J. (2017). Effect of a single bout of aerobic exercise on high-fat meal-induced inflammation. *Metabolism: Clinical and Experimental*, *71*, 144–152. https://doi.org/10.1016/j.metabol.2017.03.001

Gao, S., & Liu, J. (2017). Association between circulating oxidized low-density lipoprotein and atherosclerotic cardiovascular disease. *Chronic Diseases and Translational Medicine*, *3*(2), 89–94. https://doi.org/10.1016/j.cdtm.2017.02.008

Gower, R. M., Wu, H., Foster, G. A., Devaraj, S., Jialal, I., Ballantyne, C. M., Knowlton, A. A., & Simon, S. I. (2011). CD11c/CD18 Expression is upregulated on blood monocytes during hypertriglyceridemia and enhances adhesion to vascular cell adhesion molecule-1. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(1), 160–166. https://doi.org/10.1161/ATVBAHA.110.215434

Guy, E., Kuchibhotla, S., Silverstein, R., & Febbraio, M. (2007). Continued inhibition of atherosclerotic lesion development in long term Western diet fed CD36°/apoE° mice. *Atherosclerosis*, *192*(1), 123–130. https://doi.org/10.1016/j.atherosclerosis.2006.07.015

Handberg, A., Højlund, K., Gastaldelli, A., Flyvbjerg, A., Dekker, J. M., Petrie, J., Piatti, P., & Beck-Nielsen, H. (2012). Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. *Journal of Internal Medicine*, *271*(3), 294–304. https://doi.org/10.1111/j.1365-2796.2011.02442.x

Henning, A. L., & McFarlin, B. K. (2017). Consumption of a high-fat, high-calorie meal is associated with an increase in intracellular co-localization of PPAR-γ mRNA and protein in monocytes. *Methods*, *112*, 182–187. https://doi.org/10.1016/j.ymeth.2016.07.007

Henning, A. L., Venable, A. S., Prado, E. A., Best Sampson, J. N., & McFarlin, B. K. (2015). Using image-based flow cytometry to measure monocyte oxidized LDL phagocytosis: A potential risk factor for CVD? *Journal of Immunological Methods*, *423*, 78–84. https://doi.org/10.1016/j.jim.2015.03.015

Henning, A. L., Venable, A. S., Vingren, J. L., Hill, D. W., & McFarlin, B. K. (2018). Consumption of a high-fat meal was associated with an increase in monocyte adhesion molecules, scavenger receptors, and Propensity to Form Foam Cells. *Cytometry Part B - Clinical Cytometry*, *94*(4), 606–612. https://doi.org/10.1002/cyto.b.21478

Herd, S. L., M Lawrence, J. E., Malkova, D., Murphy, M. H., Mastana, S., Hardman, A. E., & Hardman, A. E. (2000). Postprandial lipemia in young men and women of contrasting training status. *J Appl Physiol*, *89*, 2049–2056. http://www.jap.org

Herieka, M., & Erridge, C. (2014). High-fat meal induced postprandial inflammation. In *Molecular Nutrition and Food Research* (Vol. 58, Issue 1, pp. 136–146). https://doi.org/10.1002/mnfr.201300104

Insull, W. (2009). The Pathology of Atherosclerosis: Plaque Development and Plaque Responses to Medical Treatment. *American Journal of Medicine*, *122*(1 SUPPL.). https://doi.org/10.1016/j.amjmed.2008.10.013

Janabi, M., Yamashita, S., Hirano, K.-I., Sakai, N., Hiraoka, H., Matsumoto, K., Zhang, Z., Nozaki, S., & Matsuzawa, Y. (2000). *Oxidized LDL-Induced NF-B Activation and Subsequent Expression of Proinflammatory Genes Are Defective in Monocyte-Derived Macrophages From CD36-Deficient Patients*. http://www.atvbaha.org

Jenkins, N. T., Landers, R. Q., Thakkar, S. R., Fan, X., Brown, M. D., Prior, S. J., Spangenburg, E. E., & Hagberg, J. M. (2011). Prior endurance exercise prevents postprandial lipaemia-induced increases in reactive oxygen species in circulating CD31 + cells. *Journal of Physiology*, *589*(22), 5539–5553. https://doi.org/10.1113/jphysiol.2011.215277

Jia, Q., Cao, H., Shen, D., Li, S., Yan, L., Chen, C., Xing, S., & Dou, F. (2019). Quercetin protects against atherosclerosis by regulating the expression of PCSK9, CD36, PPARγ, LXRα and ABCA1. *International Journal of Molecular Medicine*, *44*(3), 893–902. https://doi.org/10.3892/ijmm.2019.4263

Jovinge, S., Ares, M. P. S., Kallin, B., & Nilsson, J. (1996). *Human Monocytes/Macrophages Release TNF-a in Response to Ox-LDL*. http://ahajournals.org

Kackov, S., Simundic, A. M., Nikolac, N., Celap, I., Dukic, L., Ruzic, D., & Bilusic, M. (2013). The effect of high-calorie meal consumption on oxidative stress and endothelial dysfunction in healthy male adults. *Physiological Research*, *62*(6), 643–652. https://doi.org/10.33549/physiolres.932493

Kennedy, A., Spiers, J. P., Crowley, V., Williams, E., & Lithander, F. E. (2015). Postprandial adiponectin and gelatinase response to a high-fat versus an isoenergetic low-fat meal in lean, healthy men. *Nutrition*, *31*(6), 863–870. https://doi.org/10.1016/j.nut.2015.01.009

Khan, I. M., Pokharel, Y., Dadu, R. T., Lewis, D. E., Hoogeveen, R. C., Wu, H., & Ballantyne, C. M. (2016). Postprandial monocyte activation in individuals with metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism*, *101*(11), 4195–4204. https://doi.org/10.1210/jc.2016-2732

Kračmerová, J., Czudková, E., Koc, M., Mališová, L., Šiklová, M., Štich, V., & Rossmeislová, L. (2014). Postprandial inflammation is not associated with endoplasmic reticulum stress in peripheral blood mononuclear cells from healthy lean men. *British Journal of Nutrition*, *112*(4), 573–582. https://doi.org/10.1017/S0007114514001093

Lassenius, M. I., Mäkinen, V. P., Fogarty, C. L., Peräneva, L., Jauhiainen, M., Pussinen, P. J., Taskinen, M. R., Kirveskari, J., Vaarala, O., Nieminen, J. K., Hörkkö, S., Kangas, A. J., Soininen, P., Ala-Korpela, M., Gordin, D., Ahola, A. J., Forsblom, C., Groop, P. H., & Lehto, M. (2014a). Patients with type 1 diabetes show signs of vascular dysfunction in response to multiple high-fat meals. *Nutrition and Metabolism*, *11*(1). https://doi.org/10.1186/1743-7075-11-28

Lassenius, M. I., Mäkinen, V. P., Fogarty, C. L., Peräneva, L., Jauhiainen, M., Pussinen, P. J., Taskinen, M. R., Kirveskari, J., Vaarala, O., Nieminen, J. K.,

Hörkkö, S., Kangas, A. J., Soininen, P., Ala-Korpela, M., Gordin, D., Ahola, A. J., Forsblom, C., Groop, P. H., & Lehto, M. (2014b). Patients with type 1 diabetes show signs of vascular dysfunction in response to multiple high-fat meals. *Nutrition and Metabolism*, *11*(1). https://doi.org/10.1186/1743-7075-11-28

Li, B., Yang, C., Jia, G., Liu, Y., Wang, N., Yang, F., Su, R., Shang, Y., & Han, Y. (2022). Comprehensive evaluation of the effects of long-term cryopreservation on peripheral blood mononuclear cells using flow cytometry. *BMC Immunology*, *23*(1). https://doi.org/10.1186/s12865-022-00505-4

Libby, P. (2021). The changing landscape of atherosclerosis. In *Nature* (Vol. 592, Issue 7855, pp. 524–533). Nature Research. https://doi.org/10.1038/s41586-021-03392-8

Luo, J., Yang, H., & Song, B. L. (2020). Mechanisms and regulation of cholesterol homeostasis. In *Nature Reviews Molecular Cell Biology* (Vol. 21, Issue 4, pp. 225–245). Nature Research. https://doi.org/10.1038/s41580-019-0190-7

Lyte, J. M., Gabler, N. K., & Hollis, J. H. (2016). Postprandial serum endotoxin in healthy humans is modulated by dietary fat in a randomized, controlled, cross-over study. *Lipids in Health and Disease*, *15*(1), 1–10. https://doi.org/10.1186/s12944-016-0357-6

Maguire, E. M., Pearce, S. W. A., & Xiao, Q. (2019). Foam cell formation: A new target for fighting atherosclerosis and cardiovascular disease. In *Vascular Pharmacology* (Vol. 112, pp. 54–71). Elsevier Inc. https://doi.org/10.1016/j.vph.2018.08.002

Marchio, P., Guerra-Ojeda, S., Vila, J. M., Aldasoro, M., Victor, V. M., & Mauricio, M. D. (2019). Targeting early atherosclerosis: A focus on oxidative stress and inflammation. In *Oxidative Medicine and Cellular Longevity* (Vol. 2019). Hindawi Limited. https://doi.org/10.1155/2019/8563845

Masuda, D., Hirano, K. I., Oku, H., Sandoval, J. C., Kawase, R., Yuasa-Kawase, M., Yamashita, Y., Takada, M., Tsubakio-Yamamoto, K., Tochino, Y., Koseki, M., Matsuuura, F., Nishida, M., Kawamoto, T., Ishigami, M., Hori, M., Shimomura, I., & Yamashita, S. (2009). Chylomicron remnants are increased in the postprandial state in CD36 deficiency. *Journal of Lipid Research*, *50*(5), 999–1111. https://doi.org/10.1194/jlr.P700032-JLR200

McFarlin, B. K., Carpenter, K. C., Henning, A. L., & Venable, A. S. (2017). Consumption of a high-fat breakfast on consecutive days alters preclinical biomarkers for atherosclerosis. *European Journal of Clinical Nutrition*, *71*(2), 239–244. https://doi.org/10.1038/ejcn.2016.242

Meher, D., Dutta, D., Ghosh, S., Mukhopadhyay, P., Chowdhury, S., & Mukhopadhyay, S. (2014). Effect of a mixed meal on plasma lipids, insulin resistance and systemic inflammation in non-obese Indian adults with normal glucose tolerance and treatment naïve type-2 diabetes. *Diabetes Research and Clinical Practice*, *104*(1), 97–102. https://doi.org/10.1016/j.diabres.2013.12.047

Milan, A. M., Nuora, A., Pundir, S., Pileggi, C. A., Markworth, J. F., Linderborg, K. M., & Cameron-Smith, D. (2016). Older adults have an altered chylomicron response to a high-fat meal. *British Journal of Nutrition*, *115*(5), 791–799. https://doi.org/10.1017/S000711451500505X

Milan, A. M., Pundir, S., Pileggi, C. A., Markworth, J. F., Lewandowski, P. A., & Cameron-Smith, D. (2017). Comparisons of the postprandial inflammatory and endotoxaemic responses to mixed meals in young and older individuals: A randomised trial. *Nutrients*, *9*(4). https://doi.org/10.3390/nu9040354

Mo, Z., Huang, S., Burnett, D. J., Rutledge, J. C., & Hwang, D. H. (2020). Endotoxin may not be the major cause of postprandial inflammation in adults who consume a single high-fat or moderately high-fat meal. *Journal of Nutrition*, *150*(5), 1303–1312. https://doi.org/10.1093/jn/nxaa003

Moniuszko, M., Kowal, K., Rusak, M., Pietruezuk, M., Dabrowska, M., & Bodzenta-Lukaszyk, A. (2006). Monocyte CD163 and CD36 Expression in Human Whole Blood and Isolated Mononuclear Cell Samples: Influence of Different Anticoagulants. *Clinical and Vaccine Immunology*, *13*(6), 704–707. https://doi.org/10.1128/CDLI.00417-05

Mukherjee, R., Kanti Barman, P., Kumar Thatoi, P., Tripathy, R., Kumar Das, B., & Ravindran, B. (2015). Non-Classical monocytes display inflammatory features: Validation in Sepsis and Systemic Lupus Erythematous. *Scientific Reports*, *5*. https://doi.org/10.1038/srep13886

Nestel, P. J., Pally, S., MacIntosh, G. L., Greeve, M. A., Middleton, S., Jowett, J., & Meikle, P. J. (2012). Circulating inflammatory and atherogenic biomarkers are not increased following single meals of dairy foods. *European Journal of Clinical Nutrition*, *66*(1), 25–31. https://doi.org/10.1038/ejcn.2011.134

Nozaki, S., Kashiwagi, H., Yamashita, S., Nakagawa, T., Kostner, B., Tomiyama, Y., Nakata, A., Ishigami, M., Miyagawa, J. I., Kameda-Takemura, K., Kurata, Y., & Matsuzawa, Y. (1995). Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects. *Journal of Clinical Investigation*, *96*(4), 1859–1865. https://doi.org/10.1172/JCI118231

Oh, E. S., Petersen, K. S., Kris-Etherton, P. M., & Rogers, C. J. (2020). Spices in a High-Saturated-Fat, High-Carbohydrate Meal Reduce Postprandial Proinflammatory Cytokine Secretion in Men with Overweight or Obesity: A 3-Period, Crossover, Randomized Controlled Trial. *Journal of Nutrition*, *150*(6), 1600–1609. https://doi.org/10.1093/jn/nxaa063

Park, E., Edirisinghe, I., & Burton-Freeman, B. (2018). Avocado fruit on postprandial markers of cardio-metabolic risk: A randomized controlled dose response trial in overweight and obese men and women. *Nutrients*, *10*(9). https://doi.org/10.3390/nu10091287

Park, Y. M. (2014). CD36, a scavenger receptor implicated in atherosclerosis. In *Experimental and Molecular Medicine* (Vol. 46, Issue 6). Nature Publishing Group. https://doi.org/10.1038/emm.2014.38

Pendyala, S., Walker, J. M., & Holt, P. R. (2012). A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology*, *142*(5). https://doi.org/10.1053/j.gastro.2012.01.034

Phillips, L. K., Peake, J. M., Zhang, X., Hickman, I. J., Briskey, D. R., Huang, B. E., Simpson, P., Li, S. H., Whitehead, J. P., Martin, J. H., & Prins, J. B. (2013). Postprandial total and HMW adiponectin following a high-fat meal in lean, obese and diabetic men. *European Journal of Clinical Nutrition*, *67*(4), 377–384. https://doi.org/10.1038/ejcn.2013.49

Piechota, M., Banaszewska, A., Dudziak, J., Slomczynski, M., & Plewa, R. (2012). Highly upregulated expression of CD36 and MSR1 in circulating monocytes of patients with acute coronary syndromes. *Protein Journal*, *31*(6), 511–518. https://doi.org/10.1007/s10930-012-9431-8

Poznyak, A. v., Bharadwaj, D., Prasad, G., Grechko, A. v., Sazonova, M. A., & Orekhov, A. N. (2021). Anti-inflammatory therapy for atherosclerosis: Focusing on cytokines. In *International Journal of Molecular Sciences* (Vol. 22, Issue 13). MDPI. https://doi.org/10.3390/ijms22137061

Poznyak, A. v., Nikiforov, N. G., Markin, A. M., Kashirskikh, D. A., Myasoedova, V. A., Gerasimova, E. v., & Orekhov, A. N. (2021). Overview of OxLDL and Its Impact on Cardiovascular Health: Focus on Atherosclerosis. In *Frontiers in Pharmacology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fphar.2020.613780

Rocha, D. M. U. P., Lopes, L. L., Da Silva, A., Oliveira, L. L., Bressan, J., & Hermsdorff, H. H. M. (2017). Orange juice modulates proinflammatory cytokines after high-fat saturated meal consumption. *Food and Function*, *8*(12), 4396–4403. https://doi.org/10.1039/C7FO01139C

Rosenkranz, S. K., Townsend, D. K., Steffens, S. E., & Harms, C. A. (2010). Effects of a high-fat meal on pulmonary function in healthy subjects. *European Journal of Applied Physiology*, *109*(3), 499–506. https://doi.org/10.1007/s00421-010-1390-1

Sawyer, A., & Flagg, L. A. (2000). *State Declines in Heart Disease Mortality in the United States, 2000-2019 Key findings Data from the National Vital Statistics System*. https://www.cdc.gov/nchs/products/index.htm.

Sieg, S. F., Bazdar, D. A., Zidar, D., Freeman, M., Lederman, M. M., & Funderburg, N. T. (2020). Highly oxidized low-density lipoprotein mediates activation of monocytes but does not confer interleukin-1β secretion nor interleukin-15 transpresentation function. *Immunology*, *159*(2), 221–230. https://doi.org/10.1111/imm.13142

Strohacker, K., Breslin, W. L., Carpenter, K. C., Davidson, T. R., Agha, N. H., & McFarlin, B. K. (2012). Moderate-intensity, premeal cycling blunts postprandial increases in monocyte cell surface CD18 and CD11a and endothelial microparticles following a high-fat meal in young adults. *Applied Physiology, Nutrition and Metabolism*, *37*(3), 530–539. https://doi.org/10.1139/H2012-034

Swirski, F. K., Libby, P., Aikawa, E., Alcaide, P., Luscinskas, F. W., Weissleder, R., & Pittet, M. J. (2007). Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *Journal of Clinical Investigation*, *117*(1), 195–205. https://doi.org/10.1172/JCI29950

Teeman, C. S., Kurti, S. P., Cull, B. J., Emerson, S. R., Haub, M. D., & Rosenkranz, S. K. (2016a). The effect of moderate intensity exercise in the postprandial period on the inflammatory response to a high-fat meal: An experimental study. *Nutrition Journal*, *15*(1). https://doi.org/10.1186/s12937-016-0134-4

Teeman, C. S., Kurti, S. P., Cull, B. J., Emerson, S. R., Haub, M. D., & Rosenkranz, S. K. (2016b). Postprandial lipemic and inflammatory responses to high-fat meals: A review of the roles of acute and chronic exercise. In *Nutrition and*

Metabolism (Vol. 13, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12986-016-0142-6

Torres, N., Guevara-Cruz, M., Velázquez-Villegas, L. A., & Tovar, A. R. (2015). Nutrition and Atherosclerosis. In *Archives of Medical Research* (Vol. 46, Issue 5, pp. 408–426). Elsevier Inc. https://doi.org/10.1016/j.arcmed.2015.05.010

Wang, L., Tao, L., Hao, L., Stanley, T. H., Huang, K. H., Lambert, J. D., & Kris-Etherton, P. M. (2020). A moderate-fat diet with one avocado per day increases plasma antioxidants and decreases the oxidation of small, dense LDL in adults with overweight and obesity: A randomized controlled trial. *Journal of Nutrition*, *150*(2), 276–284. https://doi.org/10.1093/jn/nxz231

Wiedermann, C. J., Kiechl, S., Dunzendorfer, S., Schratzberger, P., Egger, G., Oberhollenzer, F., & Willeit, J. (1999). *Association of Endotoxemia With Carotid Atherosclerosis and Cardiovascular Disease Prospective Results from the Bruneck Study*.

Xu, L., Dai Perrard, X., Perrard, J. L., Yang, D., Xiao, X., Teng, B. B., Simon, S. I., Ballantyne, C. M., & Wu, H. (2015). Foamy monocytes form early and contribute to nascent atherosclerosis in mice with hypercholesterolemia. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(8), 1787–1797. https://doi.org/10.1161/ATVBAHA.115.305609

Yu, X. H., Fu, Y. C., Zhang, D. W., Yin, K., & Tang, C. K. (2013). Foam cells in atherosclerosis. In *Clinica Chimica Acta* (Vol. 424, pp. 245–252). https://doi.org/10.1016/j.cca.2013.06.006

Zhu, M., Lei, L., Zhu, Z., Li, Q., Guo, D., Xu, J., Chen, J., Sha, H., Zhang, X., Yang, X., Song, B., Li, B., Yan, Y., & Xiong, Y. (2015). Excess TNF-α in the blood activates monocytes with the potential to directly form cholesteryl ester-laden cells. *Acta Biochimica et Biophysica Sinica*, *47*(11), 899–907. https://doi.org/10.1093/abbs/gmv092

Zingg, J. M., Vlad, A., & Ricciarelli, R. (2021). Oxidized ldls as signaling molecules. In *Antioxidants* (Vol. 10, Issue 8). MDPI. https://doi.org/10.3390/antiox10081184