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The Acute Impact of a High-Fat Load on PBMCs among Women: The Impact of Ethnicity and Weight Status

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THE ACUTE IMPACT OF A HIGH-FAT LOAD ON PBMCS AMONG WOMEN:
THE IMPACT OF ETHNICITY AND WEIGHT STATUS

A Thesis
Presented to
The Faculty of the School of Kinesiology, Recreation, and Sport
Western Kentucky University
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In Partial Fulfillment
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Master of Science

By
Regis Coby Pearson

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THE ACUTE IMPACT OF A HIGH-FAT LOAD ON PBMCS AMONG WOMEN:
THE IMPACT OF ETHNICITY AND WEIGHT STATUS

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Peripheral blood mononuclear cells (PBMCs) can respond to dietary stimuli modulating the up-regulation of pro-inflammatory cell signaling, which is associated with metabolic disease and has been seen to be elevated in African American (AA) when compared to Caucasian American (CA) women. Little is known about the response of PBMCs to a high fat meal among women and the potential impact of ethnicity and/or weight status on this response. The purpose of this study was to examine PBMC response to consuming a high fat meal and the response to culturing PBMCs in media supplemented with lipid among AA and CA women, and to determine the impact that ethnicity and/or weight status may have on this response. Twenty-one women participated in the study: 10 AA (age: 28.00±6.60), 11 CA (age: 26.91±6.28), of whom 11 were lean (BMI<27.0) and 9 were obese (BMI≥27.0). PBMCs were isolated at baseline (pre-shake) and 4-hours post high-fat shake ingestion then cultured for 3 hours in 1) RPMI-1640 media (control) or 2) RPMI-1640 media supplemented with 250uM 1:1 Oleate: Palmitate (lipid-treated); resulting in the following experimental conditions: 1) pre-shake, control; 2) pre-shake, lipid-treated; 3) post-shake, control; and 4) post-shake, lipid-treated. Using RT-PCR, we evaluated gene expression of IL-6, IL-8, IL-10, and TNF-α. Repeated-measures ANOVA were performed with emphasis on a time (pre- vs. post-shake) X treatment (control vs. lipid-treated culture) X ethnicity or weight status interactions. PBMC IL-8 gene expression significantly increased post-shake in both the control (p=0.05) and lipid-
treated \((p=0.01)\) conditions for all subjects. In the post-shake, control condition IL-8 gene expression was significantly higher among the AA women \((p=0.03)\). Among the AA women there was a significant decrease in IL-6 gene expression post-shake in the lipid-treated condition \((p=0.04)\). The expression of IL-6 and IL-8 significantly decreased in the lipid \((p<0.05)\) condition in obese women, post-shake. The expression of IL-8 significantly increased in both the control \((p=0.04)\) and lipid \((p=0.05)\) conditions in lean women, post-shake. This study showed that the ingestion of a high fat shake does effect PBMC gene expression response in women with specific gene expression differences noted between different ethnicities and weight statuses.
Chapter 1. Introduction

The immune system plays a vital role in the prevention and development of many diseases, including cardiovascular and metabolic disease (Drew, 2012). The control of the immune system and other cellular defense systems (e.g. metabolism) are interlinked and require a network of communication between immune cells to direct defense and respond to environment (Drew, 2012). These systems are highly responsive to an acute dietary intake, such as a meal in a typical Western high fat diet, which can modulate a variety of physiological processes (Drew, 2012), such as the upregulation of pro-inflammatory cell signaling (Monterio & Azevedo, 2010). An essential step in the maturation of immune cells is the reprogramming of their metabolic profiles as a result of an extracellular stimuli (Ganeshan & Chawla, 2014), which can be a representation of a whole body systemic response.

Peripheral blood mononuclear cells (PBMCs) collectively refer to a heterogeneous mixture of mononuclear immune cells (Koncarevic et al., 2014) that are an imperative component of the immune system (Arosio et al., 2014). In recent years, the investigation of how PBMCs, as a representor of systemic immune response, are related to metabolic biomarkers and disease has ensued (Bouwens et al., 2010; Stepien et al., 2014) in relation to the unique gene expression seen in PBMCs from patients with systemic inflammatory diseases (Aune, Maas, Parker, Moore, & Olsen, 2004; Edwards et al., 2007; Mandal, Gurevich, Pauzner, Kaminski, & Achiron, 2004; van ‘t Veer et al., 2002). The investigation of PBMCs could serve as a model to investigate the link between immune function, inflammation, and metabolic disease. The inflammatory state of circulating PBMCs elicits alterations to their gene expression, affecting other cellular
function (Aune et al., 2004; Edwards et al., 2007; Mandal et al., 2004; O’Rourke et al., 2006; van ‘t Veer et al., 2002). Cytokines can be produced and released by peripheral blood mononuclear cells (Friberg et al., 1994) and in an obese population can play a role in insulin resistance (Rodriguez-Hernandez et al., 2013).

The predominance of cardiovascular disease risk, obesity, high cholesterol, and hypertension is increased among African American (AA) women compared to Caucasian American (CA) women (Malayala & Raza, 2016). African Americans also have increased insulin resistance when compared to CAs (Meis, Schuster, Gaillard, & Osei, 2006), which is a direct indication that the action of insulin at the tissue level is impaired (Kasim-Karakas, 2000). Additionally, AAs have a disproportionately higher coronary heart disease mortality and morbidity rate (Clark et al., 2001).

With an increase in disease risk among AA women (Taylor et al., 2008) the likelihood of an elevated pro-inflammatory cellular environment is increased as well (Park & Kang, 2013). However, the causes of increased disease risk, and associated pro-inflammatory environment, among AA women have not been fully explained. Some studies have shown that AAs have a higher level of IL-6 (a pro-inflammatory cytokine) compared to CAs (Kalra et al., 2005; Walston et al., 2007). Another potential explanation may be due partially to socioeconomic status; a low socioeconomic status has been correlated with an increase of C-reactive protein, IL-6, and TNF-α (Paalani, Lee, Haddad, & Tonstad, 2011).

Over-nutrition promotes a dysregulation of energy storage and utilization, causing an activation of local inflammatory pathways and compromised metabolic function (Calcada et al., 2014; Hotamisligil & Erbay, 2008). This compromise in metabolic
function can lead to the inability to switch between different macronutrition oxidation, this inability is known as metabolic inflexibility and can lead to inflammatory disease states (Muio, 2014). Berk et al. (2006) found that obese AA women were not able to efficiently switch between fat oxidation (fasting state) and carbohydrate oxidation (fed state); suggesting that this AA population may have decreased metabolic flexibility. Given the responsiveness of the immune system to dietary stimuli, one potential explanation may be that certain immune cells may be more pro-inflammatory in response to certain types of dietary stimuli, among AA women compared to CA women. If immune cells, such as PBMCs, from AA women display a more pro-inflammatory profile in response to dietary stimuli, such as lipid incubation or consumption of a high fat meal, this may contribute to increased disease risk.

While it is seen that immunity can affect overall disease state, weight status is gaining acceptance as a predictor of severe health complications. Between 2011 and 2014 it is estimated that 36.5% of Americans were categorized as obese (Ogden et al., 2015). Obesity has been associated with several health effects, such as mortality, hypertension, type-2 diabetes, coronary heart disease, low quality of life (Bhaskarn et al., 2014; NHLBI, 1998; NHLBI, 2014); as well as mental illness (Kasen et al., 2008; Luppino et al., 2010) and difficulty with physical functioning (Roberts et al., 2003). Epidemiological studies have shown a foundational association between obesity, type-2 diabetes, and cancer (LeRoith et al., 2008). With greater prevalence of obesity-driven type 2 diabetes (Ogden et al., 2006), the prediction of increased cancer rates and subsequently an increase of cancer deaths can be plausible (LeRoith et al., 2008).
Along with the systemic complications that an elevated weight status can cause, there is also a state of low-grade inflammation due to an increase in pro-inflammatory cells (Lumeng & Saltiel, 2011). Low-grade inflammation is often measured by increased levels of C-reactive protein and other pro-inflammatory cytokines (Hotamisligil, 2006). A greater presence of pro-inflammatory cells increases the probability of disruptions in metabolic health (Phillips & Perry, 2013), such as the pathogenesis of insulin resistance. Additionally, the chronic low-grade inflammatory state is associated with an increased prevalence of cardiovascular disease and many other maladies (Rodriguez-Hernandez, Simental-Mendia, Rodriguez-Ramierz, & Reyes-Romero, 2013).

Altered levels of metabolic genes and RNA transcripts have been associated with obesity, insulin resistance, and weight gain (Gao & Liu, 2014); due to obesity being associated with an increased risk of hyperlipidemia, hyperglycemia, hypertension, atherosclerosis, and chronic heart disease (Adams et al., 2006; Gade, Schmit, Collins, & Gade, 2010; Hedley et al., 2004; Hossain, Kawar, & El Nahas, 2007; Kolovou, Anagostopoulu, & Cokkinos, 2005). Obesity has serious metabolic consequences such as developing resistance to insulin and leptin, dyslipidemia related to increased visceral adipose tissue, chronic inflammation leading to atherosclerosis, and lipotoxic damage to tissues and vasculature; all of which result in a dramatic increase in cardiovascular disease (Gade et al., 2010).

Gene expression alterations among PBMCs may represent overall systemic health (Afman, Milenkovic, & Roche, 2014). Because of the representation of systemic health and the effects of long-term dietary interventions on lipid metabolism (Bouwens et al., 2009; De Mello et al., 2009; Myhrstad et al., 2014; van Dijk, Feskens, et al., 2012),
PBMCs can serve as a model for examining early risk markers to dietary changes (Leder et al., 2016; Visvikis-Siest et al., 2007). Changes in lipid homeostasis due to an acute challenge are linked to inflammatory status (Leder et al., 2016). Thus, PBMC gene expression changes may represent metabolic health changes (O’Grada et al., 2014).

Obesity is related to immune and inflammatory system responses contributing to an abnormal regulation of pro-inflammatory cytokines (Bastard et al., 2000; Juge-Aubry et al., 2004; Meier et al., 2002; Moon, Kim, & Song, 2004; Park, Park, & Yu, 2004). Alterations in gene expression of PBMCs, in obese individuals, have been found to affect lymphocyte function due to the abnormal metabolism of cytokines (O’Rourke et al., 2006). Metabolic diseases, such as obesity, metabolic syndrome, and type-2 diabetes, are associated with modified immune response and lipid metabolism; food challenges that alter these responses may play an important role in the detection and prevention of these diseases (van Ommen et al., 2009). Regarding metabolic regulatory genes, PBMCs could serve as a potential source for inflammatory biomarkers in early homeostatic imbalance (Oliver, Reynes, Caimari, & Palou, 2013). While most studies, similar to Bruun et al. 2006, investigating weight and inflammatory status involve a prolonged dietary and/or exercise intervention, there is limited research examining the effects of an acute change on inflammatory status of PBMCs.

Along with weight status contributing to altered systemic inflammation, ethnic differences have been found to effect gene expression in inflammatory disease. It has been found that gene expression of PBMCs differs between AA and CA women, suggesting molecular factors attributing to inflammatory disease, such as hypertension
and atherosclerosis (Dluzen et al., 2016). Currently, there is limited research investigating cytokine gene expression of PBMCs, particularly in response to an acute dietary stimulus.

To the principal investigator’s knowledge, there is no current data on the PBMCs response (in vivo) to an acute high fat meal among lean and obese AA and CA women. In addition, no study has investigated the (in vitro) lipid stimulated response of cultured PBMCs from lean and obese AA and CA women. Therefore, the purpose of this study was to examine PBMC response to lipid oversupply in vivo (in response to consuming a high fat meal) and in vitro (in response to culturing PBMCs in media supplemented with lipid) among AA and CA women. The secondary purpose was to determine the impact that weight status may have on this response.

These experiments will provide insight into how the immune system is linked to lipid metabolism, inflammation, and dietary intake. More specifically, these results may uncover ethnic and/or weight status differences in how certain immune cells respond to a dietary intake and/or lipid stimulus. If ethnic differences are discovered, this may help explain why AA women have disproportionately higher rates of obesity, metabolic disease, and other chronic inflammatory-related diseases. Additionally, if weight status differences are found this may aid in the current knowledge of how chronic low-grade inflammation and the immune system are associated with weight statuses of women.
Chapter 2. Literature Review

Obesity

Weight status is gaining acceptance as a predictor of severe health complications. Between 2011 and 2014 it is estimated that 36.5% of Americans were categorized as obese (Ogden et al., 2015). As a means of measuring an individual’s weight status, body mass index (BMI) is now considered a standard measure of obesity. BMI is found by dividing weight in kilograms by height in meters squared (BMI=kg/m$^2$) (Pescatello & American College of Sports Medicine (ACSM), 2014), and although this method of categorizing weight status is an easy tool, it does not properly display the location of adipose tissue, which may be a determinant in risk stratification (Eckel, 1997).

Obesity has been associated with several health effects, such as mortality, hypertension, type-2 diabetes, coronary heart disease, low quality of life (Bhaskarn et al., 2014; NHLBI, 1998; NHLBI, 2014); as well as mental illness (Kasen et al., 2008; Luppino et al., 2010) and difficulty with physical functioning (Roberts et al., 2003). The association between obesity and coronary heart disease is well established (Manson et al., 1990; Prospective Studies Collaboration et al., 2009) primarily due to the exacerbated rate of atherosclerosis (Shimano, 2009). Epidemiological studies have shown a foundational association between obesity, type-2 diabetes, and cancer (LeRoith et al., 2008). With greater prevalence of obesity-driven type-2 diabetes (Ogden et al., 2006), the prediction of increased cancer rates and subsequently an increase of cancer deaths can be plausible (LeRoith et al., 2008).

Along with the large systems complications that an elevated weight status can cause, there is also a state of low-grade inflammation due to an increase in pro-
inflammatory cells (Lumeng & Saltiel, 2011). Low-grade inflammation is often measured by increased levels of C-reactive protein and other pro-inflammatory cytokines (Hotamisligil, 2006). This increased amount of pro-inflammatory cells increases the probability of disruptions in metabolic health (Phillips & Perry, 2013) such as the pathogenesis of insulin resistance. Additionally, the chronic low-grade inflammatory state is associated with an increased prevalence of cardiovascular disease, diabetes, metabolic syndrome, and many other maladies (Rodriguez-Hernandez, Simental-Mendia, Rodriguez-Ramierz, & Reyes-Romero, 2013). Consequently, the reduction of pro-inflammatory biomarkers is associated with increased likelihood of a metabolically healthy individual (Phillips & Perry, 2013).

The elevated prevalence of obesity, coupled with other socio-economic burdens associated with obesity, emphasizes the critical need for understanding the molecular mechanisms associated with the obese state. Altered levels of metabolic genes and RNA transcripts are associated with obesity, insulin resistance, and weight gain (Gao & Liu, 2014). Due to obesity being associated with an increased risk of hyperlipidemia, hyperglycemia, hypertension, atherosclerosis, and chronic heart disease (Adams et al., 2006; Gade, Schmit, Collins, & Gade, 2010; Hedley et al., 2004; Hossain, Kawar, & El Nahas, 2007; Kolovou, Anagostopoulu, & Cokkinos, 2005). Obesity has serious metabolic consequences such as developing resistance to insulin and leptin, dyslipidemia related to increased visceral adipose tissue, chronic inflammation leading to atherosclerosis, and lipotoxic damage to tissues and vasculature; all of which result in a dramatic increase in cardiovascular disease (Gade et al., 2010).
Gender

The United States showed a greater prevalence of obesity prevalence in 1999-2002 compared to other developed countries, with the variance largely due to the higher prevalence seen among women (Flegal, Carroll, Ogden, & Curtin, 2010; Tjepkema, 2006). Between 2011 and 2014, women have shown an increased prevalence of obesity, 38.3%, compared to men, 34.3% (Ogden et al., 2015). With this increasing trend of obesity among women, research examining possible physiological changes aiding in the increasing obesity prevalence among women is warranted.

African American Health

African Americans and Overall Health Differences

The predominance of cardiovascular risk, obesity, high cholesterol, and hypertension is increased among AA women compared to CAs (Malayala & Raza, 2016). African Americans also have increased insulin resistance when compared to CAs (Meis, Schuster, Gaillard, & Osei, 2006), which is a direct indication that the action of insulin at the tissue level is impaired (Kasim-Karakas, 2000). Additionally, AAs have a disproportionately higher coronary heart disease mortality and morbidity (Clark et al., 2001).

AA and Inflammation

With an increase in disease risk among AA women (Taylor et al., 2008) the likelihood of an increased pro-inflammatory cellular environment is increased as well (Park & Kang, 2013). However, the causes of increased disease risk and associated pro-inflammatory environment among AA women have not been fully explained. Some studies have shown that AAs have a higher level of IL-6 (as a pro-inflammatory
cytokine) compared to CAs (Kalra et al., 2005; Walston et al., 2007). Another potential explanation may be due partially to socioeconomic status; a low socioeconomic status has been link with an increase of C-reactive protein, IL-6, and TNF-α (Paalani, Lee, Haddad, & Tonstad, 2011).

AA, Diet, and Immunity

Over-nutrition promotes a dysregulation of energy storage and utilization, causing an activation of local inflammatory pathways and compromised metabolic function (Calcada et al., 2014; Hotamisligil & Erbay, 2008). This compromise in metabolic function can lead to the inability to switch between different macronutrition oxidation, this inability is known as metabolic inflexibility and can lead to inflammatory disease states (Muio, 2014). Berk et al. (2006) found that obese AA women were not able to efficiently switch between fat oxidation (fasting state) and carbohydrate oxidation (fed state); demonstrating that the AA population has decreased metabolic flexibility. Given the responsiveness of the immune system to dietary stimuli, it is plausible that certain immune cells may be more pro-inflammatory in response to certain types of dietary stimuli, particularly in AA women when compared to CA women. If immune cells, such as PBMCs, from AA women display a more pro-inflammatory profile in response to dietary stimuli, such as lipid incubation or consumption of a high fat meal, this may contribute to increased disease risk.

Metabolism and Immunity

The immune system plays a vital role in the prevention and development of many diseases, including cardiovascular and metabolic disease (Drew, 2012). The control of the immune system and other cellular defense systems, e.g. metabolism, are interlinked
and require a network of communication between immune cells to direct defense and respond to the environment (Drew, 2012). These systems are highly responsive to an acute high fat dietary intake, which can modulate a variety of physiological processes (Drew, 2012), such as the upregulation of pro-inflammatory cell signaling (Monterio & Azevedo, 2010).

While there is a split seen when discussing the metabolic states of innate or adaptive immune cells, both groups transition from naive to activated states in response to stimuli (Ganeshan & Chawla, 2014). The innate immune system consists of neutrophils, mast cells, macrophages, and dendritic cells. Neutrophils make up approximately 50-60% of circulating leukocytes and perform host defense functions, such as the phagocytosis of invading pathogens (bacteria and/or fungi) (Segal, 2005). This phagocytosis phases result in changes to the metabolic state of neutrophils (Ganeshan & Chawla, 2014). Mast cells are responsible for functions that involve wound healing and defense against pathogens, venoms, and toxins (Abraham & St John, 2010); these functions require mast cells to rapidly degranulate (Ganeshan & Chawla, 2014). While the energy demands of mast cell degranulation and regranulation are likely to be high, little is known about how the metabolic demand is met (Ganeshan & Chawla, 2014). Macrophages serve as a first line of defense against foreign bodies and participate in tissue homeostasis (Wynn, Chawla, & Pollard, 2013). Macrophages can undergo distinct activation programs resulting in two characterized states, classical (M1) and alternative (M2) (Ganeshan & Chawla, 2014). Classically activated macrophages aid in the protection against pathogens by escalating a pro-inflammatory response by releasing cytokines like IL-6 and TNF-α (Ganeshan & Chawla, 2014). Alternatively activated
macrophages act as a hot defense system against infections by regulating the innate and adaptive immune system responses in addition to facilitating the formation of granulation (Ganeshan & Chawla, 2014). The glycolytic and oxidative metabolism is crucial in the promotion of the maturation of classically and alternatively activated macrophages (Ganeshan & Chawla, 2014). However, in dendritic cells, glycolysis may be necessary to provide intermediates required for the early stages of cellular activation (Ganeshan & Chawla, 2014). Studies show that the metabolic reprogramming of dendritic cells by toll-like receptor ligands may be responsible for functions that drive the maturation of an immune response (Ganeshan & Chawla, 2014).

The main roles of adaptive immune cells are cytokine and chemokine production. These cells also have functions that must be supported by cataplerotic and anaplerotic reactions of cell level metabolism (Ganeshan & Chawla, 2014). Most research that focuses on adaptive immune system is geared towards CD4+ T cells, but similarities can be associated with CD8+ T cells and B cells. Naïve CD4+ T cells are metabolically active and require ATP synthesis for migration where they actively sample antigens (Ganeshan & Chawla, 2014). The formation of CD8+ T cells, or memory T cells, are dependent on fatty acid oxidation and mitochondrial oxidative metabolism (Pearce et al., 2009; Van der Windt et al., 2012). B cells are the sole producers of antibodies that establish and maintain humoral immunity (Ganeshan & Chawla, 2014). Though, the unique features of B cells, like antibody class switching, affinity maturation, and plasma cell formation, have not yet been thoroughly investigated (Ganeshan & Chawla, 2014).

An essential step in the maturation of immune cells is the reprogramming of their metabolic profiles, which is a result of a response to extracellular stimuli (Ganeshan &
Chawla, 2014). Metabolic control of circulating immune cells can be a representation of a systemic response of the whole body. Despite this knowledge, the understanding of immune cell metabolism is still not fully clear and warrants further investigation.

**Peripheral Blood Mononuclear Cells**

Peripheral blood mononuclear cells collectively refer to a heterogeneous mixture of mononuclear immune cells (Koncarevic et al., 2014), and are defined as any blood cell having a round nucleus; this includes T cells, B cells, natural killer cells and monocytes and are an imperative component of the immune system (Arosio et al., 2014). In recent years, the investigation of how PBMCs, as a representor of systemic immune response, are related to metabolic biomarkers and disease has ensued (Bouwens et al., 2010; Stepien et al., 2014). Recent studies have provided information about unique gene expression from PBMCs from patients with lupus, multiple sclerosis, cancer, and rheumatoid arthritis (Aune, Maas, Parker, Moore, & Olsen, 2004; Edwards et al., 2007; Mandal, Gurevich, Pauzner, Kaminski, & Achiron, 2004; van ‘t Veer et al., 2002); all of which are systemic inflammatory diseases. The investigation of PBMCs could serve as a model to investigate the link between immune function, inflammation, and metabolic disease.

**Cytokines**

It is important to assess the inflammatory state of circulating immune cells because of the alterations in gene expression, found specifically in PBMCs, affecting other cellular function (Aune et al., 2004; Edwards et al., 2007; Mandal et al., 2004; O’Rourke et al., 2006; van ‘t Veer et al., 2002). Cytokines are a broad category of small proteins that play an important role in immune function, metabolism, life-style related-
disease, and overall health (Duque & Descoteaux, 2014; Friberg, Bryant, Shannon, & Whiteside, 1994; Katial, Sachanandanim, Pinney, & Lieberman, 1998). These proteins can be produced and released by peripheral blood mononuclear cells (Friberg et al., 1994). In an obese population, it is believed that the higher concentration of pro-inflammatory cytokines play a role in insulin resistance (Rodriguez-Hernandez et al., 2013).

Interleukin 6 (IL-6) is a cytokine that is synthesized by adipose tissue, endothelial cells, macrophages, and lymphocytes. This cytokine plays a vital role in multiple systems and processes, to include bone metabolism, inflammation, and acute phase reactions; while regulating energy homeostasis and inflammation (Rodriguez-Hernandez et al., 2013). Interleukin 6 can act as a pro- or anti-inflammatory cytokine; where as a pro-inflammatory cytokine is mediated by trans-signaling (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Cells which only expression the signaling receptor protein gp130 are not response to IL-6 alone, but can response to a complex that is naturally bound to the soluble form of the IL-6 receptor, this adaptability can greatly enlarge the spectrum of IL-6 target cells that can be activated through trans-signaling (Scheller et al., 2011). Conversely, IL-6 can act as an anti-inflammatory, or regenerative, cytokine which is mediated by classic signaling (Scheller et al., 2011). This can occur once IL-6 stimulates the target cells via a membrane bound receptor, then signal transducers and activators of transcription factors are recruited which are dimerized, where they translocate into the nucleus and activate the target genes (Scheller et al., 2011). This cytokine is important in the transition from acute inflammation to chronic inflammation, which plays a pivotal role in the promotion of disease such as obesity, insulin resistance, and inflammatory
arthritis (Naugler & Karin, 2008; Rodriguez-Hernandez et al., 2013), by changing the nature of leucocyte infiltration from polymorphonuclear neutrophils to macrophages (Gabay, 2006). Additionally, IL-6 increase hepatic lipogenesis (Feingold & Grunfeld, 1992) and can trigger an acute-phase response (Baumann & Gauldie, 1994).

Interleukin 8 (IL-8) is one of the most widely studied pro-inflammatory cytokines (Turner, Nedjai, Hurst, & Pennington, 2014), which is responsible for the targeted recruitment of neutrophils (Bickel, 1993; Hammond et al., 1995). While having roles in chemotactic migration and activation of monocytes, lymphocytes, basophils, and eosinophils at sites of inflammation (Miller et al., 1992), IL-8 is strongly stimulated by IL-1 and TNF-α (Qazi, Tang, & Qazi, 2011). Interleukin 8 can also recruit T cells and other nonspecific inflammatory cells to the sites of inflammation (Schroder, 1992). IL-8 plays a pivotal role by attracting mononuclear cells to the sites of infection or inflammation (Shin, Szuba, & Rockson, 2002), leading to the initiation and progression of inflammatory statuses such as atherosclerosis (Niculescu & Rus, 1999) which it has been suggested to have a common inflammatory basis as other metabolic disease (Pradhan & Ridker, 2002). Additionally, an increase of glucose concentrations has been seen to induce the expression of IL-8, which eludes to the development of hyperglycemia, inflammatory statuses, and atherosclerosis (Temaru et al., 1997).

Interleukin 10 (IL-10) is an anti-inflammatory cytokine produced by leukocytes, dendritic cells, macrophages, mast cells, natural killer cells, eosinophils, neutrophils, CD4 and CD8 T cells and B cells and represses extreme inflammatory responses (Ouyang, Rutz, Crellin, Valdez, & Hymowitz, 2011). Interleukin 10 derived from macrophages inhibit the differentiation of neighboring cells into classically activated
macrophages; this allows these macrophages to be self-regulated (Sing et al., 2002). Additionally, IL-10 inhibits innate and adaptive immune system responses which limit possible tissue damage caused by inflammation (Ouyang et al., 2011).

Tumor necrosis factor alpha (TNF-α) is a pleiotropic molecule that promotes the secretion of other pro-inflammatory cytokines and reduces anti-inflammatory responses, due to this it plays an essential function in inflammation, the immune system, and metabolism (Rodriguez-Hernandez et al., 2013). This being said, TNF-α is increased in an obese population and promotes insulin resistance by inhibiting insulin receptor substrate signaling pathways (Hotamisligil et al., 1996; Rodriguez-Hernandez et al., 2013; Wang & Trayhurn, 2006).

**Population Differences in Cytokine and PBMC Expression**

Gene expression alterations among PBMCs may represent overall systemic health (Afman, Milenkovic, & Roche, 2014). Because of the representation of systemic health and the effects of long-term dietary interventions on lipid metabolism (Bouwens et al., 2009; De Mello et al., 2009; Myhrstad et al., 2014; van Dijk, Feskens, et al., 2012). PBMCs can serve as a model for examining early risk markers to dietary changes (Leder et al., 2016; Visvikis-Siest et al., 2007). Changes in lipid homeostasis due to an acute challenge are linked to inflammatory status (Leder et al., 2016). Thus, PBMC gene expression changes may represent metabolic health changes (O’Grada et al., 2014).

Obesity has been connected with immune and inflammatory system responses contributing to an abnormal regulation of pro-inflammatory cytokines (Bastard et al., 2000; Juge-Aubry et al., 2004; Meier et al., 2002; Moon, Kim, & Song, 2004; Park, Park, & Yu, 2004). Alterations in gene expression of PBMCs, in obese individuals, have been
found to affect lymphocyte function due to the abnormal metabolism of cytokines (O’Rourke et al., 2006). Metabolic diseases, such as obesity, metabolic syndrome, and type-2 diabetes are associated with immune response and lipid metabolism; food challenges that alter these responses may play an important role in the detection and prevention of these diseases (van Ommen et al., 2009). Regarding metabolic regulatory genes, PBMCs could serve as a potential source for inflammatory biomarkers in early homeostatic imbalance (Oliver, Reynes, Caimari, & Palou, 2013). While most studies, similar to Bruun et al. 2006, investigating weight and inflammatory status involve a prolonged dietary and/or exercise intervention, there is limited research examining the effects of an acute change on inflammatory status of PBMCs.

Along with weight status contributing to systemic inflammatory changes, ethnic differences have been found to effect gene expression in inflammatory disease. Research suggests that gene expression of PBMCs differs between AA and CA women, suggesting molecular factors attributing to inflammatory disease, such as hypertension and atherosclerosis (Dluzen et al., 2016). Currently, there is limited research investigating cytokine gene expression of PBMCs, particularly in response to an acute dietary stimulus.
Chapter 3. Methods

Informed Consent and IRB Approval

This study received Western Kentucky University (WKU) Institute Review Board (IRB) approval (IRB # 17-021) on August 28th, 2016. All subjects were recruited from WKU and the Bowling Green, KY community via paper flyers and targeted emails. Before participations, subjects signed an IRB approved Informed Consent (Appendix A), in a private location, to maintain confidentiality. A principal investigator or co-investigator verbally guided each subject through the informed consent, while allowing time for the subject to ask any questions. Each subject received a copy of the informed consent in which they signed.

Subject Recruitment

All subjects that participated in the study were recruited from WKU and the greater Bowling Green, KY area. Initially AA subjects were recruited, then CA subjects were matched via age, weight, BMI, and health status. This method of subject recruitment resulted in 21 subjects, with 10 AA and 11 CA women participating in the study. All subjects were women between the ages of 20-37 years old, non-smokers, and not currently using any drugs to alter insulin resistance and/or metabolic profiles.

Subject Involvement

Initial Shake Ingestion

All data collection for initial shake ingestion occurred at the Exercise Research Laboratory, housed within the Department of Physical Therapy, at the WKU Medical Center Health Complex. All recruited subjects were asked to consume a standardized meal the evening prior to their appointment (Appendix B). This meal consisted of a
standardized diet consisting of approximately 50% carbohydrate, 30% fat, and 20% protein. Subjects were asked to select a meal category based on initial BMI assessment of lean, overweight, or obese. Subjects prepared this meal on their own/at-home and to fast after consuming the meal.

Subjects reported to the Exercise Research Laboratory after an approximate 10-hour fast. The subject’s weight, height, and vital signs were taken upon arrival to the laboratory. Body composition was measured using skinfold anthropometry, using a seven-site protocol to determine percent body fat (Pescatello & ACSM, 2014). A Harpenden Skinfold Caliper was used to measure skinfolds, which at a later point was entered into a standardized equation to estimate body fat percentage of each subject.

A baseline blood draw (~20 mL) was obtained via butterfly angiocatheter in the antecubital region of the arm or the posterior surface of the hand. The angiocatheter was flushed with saline to maintain patency at each time point blood draw. After the baseline blood draw, subjects consumed a standard ~1000-kcal high fat shake, similar in composition to previous studies (Heilbroon, Gregersen, Shirkhedkar, Hu, & Campbell, 2007; Jakulj et al., 2007). The high-fat shake consisted of 1062 calories, 55.93% fat, which was obtained from a local smoothie shop. The smoothie contained almond milk, banana, apple, peanut butter, super grains, cocoa, vegan protein, and almond. This smoothie was similar in fat content to an average high fat meal found in the standard western diet, but compiled from healthier food sources to reduce the risk of gastrointestinal discomfort. An additional blood draw (~20 mL) was taken from the same angiocatheter, four hours after the high fat shake was ingested. At the end of the study, the angiocatheter was removed by the attending nurse practitioner. During the four hours
post shake ingestion, the subject was asked to sit or recline comfortably, while being allowed to talk on the phone, work on a portable device, or watch TV.

Additionally, during the four-hour study period, subjects were asked to complete several surveys, including the National Institutes of Health’s validated Dietary History Questionnaire II to determine potential differences in normal diet and the International Physical Activity Questionnaire to determine domain and mode of physical activities.

All study procedures were supervised by a doctor of nursing practice or a physician trained in Syria with 5 years experienced working in diagnostics. Dr. Blankenship and Nuha Shaker provided clinical expertise in supervising the subject to ensure patient safety. Dr. Blankenship and Nuha Shaker were responsible for venous blood draws conducted in this study.

Before the conclusion of the subject’s appointment and after the angiocatheter was removed, subjects were given an ActiGraph Link Accelerometer (ALA) (ActiGraph LLC, Pensacola, FL) to objectively confirm their self-reported physical activity levels. The ALA was placed on the non-dominant wrist with non-removable hospital-grade wristbands. This specific tri-axel accelerometer was chosen because it can be used as a valid measure of physical activity energy expenditure in women (Plasqui & Westerterp, 2007). Data was collected for seven consecutive days at 30 Hz. The accelerometer output was sampled by a 12-bit analog-to-digital converter. The percentage of time spent in sedentary activity, as well as the amount of time spent participating in different categories of physical activity ranging from light and lifestyle to moderate, was calculated using algorithms corresponding to the following activity counts: sedentary: 0 - 99 counts/min,

Sample Transportation and Subsequent Procedures

Transportation of Biological Samples

All biological samples were transported to the WKU Exercise Biochemistry Laboratory (Biosafety Level 2) at approximately +20°C to preserve the integrity of the sample. Once samples arrived at the WKU Exercise Biochemistry Laboratory, they were immediately processed; time between blood draw and initiation of isolation was less than 2 hours.

PBMC Isolation

All blood samples were captured in a 10 mL EDTA vacutainer and immediately transported to begin processing for isolation and culturing of PBMCs using a ficoll gradient protocol (Histopaque-1077, Sigma-Aldrich, St. Louis, MO). The specific protocol used is as follows:

- Add two 5 mL aliquots to 5 mL phosphate buffered saline (PBS) and gently homogenized.
- Layer whole blood: PBS mixture (~10 mL) onto 3 mL Histopaque-1077.
- Spin @ 400 xg for 20 min @ room temperature (RT).
- Transfer PBMC layer to a new 15 mL conical tube.
- Add 9 mL PBS and gently homogenized.
- Spin @ 100 xg for 10 min @ RT, discarded the supernatant and resuspended pellet.
- Add 9 mL PBS and gently homogenized.
• Spin @ 100 xg for 10 min @ RT, discarded the supernatant and resuspended pellet.

**PBMC Culture and Storage of Supernatant**

PBMCs were cultured in two different dishes for three hours in one of two different conditions: 1) in RPMI-1640 media supplemented with 10% FBS (control condition) and 2) in RPMI-1640 media supplemented with 10% FBS and 250 micromolar 1:1 Oleate Palmitate lipid mixture (lipid-treated condition) (Maples et al., 2015). The specific protocol used is as follows and continues with PBMC Isolation Protocol:

• For one sample, add 2 mL of lipid incubation media and transfer to a 35-mm cell culture dish.
• For one sample, add 2 mL of RPMI-1640 control media and transferred to a 35-mm cell culture dish.
• Incubate @ +37°C for 3 hours.
• Remove supernatant and transfer to a 2 mL microcentrifuge tube, then store @ -80°C.
• Add 400 μL PBS, scrap cells, and transfer cell suspension to a 2 mL microcentrifuge tube for RNA isolation.

**RNA Isolation and Storage**

PBMC RNA isolation was performed according to manufacturer’s protocol using the TRIzol Reagent method to isolate RNA (Thermo Fisher Scientific Inc., Waltham, MA). RNA isolation immediately followed PBMC isolation and culture. The specific protocol used is as follows and continues with PBMC Culture and Storage of Supernatant:
• Add 400 μL PBMC sample to 1,200 μL TRIzol, lysis by pipetting and incubate @ RT for 5 min.

• Add 240 μL chloroform and vortex for 15 secs, incubate for 3 min @ RT.

• Spin @ 12,000 xg for 15 min @ +4°C.

• Remove and transfer the colorless upper aqueous phase to a new 2 mL microcentrifuge tube.

• Add 600 μL 99.5% isopropanol.

• Incubate @ -20°C for overnight.

• Spin @ 12,000 xg for 10 min @ +4°C.

• Remove supernatant and add 1,200 μL 75% ETOH.

• Spin @ 7,500 xg for 5 min @ +4°C.

• Remove supernatant and let air dry for 15 min @ RT.

• Resuspend in 20 μL of RNase-free water.

• Incubate @ +60°C for 15 min.

• Store @ -80°C, allowing to let warm to RT before quantifying, making sure to keep on ice.

*RNA Quantitation*

RNA was tested for quantity and purity using ultraviolet absorbance at 230nm, 260nm, and 280nm Spectrophotometry was performed in triplicate using a Synergy HTX Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT).

*cDNA Reverse Transcription*

Reverse transcription of RNA to cDNA was conducted using an Applied Biosystems High-Capacity cDNA Reverse Transcription Kit. Reverse transcription of
performed by manufacture’s protocol to yield 1 μg per 20 μL using a Mastercycler thermocycler (Eppendorf, Hauppauge, NY) which is as follows:

- Prepare 2x RT master mix (without RNase Inhibitor) by combining:
  - 2.00 μL 10x RT Buffer
  - 0.80 μL 25x dNTP Mix (100 mM)
  - 2.00 μL RT Random Primers
  - 1.00 μL MultiScrib Reverse Transcriptase
- Add 2x RT master mix with appropriate volume of RNA to yield 1ug.
- Add remaining amount of Nuclease-free H2O to yield a total volume of 20 μL.
- Briefly centrifuge the spin down contents.
- Program thermal cycler conditions listed below:
  - Step 1: 25°C for 10 min
  - Step 2: 37°C for 120 min
  - Step 3: 85°C for 5 min
  - Step 4: Hold at 4°C
- Set reaction volume to 20 μL.
- Start the reverse transcription run.
- After the reverse transcription run is complete, store samples at -20°C.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

PCR was performed in triplicate using the Applied Biosystems ABI 7300 sequence detection instrument and software with TaqMan Universal PCR Master Mix and TaqMan gene expression assays (Applied Biosystems, Foster City, California) in accordance with manufacturer’s instructions. Using standard techniques, reactions were
run with the following thermal cycling conditions: 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 s; followed by 60°C for 1 min. mRNA content was measured using the relative Ct method, compared to as endogenous control (B2M) and converted to a linear function by using a base 2 antilog transformation.

**Statistical Analysis**

Statistical analyses were performed using SPSS Statistics 23 Software (SPSS, Chicago, IL) on log-transformed data. Using RT-PCR, we evaluated gene expression of IL-6, IL-8, IL-10, and TNF-α. To determine the impact that ethnicity may have on PBMC gene expression in response to the high fat shake consumption and lipid-treatment of cultured PMBCs, a repeated-measures ANOVA with emphasis on a time (pre- vs. post-shake) X treatment (control, lipid-treated) X ethnicity (AA vs. CA) interaction were performed. To determine the impact of weight status on PBMC response, a repeated-measures ANOVA with emphasis on a time (pre- vs. post-shake) X treatment (control, lipid-treated) X weight status (lean <27 BMI vs Obese >27 BMI) interaction were performed. All data met assumptions of sphericity and homogeneity of variance. Data are presented as means +/- SD.
Chapter 4. Results

Subject Characteristics

Subject characteristics are presented in Table 1. Table 1 describes an ethnicity subset to include 21 women, 10 AA (age: 28.00±6.60) and 11 CA (age: 26.91±6.28), which were used when analyzing ethnical difference between women, and weight status subset to include 21 women, 11 lean (age: 26.27±5.10; BMI<27.0) and 9 obese (age: 28.67±7.92, BMI≥27.0), which were used to examine weight status differences between women. There were no significant differences seen between lean and obese AA and CA in age, SBP, DBP, resting glucose; while BMI between lean and obese AA and CA were significantly different.

Table 1. Subject Characteristics

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<th>All</th>
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<th>Obese</th>
<th>P-Value</th>
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<tr>
<td>Age</td>
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<td>BMI</td>
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<td>SBP</td>
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<td>DBP</td>
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<tr>
<td>Glucose</td>
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<td>95.36±67.30</td>
<td>97.56±7.92</td>
<td>0.71</td>
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<th></th>
<th>AA</th>
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<th></th>
<th></th>
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</thead>
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<tr>
<td>Age</td>
<td>28.00±6.60</td>
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<td>BMI</td>
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<td>SBP</td>
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<td>117.00±14.94</td>
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<tr>
<td>DBP</td>
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<td>74.25±11.35</td>
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<tr>
<td>Glucose</td>
<td>94.50±12.95</td>
<td>97.00±6.93</td>
<td>92.00±17.71</td>
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<th></th>
<th>CA</th>
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<tr>
<td>Age</td>
<td>26.91±6.28</td>
<td>26.14±5.30</td>
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<td>BMI</td>
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<td>SBP</td>
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<td>DBP</td>
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<tr>
<td>Glucose</td>
<td>97.81±14.37</td>
<td>94.00±7.26</td>
<td>104.50±22.13</td>
<td>0.27</td>
</tr>
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Gene Expression

Time and Treatment

First, the response of PBMCs to lipid oversupply was assessed among all participants in the study. Overall, there was no significant change in IL-6 expression for all participants in the control condition pre- vs. post-shake consumption (Figure 1 Panel A); however, there was an overall trend for a decrease in the expression of IL-6 post-shake (compared to pre-shake) in the lipid-treated condition (pre- 3.11±0.61 vs. post- 2.77±0.59; p=0.06) (Figure 1 Panel B). There was a significant time effect for IL-8 gene expression (p=0.04), where IL-8 expression significantly increased post-shake in both the control (pre- 5.28±0.36 vs post-5.46±0.27; p=0.05) and lipid-treated (pre- 5.33±0.31 vs. post- 5.60±0.35; p=0.01) conditions for all subjects (Figure 2). There was no significant treatment effect (control vs. lipid) for TNF-α (pre- p=0.95; post- p=0.45), IL-6 (pre- p=0.25; post- p=0.49), IL-8 (pre- p=0.88; post- p=0.12), or IL-10 (pre- p=0.99; post- p=0.59).

Ethnic Differences

To assess the impact that ethnicity has on PBMC response to lipid oversupply, all AA women were collapsed into one group (AA) and all CA women were collapsed into one group (CA). There were no baseline differences between ethnic groups in TNF-α, IL-6, IL-8, and IL-10 expression (pre-shake, control and lipid-treated) (Table 2). For IL-6 gene expression, the interaction between time X treatment X ethnicity approached significance (p=0.07) and a significant time X treatment interaction (p=0.04) was observed. Among the AA women only there was a significant decrease in IL-6 gene expression post-shake (compared to pre-shake) in the lipid-treated condition (pre-
3.22±0.64 vs. post- 2.54±0.59; \( p=0.04 \) (Figure 1 Panel B). In the post-shake, control condition IL-8 gene expression was significantly higher among the AA women (AA 5.84±0.38 vs. CA 5.48±0.30; \( p=0.03 \) (Figure 2 Panel A).

Table 2. Baseline Differences in AA and CA Gene Expression

<table>
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<th>AA</th>
<th>CA</th>
<th>P-Value</th>
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<tr>
<td>IL-6</td>
<td>Lipid</td>
<td>3.23±0.60</td>
<td>3.13±0.71</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.14±0.43</td>
<td>3.11±0.62</td>
</tr>
<tr>
<td>IL-8</td>
<td>Lipid</td>
<td>5.34±0.31</td>
<td>5.30±0.47</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.49±0.22</td>
<td>5.29±0.36</td>
</tr>
<tr>
<td>IL-10</td>
<td>Lipid</td>
<td>3.26±0.19</td>
<td>3.10±0.35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.20±0.38</td>
<td>3.09±0.37</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>Lipid</td>
<td>4.64±0.20</td>
<td>4.64±0.35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.67±0.08</td>
<td>4.59±0.32</td>
</tr>
</tbody>
</table>
Figure 1. IL-6 Gene Expression

Panel A IL-6 Control Culture

Panel B IL-6 Lipid-Treated Culture

Note: * represents significant time effect in AA women, $p=0.04$. + represents a trending time effect in all women, $p=0.06$. 
Figure 2. IL-8 Gene Expression

Note: a) represents a significant time effect in all women, lipid-treated: p=0.01; control: p=0.05. b) represents a significant increase in AA women when compared to CA women, p=0.03.
**Weight Status Differences**

To assess the impact that weight status has on PBMC response to lipid oversupply, lean AA and CA women were collapsed into one group (lean) and the obese AA and CA women were collapsed into one group (obese). In the post-shake, lipid-treated condition IL-6 gene expression was significantly lower among obese women (lean- 3.05±0.56 vs. obese- 2.48±0.50; \(p=0.04\)) (Figure 3 Panel A). Post-shake, the expression of IL-8 significantly increased in both the control (pre- 5.32±0.28 vs. post-5.60±0.27; \(p=0.04\)) and lipid (pre- 5.31±0.33 vs. post- 5.58±0.20; \(p=0.05\)) conditions in lean women only (Figure 4); this likely drove the significant increase in IL-8 gene expression seen among all subjects (Figure 2). Additionally, post-shake, the expression of IL-8 was significantly lower in the lipid (lean- 5.63±0.27 vs. obese- 5.31±0.27; \(p=0.02\)) condition in obese women compared to the lean (Figure 3 Panel B).
Figure 3. Gene Expression in Lipid-Treated Cultures of Obese Women

**Panel A IL-6 Gene Expression**

Panel A note: * represents a significant decrease in obese women when compared to lean women, $p=0.04$. Panel B Note: * represents a significant decrease in obese women when compared to lean women, $p=0.02$. 
Figure 4. IL-8 Gene Expression in Lean Women

Note: + represents significant increase in lipid-treated (p=0.05) and * represents significant increase in control (p=0.04) after shake ingestion in lean women.
Chapter 5. Discussion

This is the first study to describe an examination of PBMC response to lipid oversupply in vivo (in response to ingestion of a high fat shake) and in vitro (in response to culturing PBMCs in media supplemented with 250uM oleate: palmiate) among women. The purpose of the study was to determine the impact that ethnicity and/or weight status has on PBMC response to lipid oversupply in terms of the expression of genes that are important in the immune response (inflammation) and metabolism. Our results indicate that the (in vivo) consumption of a high fat shake significantly impacts the expression of IL-6 and IL-8 genes expression profiles (Figure 1 and 2) and that differential expression patterns for IL-6 and IL-8 exist when the impact of ethnicity and/or weight status are considered.

IL-6, synthesized by adipose tissue, endothelial cells, macrophages, and lymphocytes, plays a vital role in multiple systems and processes, including bone metabolism, inflammation, and acute phase reactions; while regulating energy homeostasis and inflammation (Rodriguez-Hernandez et al., 2013). In the present study, we found a decreased gene expression of IL-6 after the ingestion of a high fat shake in the lipid-treated condition among AA (Figure 1 Panel B). Multiple studies have reported an increased expression of IL-6 four hours after the ingestion of a high fat meal within adipose tissue (Dordevic et al., 2015; Kruse et al., 2015; Meneses et al., 2011; Pietraszek, Gregersen, & Hermansen, 2011); this contradicts our findings, suggesting what is occurring in adipose tissue does not represent what is happening systemically from PBMCs. Our study provided an additional dynamic by revealing AA IL-6 expression to be contrasting with previous research. One possible explanation may be that PBMCs
showed an additive effect to the fatty acids used in the lipid-treated culture, though researchers are unsure of how this decreased expression of IL-6 affected the systemic inflammatory status. One probable explanation for the decrease in IL-6 is it is acting as an anti-inflammatory cytokine, but at the time point post-shake ingestion IL-6 was not yet activated; this rationale may explain why we saw a decrease in the expression of IL-6 post-shake ingestion. These findings suggest that ethnic differences may be an important consideration with examining systemic inflammatory maker response due to a lipid overdose.

IL-8, a pro-inflammatory cytokine, is responsible for the targeted recruitment of neutrophils (Bickel, 1993; Hammond et al., 1995); while having roles in chemotactic migration and activation of monocytes, lymphocytes, basophils, and eosinophils at sites of inflammation (Miller et al., 1992). The present study found that there was an increased gene expression of IL-8 four hours after the ingestion of a high fat shake among all women (Figure 2). Additionally, the expression of IL-8 in PBMCs isolated from lean women was also higher after the ingestion of a high fat shake in both lipid-treated and control conditions (Figure 4). These findings parallel with multiples studies that have found significant increases in IL-8 post-acute high fat ingestion (Esser et al., 2013; Myhrstad et al., 2011; van Dijk, Mensink, et al., 2012). A possible explanation for this increased expression of IL-8 is that oleic acid, which was used in the lipid culture and the high fat shake, poses higher gene expression changes in inflammatory genes due to unsaturated fatty acids being more prone to oxidation; thus, may induce more oxidative stress and as a result affect inflammatory status of PMBCs (van Dijk, Mensink, et al., 2012).
Additionally, we found that PBMC gene expression of IL-8 was higher in lipid treatment culture conditions vs control culture conditions (Figure 2). Another explanation may be that PBMCs showed an additive effect of the saturated and unsaturated fatty acids, resulting in an increased pro-inflammatory environment, due to the higher expression of IL-8 in a lipid-treated culture. This may be indicative of why women show a higher prevalence in pro-inflammatory cellular environment, thus aiding to the development of obesity, type-2 diabetes, and other inflammatory diseases.

Moreover, AA showed an elevated gene expression of IL-8 after the ingestion of a high fat shake in the control condition when compared to CA gene expression (Figure 2 Panel A). One explanation of this increased IL-8 expression is the type of fat used in the shake may pose higher gene expression changes in inflammatory genes due to the type of fatty acids in the shake being more prone to oxidation; thus, may induce more oxidative stress and as a result affect inflammatory status of PMBCs (van Dijk, Mensink, et al., 2012). This increased PBMC expression of IL-8 supports previous speculation that AA have an increased pro-inflammatory cellular environment (Park & Kang, 2013) aiding in an increased disease risk (Taylor et al., 2008) and predominance of cardiovascular risk, obesity, high cholesterol, and hypertension (Malayala & Raza, 201).

PBMCs from obese women expressed a lower level of IL-6 and IL-8 after the ingestion of high fat shake in the lipid-treated condition, when compared to lean women (Figure 3). These findings suggest lean women’s PBMC response may be more sensitive to an acute dietary stimulus or may be more sensitive to lipid post-shake ingestion due to changes being seen in lipid-treated culture conditions. It is notable that researchers found no shake effect on obese women’s IL-6 or IL-8 gene expression. A similar study found
that there is no high fat meal effect for the expression of IL-8 in overweight and obese men (Teng, Chang, Chang, & Nesaretnam, 2014). These findings, along with other IL-8 expression pattern findings of this study, suggest that IL-8 could be involved with the development of obesity related disorders (Herder et al., 2005).

A major limitation of our study was the small sample size which could affect the power, thus not being able to detect some statistical differences. Another limitation was that researchers did not know the specific fatty acid makeup of the high fat shake given to subjects. If researchers knew the specific types of fats within the shake, this could have help developed a greater rationale for why some of the inflammatory markers were significantly changed. Lastly, researchers did not stimulate PBMCs while in culture. This lack of stimulation could have prevented researchers from elucidating specific gene expression changes due to the time of PBMC culture.

This study includes many strengths that have not been previously examined. Specifically, PBMC inflammatory gene expression response among AA and CA women has not been fully developed. This study aids in the knowledge of how AA women response to an acute high fat shake and compares them to CA women. Additionally, this study divides all women into weight status categories of obese and lean. These findings can aid in the explanation of how obesity is effected by a high fat overload, thus explaining how the inflammatory status of these individuals is affected. Specifically, the elevated expression of the pro-inflammatory cytokine IL-8 can further explain why women, particularly AA women, are at higher prevalence for metabolic/ inflammatory disease. Researchers speculate the elevated expression of IL-8, seen in this study, aids to
chronic pro-inflammatory status of these individuals which results in higher likelihood of developing these inflammatory diseases (Park & Kang, 2013).
Chapter 6. Conclusion

In this study, we demonstrated that PBMCs express unique gene changes in different ethnic and weight status subsets of women. The purpose of this study was to examine PBMC response to lipid oversupply in vivo (in response to consuming a high fat meal) and in vitro (in response to culturing PBMCs in media supplemented with lipid) among AA and CA women. The secondary purpose was to determine the impact that weight status may have on this response. Among all women, there was a trend for a decreased expression of IL-6, post-shake ingestion, in the lipid-treated culture condition. Additionally, all women showed a significant increase in IL-8 expression, post-shake ingestion, in both the control and lipid-treated culture conditions. AA women showed a significant decrease in the expression of IL-6, post-shake ingestion, in the lipid-treated culture condition. While AA women showed a significantly higher expression of IL-8, post-shake ingestion in the control culture condition, when compared to CA women. Obese women showed a significantly lower expression of IL-6 and IL-8, post-shake ingestion in the lipid-treated culture condition, when compared to lean women. While Lean women showed a significant increase in the expression of IL-8, post-shake ingestion, in both the control and lipid-treated culture conditions. Our data revealed specific gene expression differences of PBMC in regards to IL-6 and IL-8, with time, ethnicity, and weight status effects. In summary, our study showed that PBMCs are sensitive enough to reveal the inflammatory status of women in response to the ingestion of an acute high fat shake, with specific gene expression differences noted among women of different ethnicities (AA vs CA) and weight statuses (lean vs obese). While this PBMC response seen in our study necessitates a greater examination, PBMC response to a high
fat shake elicits an inflammatory response which researchers speculate that this response may lead to chronic inflammatory disease due possible chronic nature of this inflammatory status.
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Appendix A

Informed Consent

INFORMED CONSENT DOCUMENT

Project Title: Metabolic Flexibility among Women in Response to an Acute High Fat Load
Investigators:
Alyssa Olenick - School of Kinesiology, Recreation, and Sport, (724) 504 - 8403
Dr. Jill Maples - School of Kinesiology, Recreation, and Sport, (270) 745 - 4339

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigators will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:**
Metabolic syndrome is often linked to obesity and is a rising concern. It is the combination of a variety of health conditions, such as excess body fat, high blood pressure, high cholesterol, and abnormal triglyceride levels. These risk factors can lead to even more major health conditions such as cardiovascular disease, stroke, diabetes or obesity. Obesity, or excessive weight gain, is self is also a serious public health concern as it contributes to inflammation, and insulin resistance - all of which negatively affect health. Metabolic flexibility is a person’s ability to adapt to utilize the available fuel source, such as fat or carbohydrate, found in the human diet. Contrastingly, metabolic inflexibility is the inability to utilize a given fuel source, such as the switch into fat metabolism after the consumption of a high fat meal. In addition, physical activity improves unfavorable outcomes associated with obesity. African American women have a higher predisposition to certain disease states like obesity, coronary heart disease and metabolic syndrome. However, the connections between physical activity, ethnicity, inflammation, and metabolic dysfunction, particularly in response to a single high-fat meal, independent of a controlled diet, (similar to that which is typically consumed in a Western diet), among Caucasian and African American women have not been studied. This study will examine the impact of ethnicity on inflammatory and metabolic responses to a high-fat meal in Caucasian and African American women.

WKU IRB# 17-021
Approval - 8/26/2016
End Date - 8/26/2017
Expedited
Original - 8/26/2016
2. **Explanation of Procedures:**
The study will involve one visit either Exercise Laboratory within the WKU Medical Sciences Complex at the Medical Center or Smith Stadium East in the Kinesiology Department. The night before the visit, you will be given a list of instructions for consuming a healthy meal that will not disturb your metabolism. You will be asked to not eat or drink anything besides water during the 10 hours prior to the study. Upon arrival, a medical professional will take your vital signs and ask you some questions. After that, you will be asked to lay comfortably on a bed. A clear hood-like device will be placed over your head to measure your metabolism for ~40 minutes (total throughout the study session). You will be asked to sit quietly and relax during this measurement. After this measurement is complete, the nurse practitioner will do a blood draw by inserting a catheter (soft, plastic tube) in the bend of your arm or your hand. Following this blood draw, you will be provided with a high-fat meal consisting of a pre-made smoothie from Smoothie King Inc. Following the ingestion of the meal, you will be asked to remain resting in the lab for four hours. During these four hours, you will be able to work, read, watch TV, use your phone, etc. Blood will be drawn three times over the course of the four hours by the nurse from the same catheter (i.e. you will only be “stuck” one time). During the last half hour, the hood-like device will be used again to measure metabolism (for approximately 10 minutes). At the end of the study visit, the catheter will be removed. After this visit, you will be given a wristwatch that monitors your physical activity levels. You will wear this watch for a week, and then return it to the lab.

3. **Discomfort and Risks:**

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<th>Likely/Common</th>
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<tr>
<td>Mild</td>
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<tr>
<td>- Pain from the blood draws</td>
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<tr>
<td>- Gastrointestinal discomfort from the high-fat meal</td>
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<th>Less Likely/Less Common</th>
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<tr>
<td>Serious</td>
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<td>- Bruising or fainting from the blood draws</td>
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<td>- Anxiety from the hood measurement if claustrophobic</td>
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<td>Serious</td>
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<td>- Infection from blood draws</td>
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4. **Benefits:**
You will not benefit directly from being in the study. However, you will learn information about your metabolism and other baseline health measures (e.g., body fat %, resting blood pressure, etc.). We hope the knowledge you gain will help understand factors that influence the overall metabolic health of women. You will be paid $50 for your participation in this study. This sum will be paid upon return of the watch used in this study.

**Institutional Review Board**

WKU IRB# 17-021
Approval - 8/26/2016
End Date - 8/26/2017
Exempted
Original - 8/26/2016

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5. **Confidentiality:**

To help protect your confidentiality, we will do everything we can to keep your information private and protected. Your research file will contain identifiable information such as your name, patient ID#, and birthday. Protected Health Information (PHI) will be created by the study. Study PHI will be kept in your research record and only the research team will have access to the information. The data obtained from this study will be kept confidential. Patients are assigned a study specific identifying number (PID) upon entry to the study, after which all medical information is referenced by this number. Databases that contain private health, medical or research information are behind firewalls, require password/username for access, are maintained using the PID, and only the PI and Co-PIs, have access to the code that matches the PID with other patient identifiers. All hardcopy data records are stored in locked file cabinets and kept in a locked office. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be identified.

6. **Refusal/Withdrawal:**

Refusal to participate in this study will have no effect on any future services you may be entitled to from the University or from your physician. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

*You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks. If a medical emergency does occur, you understand that you are responsible for any costs incurred, including but not limited to the services of Emergency Medical Technicians, emergency room care, hospitalization, etc. We strongly encourage you to ensure that you have adequate health insurance coverage or other means of satisfying any costs for which you will be liable.*

____________________  ____________________
Signature of Participant  Date

____________________  ____________________
Witness  Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT
THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY
THE WESTERN KENTUCKY UNIVERSITY INSTITUTIONAL REVIEW BOARD
Paul Mooney, Human Protections Administrator
TELEPHONE: (270) 745-2129

WKU IRB# 17-021
Approval - 8/26/2016
End Date - 8/26/2017
Expedited
Original - 8/26/2016
Appendix B

Adapted from Pretest meals created by Registered Dieticians at the Washington University School of Medicine’s Metabolic Kitchen as part of the Clinical Research Unit.

Pretest Meals

Lean (800 calories), over weight (1000 calories), and obese (1200 calories) women

Lean (800 calories) Meal:

In order to prepare for the testing on the WKU, we would like to ask for you to follow a specific diet for the dinner the night before you come into the Lab.

Begin your dinner meal around 6 pm, have an evening snack around 9 pm, and then only drink water after that.

Eat all of the food on the menu to get adequate amounts of food.

Avoid alcohol for 24 hours before coming in for the testing.

Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper

Do not consume any caffeine or chocolate

Dinner:
3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, beef, pork, fish or tofu
1 medium baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice
1 ounce sour cream or ¼ cup of gravy or sauce (like Alfredo) for potato, pasta or rice
½ cup carrots, green beans, asparagus, or other non-starchy vegetable
1 dinner roll, slice of bread or equal amount of pita, naan or corn bread
2 teaspoons margarine or butter
½ cup grapes or one small peach, orange, apple or other desired fruit
½ cup ice cream, sherbet or frozen yogurt or 1 serving vanilla wafers or shortbread cookies
½ cup apple or other fruit juice

Evening Snack- to be eaten at 9 pm:
3 graham cracker squares
1 cup 2% milk

Or
½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after the health professional tells you may eat at the Exercise Laboratory.
Overweight (1000 calories) Meal:

In order to prepare for the testing at the Medical Center, we would like to ask for you to follow a specific diet for the dinner the night before you come to visit us.

Begin your dinner meal around 6 pm, have an evening snack around 9 pm, and then only drink water after that.

Eat all of the food on the menu to get adequate amounts of food.

Avoid alcohol for 24 hours before coming in for the testing.

Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper

**Do not consume any caffeine or chocolate**

**Dinner:**

- 3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, beef, pork, fish or tofu
- 1 baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice
- 1.5-ounce sour cream or ¼ cup of gravy or sauce (like Alfredo) for potato, pasta or rice
- ¾ cup carrots, green beans, asparagus, or other non-starchy vegetable
- 1.5 dinner roll, slice of bread or equal amount of pita, naan or corn bread
- 1.5 teaspoons margarine or butter
- ¾ cup grapes or one small peach, orange, apple or other desired fruit
- ½ cup ice cream, sherbet or frozen yogurt or 1 serving vanilla wafers or shortbread cookies
- ¾ cup apple or other fruit juice

**Evening Snack- to be eaten at 9 pm:**

- 3 graham cracker squares
- 1 cup 2% milk
  
  **Or**
  
- ½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after your nurse tells you may eat at the Exercise Laboratory.
Obese (1200 calories) Meal:

In order to prepare for the testing at WKU, we would like to ask for you to follow a specific diet for the dinner the night before you come into the Lab.

Begin your dinner meal around 6 pm, eat your evening snack around 9 pm, and then drink water only after that. Eat all of the food on the menu to get adequate amounts of food. Avoid alcohol for 24 hours before coming in for the testing. Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper.

**Do not consume any caffeine or chocolate**

**Dinner - to be eaten around 6 pm:**
3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, turkey, beef, pork, fish or tofu
1 large baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice
2 ounces sour cream or ½ cup of gravy or sauce (like Alfredo) for potato, pasta or rice
1 cup carrots, green beans, asparagus, or other non-starchy vegetable
2 dinner rolls, 2 slices of bread or equal amounts of pita, naan or corn bread
1 tablespoon margarine or butter
1 cup grapes or one large peach, orange, apple or other desired fruit
1 slice cheese or small carton of yogurt
½ cup ice cream, sherbet or frozen yogurt or 1 serving vanilla wafers or shortbread cookies
1 cup of skim or low fat milk

**Evening Snack - to be eaten at 9 pm:**
3 graham cracker squares
1 cup 2% milk

**Or**
½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after your nurse tells you may eat at the Exercise Laboratory.