MONITORING MONOCYTE \textit{ox}LDL PHAGOCYTOSIS AS A CARDIOVASCULAR DISEASE RISK FACTOR FOLLOWING A HIGH-FAT MEAL

Andrea L. Henning, BS

Thesis Prepared for the Degree of
MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

December 2014

APPROVED:

Brian McFarlin, Major Professor
Jakob Vingren, Committee Member
David Hill, Committee Member
Ed Dzialowski, Committee Member
Art Goven, Chair of the Department of Biological Science
Mark Wardell, Dean of the Toulouse Graduate School
Macrophage-derived foam cells play a predominant role in the deposition of arterial plaques during the early stages of atherosclerosis. The deposition of arterial plaques is known to be effected by several factors, including a person’s dietary habits. The consumption of a high-fat (>60% of calories from fat) meal is known to elevate serum LDL and triglycerides, which have been previously implicated in the formation of foam cells. One limitation of current research models is that it is not possible to directly measure foam cells in vivo. Thus, the purpose of the present study was to validate the use of blood derived monocytes as a proxy measure of foam cells. In order to complete this objective, we evaluated monocyte oxLDL phagocytosis capacity following consumption of a high-fat meal. Eight men and women participated in the present study and venous blood samples were collected prior to the meal, 1-h, 3-h, and 5-h post-meal. Monocytes (CD14+/16- and CD14+/16+) were evaluated for adhesion molecule expression (CD11a, CD11b, and CD18), scavenger R (CD36) expression, and oxLDL phagocytosis using an image-based flow cytometry method developed in our laboratory for this purpose. Data was statistically analyzed for significance using a single-factor ANOVA with repeated measures and a $p < 0.05$. Consumption of a high-fat meal caused an increase significant increase in the proportion of pro-inflammatory monocytes (CD14+/16+) and a decrease in classic monocytes (CD14+/16-), with the greatest difference occurring at 5 h post prandial ($p = 0.038$). We also found that pro-inflammatory monocyte expression of adhesion molecules and CD36 increased in a
manner that would promote in vivo movement of monocytes into the subendothelial space. Finally, over the course of the 5 h postprandial period, the majority of oxLDL uptake occurred in pro-inflammatory compared to classic monocytes. These results suggest that consuming a high-fat meal increases the potential of monocytes to become foam cells for at least 5 h postprandial.
Copyright 2014

by

Andrea L. Henning
ACKNOWLEDGEMENTS

The development of this protocol was funded in part by a grant from the TACSM-SRDA program.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 2 METHOD</td>
<td>4</td>
</tr>
<tr>
<td>CHAPTER 3 RESULTS</td>
<td>13</td>
</tr>
<tr>
<td>CHAPTER 4 DISCUSSION</td>
<td>19</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>23</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Subject Characteristics. Data Represented as Mean ± SEM ..................... 5
Table 2. Meal Composition. Data Represented as Mean ± SEM ............................. 8
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Experimental timeline</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>IDEAs gating</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Monocyte concentration</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Adhesion molecule expression</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>oxLDL phagocytosis</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>Phagocytosis by phenotype</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>CD36 expression</td>
<td>18</td>
</tr>
</tbody>
</table>
Atherosclerosis is a disease that can be described as a form of chronic inflammation that ultimately leads to the development of arterial plaques (Glass, 2001). In the early stages of atherogenesis, the predominant component contributing to arterial plaques are macrophage-derived foam cells that have accumulated large amounts of modified lipids (Pordez, 2002). Several studies have demonstrated that high concentrations of low-density lipoproteins (LDL) in the plasma pose a major risk for plaque development and cardiovascular disease (den Hartigh, 2001; Graner, 2006; Hyson, 2003). If concentrations of plasma LDL exceed the endocytotic capacity of endothelial cells, the surplus LDL particles become trapped in the intima where they are susceptible to many forms of oxidative stress (Glass, 2001). Extensive oxidation of LDL results in the production of oxidized LDL (oLDL), the primary constituent responsible for initiating the process that leads to arterial plaque deposition (Jialal, 1996). When oLDL particles are present in the intima, they act as an inflammatory stimulus, which increases adhesion molecule expression on the surface of endothelial cells. In response to the inflammatory stimulus, monocytes are recruited to the lesion-prone site where they adhere to the endothelial wall and receive signals for transmigration into the intima (Glass, 2001). Subsequently, these monocytes differentiate into macrophages for the removal of oLDL particles through phagocytosis. The macrophages continue to phagocytize oLDL until they induce cytoplasmic overload, resulting in macrophage death and the formation of a lipid laden foam cell (Schlitt, 2004).
The formation of these macrophage-derived foam cells, and eventually arterial plaques, can be directly attributed to higher quantities of fat in an individual’s diet (Maffeis, 2012). There are several different genetic and environmental risk factors that can contribute to elevated LDL levels, but diet is one of the few risk factors that can be controlled. The postprandial lipoprotein profile of an individual is important to consider since the typical American consumes 20-70 g of fat per meal, three or more times a day (Hyson, 2003). Meals with a high fat content (> 52% of total calories) result in an elevated postprandial lipid profile for several hours after ingestion. This causes an increased and prolonged concentration of triglyceride-rich LDL (TGRL) in the plasma (Maffeis, 2012). An elevation of TGRL causes an increase in monocyte activation and adhesion molecule expression (den Hartigh, 2013), potentially representing a transient shift toward the formation of foam cells and arterial plaque.

While atherosclerosis is generally considered to be a chronic disease, recent research from our laboratory and others has demonstrated that a single high-fat meal increases monocyte adherence by altering cell-surface adhesion molecule expression (Schrijvers, 2005). It is reasonable to suspect that a single high-fat meal must also alter monocyte/macrophage interaction with oxLDL. Due to the location of endothelial tissue, the examination of foam cell formation in vivo is impossible without using very invasive means. Since circulating monocytes are essentially naïve foam cell precursors, they represent a suitable proxy measure for measuring oxLDL uptake and foam cell formation in vitro.

In order to better understand foam cell formation and plaque development, it is important to first identify patterns of change in general monocyte/macrophage activity.
and function. Monocytes and macrophages express cell-surface adhesion molecules, including CD11a, CD11b, and CD18, which play a major role in arterial wall adhesion and transmigration into the intima (Kostidou, 2009). Several studies have also demonstrated the importance of the αMβ2 (CD11b/CD18) receptor complex in monocyte rolling and adherence to the endothelial wall (Husemann, 2001; Kostidou, 2009). The examination of postprandial changes in these adhesion molecules could reveal important information about the effects of diet on the potentiality of monocytes to adhere to the vascular wall and then transmigrate into the intima. The scavenger receptor CD36 is also important to examine because of its role in mediating internalization of oxLDL. After LDL particles have been oxidized they are no longer bound by LDL receptors; instead, oxLDL is recognized by CD36 on the surface of monocytes and macrophages (Pordez, 2002). Changes in postprandial CD36 expression may demonstrate that diet also transiently alters the macrophages’ potential to uptake oxLDL. It has also been suggested that monocytes with high expression of CD11b/CD18 as well as CD36 that have transmigrated into the subendothelial space can signal for reactive oxygen species (ROS) production upon ingestion of oxLDL (Husemann, 2001). The purpose of this study is to determine the effects of a single high-fat meal on monocyte concentration, adhesion molecule expression, and phagocytosis of oxLDL. To accomplish this, meal induced changes in monocyte (CD14+) activity will be measured using CD11a, CD11b, CD18, and CD36.
CHAPTER 2

METHOD

Subjects

Prior to beginning the present study, we obtained approval from the UNT IRB and all subjects gave their written consent to participate. Young (18-35y) men (n = 6) and women (n = 6) were recruited for the present study. Once subjects had been screened for inclusion/exclusion criteria, they were scheduled for a testing appointment. Subjects were of a body composition that does not exceed 25% body fat for men and 35% body fat for women and did not have physician-diagnosed diabetes mellitus or any physician-diagnosed form of cardiovascular disease. Subjects also had average or below average cardiovascular fitness level (VO\textsubscript{2peak} of 36-46 mL/kg/min for men, 32-42 mL/kg/min for women). We limited our study population to individuals of average fitness because fitness level may influence the response to a high-fat meal. According to ACSM standards (Thompson, 2010), subjects were considered to be “apparently healthy” (Table 1). After enrollment in the study, subjects had their resting energy expenditure (REE), body composition, and peak oxygen consumption measured. These tests are described with additional detail in subsequent sections.
Table 1.

Subject Characteristics. Data Represented as Mean ± SEM

<table>
<thead>
<tr>
<th></th>
<th>Male (n=4)</th>
<th>Female (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>23 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Height (inches)</td>
<td>71 ± 2</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82 ± 3</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>%BF</td>
<td>16 ± 4</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>BMI</td>
<td>26 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>VO₂ (ml/kg/min)</td>
<td>44 ± 3</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>1689 ± 95</td>
<td>1226 ± 53</td>
</tr>
<tr>
<td>Daily Caloric Requirement (kcal/day)</td>
<td>2196 ± 124</td>
<td>1593 ± 69</td>
</tr>
</tbody>
</table>

Resting Energy Expenditure Test

Subjects arrived in the laboratory between 700 h and 1000 h following an overnight fast (>12 h) and abstention from exercise (>24 h). REE was measured using an automated metabolic cart (ULTIMA™ PF system; Medical Graphics Co.; St. Paul, MN). Approximately 30 min after arrival and quiet resting, the subjects were fitted with a pre-vent mask to collect expired gas while at rest. The subject was then instructed to lie motionless and breathe into the calorimeter for 20 min.

Body Composition Test

Body composition was determined using a standard whole body dual-energy X-ray absorptiometry (DXA) scan (GE Lunar Prodigy; Baltimore, MD). Results were interpreted using GE Adult Whole Body Software (GE Lunar Prodigy; Baltimore, MD).
VO\textsubscript{2peak} Test

VO\textsubscript{2peak} was measured during a cycle ergometer test using an automated metabolic cart (ULTIMA\textsuperscript{TM} PF system). Prior to the start of the exercise test, there was a 1 min warm-up stage consisting of a 50 W work rate. The work rate for the first stage is 75 W. After 3 min, and every 3 min thereafter, the work rate was increased by 25 W for women and 50 W for men and subjects continued until volitional fatigue (~12-15 min). VO\textsubscript{2peak} was determined as the average of the three highest VO\textsubscript{2} values measured (15 s averages).

Experimental Testing Timeline

Subjects were scheduled to arrive at the laboratory between 1000 h and 1100 h following an overnight fast (>12 h) and abstention from exercise (>24 h). Subjects were then seated for 30 min of rest in a quiet room prior to collection of the baseline blood sample. After sample collection, subjects rested for 30 min before they were given their meal for which they had 30 min to consume. Additional blood samples were collected at 1, 3, and 5 h postprandial. Subjects were allowed to drink water but no food other than the high-fat meal or any drink other than water was consumed during the experimental trial. The timeline below highlights the key aspects of the experimental treatment session (Figure. 1).
Blood Collection

Venous blood was collected using venipuncture at each time point into 3 separate collection tubes. The collected tubes were treated with either EDTA, a clot activator (SST), or lithium heparin. Serum was harvested from the clotted tube after centrifugation for 20 min at 500 x g and frozen at -80°C until analysis of serum triglyceride, cholesterol, and glucose concentration via an enzymatic assay (Pointe Scientific; Canton, MI). EDTA-treated blood was used for complete blood count (BD-3200; Mindray; Mahwah, NJ). Heparinized blood was held at room temperature (~22°C) and processed within 3-h of collection to measure monocyte activity.

High-Fat Meal

The subjects consumed a meal that contained ~65% of their estimated daily caloric needs and ~85% of their daily fat allowance. Composition of each subject’s meal was determined partially based on collected REE data. To account for activities of daily living, REE was multiplied by 1.3 (activity factor adjustment) to provide the subjects’ daily caloric requirement. For example, a typical 70 kg man with an REE of 1750 kcal/d and a daily caloric requirement of 2275 kcal/d (1750 kcal x 1.3) would consume a 1,479 kcal (2275 kcal x 0.65) meal containing 75 g of fat (daily fat allowance of 88.5 g x 0.85).
The high-fat meal was provided in the form of a commercially available thin crust cheese pizza. One serving of the pizza (90 g) contained 230 kcal, 12 g fat, 25 g cholesterol, 9 g protein, and 22 g carbohydrates (Table 2).

Table 2.

*Meal Composition. Data Represented as Mean ± SEM*

<table>
<thead>
<tr>
<th></th>
<th>Male (n=4)</th>
<th>Female (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pizza (g)</td>
<td>556 ± 32</td>
<td>405 ± 21</td>
</tr>
<tr>
<td>Calories (kcals) (65% DV)</td>
<td>1422 ± 82</td>
<td>1036 ± 53</td>
</tr>
<tr>
<td>Fat (g) (85% DV)</td>
<td>74 ± 5</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Sat Fat (g)</td>
<td>31 ± 2</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>56 ± 3</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>Carb (g)</td>
<td>136 ± 8</td>
<td>99 ± 5</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Cholesterol (g)</td>
<td>155 ± 9</td>
<td>113 ± 6</td>
</tr>
</tbody>
</table>

Antibody Cocktail Preparation

Equal parts of CD11a-FITC (eBioscience, Inc.; San Diego, CA; DF 1:20), CD11b-BV605 (BioLegend; San Diego, CA; DF1:5), CD14-APCeFluor780 (eBioscience, Inc.; DF 1:20), CD16-PE/Atto594 (DF1:20) (unconjugated CD16 was purchased from eBioscience, Inc. and conjugated using a PE/Atto594 lighting-link kit from Innova Biosciences (Cambridge, UK)), CD18-PE/Cy5 (BioLegend; DF 1:20), and CD36-
Monocyte oxLDL Phagocytosis

Using a method described previously (Henning, 2014), heparinized whole blood (150 µL) was transferred into the bottom of two, 1.2 mL polypropylene tubes. One tube was used as the treatment tube, while the other was combined with rosiglitazone (25 µL; 14.30 ng/mL final concentration; Sigma-Aldrich; St. Louis, MO) as a negative control. oxLDL-Dil (25 µL; 15 µg/mL final concentration; Life Technologies Corp; Portland, OR) was added to both tubes, covered with a breathable sealing film (Excel Scientific; St. Louis, MO), and incubated for 1 h in a 37°C, 5% CO₂ humidified incubator. After incubation, EDTA (29.2 µL; 584 µg/mL final concentration) was added to release cells that may have adhered to the tube. The cell prep was washed and the supernatant aspirated following centrifugation (15 min at 400 x g). Cell-surface staining was completed by adding the pre-diluted antibody cocktail (60 µL) and incubating for 1 h on ice and in the dark. After cell-surface staining, 1-step fix/lyse (750 µL; eBioscience, Inc.) was used fix leukocytes and lyse RBCs. The cell fraction was washed twice and FlowSight calibration beads (25 µL) were added prior to acquisition.

Acquisition and Analysis

Samples were acquired using a FlowSight (EMD Millipore; Seattle, WA) image-based flow cytometer equipped with a violet laser (405 nm, 100 mW), blue laser (488 nm, 60 mW), red laser (642 nm, 75mW), and side scatter laser (785 nm, 8.25 mW). The samples were loaded into the autosampler and 20,000 monocyte (CD14+) events were collected for each tube. Analysis was completed using IDEAS software (EMD Millipore;
v.6.1). For each sample, monocytes were identified using a dot plot with CD14 intensity vs. CD16 intensity (Figure. 2A). The identified monocyte population was then divided into 2 phenotype populations; classic monocytes (CD14+/CD16-) and pro-inflammatory monocytes (CD14+/CD16+) (Figure. 2B). OxLDL phagocytosis was determined in each phenotype population using a dot plot with oxLDL intensity vs. CD14 intensity (Figure. 2C and 2D). The monocyte population was also used to measure adhesion molecule expression using CD11a, CD11b, and CD18 intensity (Figure. 2E), and scavenger R expression using CD36 intensity (Figure. 2F).
Figure 2. IDEAs gating (A) Monocytes (CD14+) were identified using a dot plot with CD14 intensity vs. CD16 intensity. (B) Classic monocytes (CD14+/CD16-) and pro-inflammatory (PI) monocytes (CD14+/CD16+) were identified using a daughter dot plot with CD14 intensity vs. CD16 intensity. (C and D) OxLDL phagocytosis (oxLDL+) was determined for classic (C) and pro-inflammatory (D) monocytes using a daughter dot plot with oxLDL intensity vs. CD14 intensity. (E) Adhesion molecule expression was measured for each subset using CD11a, CD11b, and CD18 intensity. (F) Scavenger R expression was measured for each subset using CD36 intensity.
Statistical Analysis

All statistical analyses were completed using SPSS v. 21.0 (Chicago, IL). Prior to formal statistical testing, data was checked for normal distribution and constant error variance using the EXPLORE function and residual/predicted plots. Non-normal data was log transformed prior to statistical analysis. Each outcome variable was analyzed using a single factor (Time: PRE, 1H, 3H, and 5H), repeated measures ANOVA. Significance was set at $p < 0.05$. All alpha values were adjusted for repeated measures using the Huynh-Feldt method. Location of significant effects was determined using individual student $t$-tests with a Bonferroni correction for multiple comparisons. All data is represented as mean ± SEM.
CHAPTER 3

RESULTS

Monocyte Concentration

Total monocyte concentration, regardless of phenotype, did not change over the postprandial period. The classic monocyte (CD14+/CD16-) proportion decreased 3% ($p = 0.031$) (Figure. 3A), while the pro-inflammatory monocyte (CD14+/CD16+) proportion increased 115% ($p = 0.038$) 5 h postprandial (Figure. 3B).
Figure 3. Monocyte concentration. The proportion of classic monocytes decreased significantly 5 h postprandial (A) while the proportion of pro-inflammatory monocytes increased significantly at 5 h postprandial (B).
Adhesion Molecule Expression

There were no significant changes in adhesion molecule expression among classic monocytes assessed in this study. Further, we observed that pro-inflammatory monocytes (regardless of oxLDL uptake status) had a 433% increase in the co-expression of CD11b/CD18 ($p < 0.001$) at 3 h postprandial, but no meal-associated change in the co-expression of CD11a/CD18 (Figure 4).

**Figure 4.** Adhesion molecule expression. Pro-inflammatory monocytes had a significant increase in CD11b/CD18 expression at 3 and 5 hour postprandial.
Monocyte oxLDL Phagocytosis

Monocyte phagocytosis of oxLDL increased by 196% and 339% at 3 and 5 h postprandial respectively ($p < 0.001$; Figure. 5). The proportion of classic monocyte that had phagocytized oxLDL increased 432% ($p < 0.001$), while the proportion of pro-inflammatory monocyte that had ingested oxLDL increased 387% ($p = 0.001$) 5 h postprandial (Figure. 6A). The mean proportion of pro-inflammatory monocytes that had ingested oxLDL (13.3%) is significantly higher than the mean proportion of classic monocytes that had ingested oxLDL (3.2%) ($p = 0.003$; Figure. 6B). Consistent with our hypothesis, pro-inflammatory monocytes that had phagocytized oxLDL had a 288% greater fluorescent intensity of CD36 than those that had not phagocytized oxLDL ($p = 0.001$; Figure. 7).

Figure 5. oxLDL phagocytosis. Monocyte phagocytosis of oxLDL significantly increased at 3 and 5 hour postprandial in meal response samples. There was relatively no change in the inhibitor control samples.
Figure 6. Phagocytosis by phenotype. (A) The concentration of classic and pro-inflammatory monocytes that had ingested oxLDL significantly increased postprandial. (B) The mean concentration of pro-inflammatory monocytes that had ingested oxLDL is significantly higher than the concentration of classic monocytes.
Figure 7. CD36 expression. Pro-inflammatory monocytes that had ingested oxLDL had a significantly higher expression of CD36 than pro-inflammatory monocytes that had not ingested oxLDL.
CHAPTER 4

DISCUSSION

The key finding of this study was that consumption of a single high-fat meal increased several pre-clinical risk factors associated with atherosclerosis and atherogenesis. We found that a high-fat meal causes an increase in monocyte phagocytosis of oxLDL and that pro-inflammatory monocytes accounted for the vast majority of oxLDL phagocytosis during the postprandial period. Along with increased uptake of oxLDL, pro-inflammatory monocytes also had increased cells-surface expression of the CD11b/CD18 complex. This latter finding indicates that these monocytes had increased capacity to transmigrate from the blood into the subendothelial space. Pro-inflammatory monocytes also had greater CD36 (oxLDL scavenger receptor) expression than classic monocytes, which may partially explain why they were responsible for the majority of postprandial oxLDL uptake.

Plaque deposition is a result of an inflammation-mediated response involving lipid accumulation, monocyte adherence to endothelial cells, monocyte transmigration into the subendothelial space, and monocyte-derived macrophage phagocytosis of modified lipoproteins (Glass, 2001). Pro-inflammatory monocytes present greater inflammatory properties than classic monocytes, which is why many researchers consider them pro-atherogenic monocytes (Jaipersad, 2014; Virella, 2002). In healthy humans, pro-inflammatory monocytes account for a very small portion (<10%) of total circulating monocytes (Ziegler-Heitbrock, 2007). Consistent with previous reports, the pre-meal proportion of pro-inflammatory monocyte was 2.4%. In patients with chronic CVD or hypercholesterolemia, pro-inflammatory monocyte proportions can range...
between 11% and 20% of total circulating monocytes (Schlitt, 2004; Tapp, 2012). In the present study, the pro-inflammatory monocyte proportion increased to 5.4% at 5 h postprandial. While the mean postprandial proportion of pro-inflammatory monocytes did not reach levels reported in patients with chronic conditions, it is important to note that 25% of the subjects’ postprandial pro-inflammatory monocyte concentration reached proportions ranging from 10% to 14%, which is indicative of transient high disease risk. Results from the present study suggest that consuming a single high-fat meal increases the concentration of pro-atherogenic monocytes for at least 5 hours postprandial to proportions that are up to twice their resting concentration.

Foam cells are characterized as lipid-laden monocyte-derived macrophages within the arterial wall. The accumulation of these foam cells in the intima is a major source of both early and late atherosclerotic lesions (Ross, 1995). Foam cells are formed when macrophages phagocytize modified lipoproteins that are trapped in the subendothelial space, such as oxLDL, resulting in macrophage death (Schrijvers, 2005). Consistent with previous reports (den Hartigh, 2013; Glass, 2001; Hyson, 2003), we demonstrated that the high-fat meal used in this study caused a 115% increase in serum triglycerides at 3 h postprandial. Other studies have also demonstrated an acute link between excessive postprandial triglyceride levels, such as those observed in the present study, and an increase in serum oxLDL concentrations (Maffeis, 2012). We demonstrated that consuming a high-fat meal caused a 339% increase in monocyte phagocytosis of oxLDL 5 h postprandial. We also observed that 80% of the monocytes that had ingested oxLDL were pro-inflammatory monocytes, supporting the notion that pro-inflammatory monocytes are pro-atherogenic and play a greater role in foam cell
formation than classic monocytes. We used the approach described in this study because one limitation of current human models is that it is not practical to collect macrophages from the subendothelial space; however, given the common lineage, blood pro-inflammatory monocytes represent a potentially useful proxy target. To our knowledge, the present work is the first published study to report the finding that consumption of a high-fat meal transiently increases pro-inflammatory monocyte phagocytosis of oxLDL. More research is needed to fully quantify what the observed changes mean with respect to risk of foam cell formation.

OxLDL is characterized as an adhesion-promoting agent involved in monocyte infiltration of the endothelial wall (Lehr, 1995). The presence of oxLDL in the subendothelial space has been demonstrated to increase expression of CD11b and CD18 adhesion receptors on monocytes which are mainly responsible for monocyte adhesion and migration (Husemann, 2001; Kostidou, 2009; Lehr, 1995). Data previously reported from our lab demonstrated that consuming a high-fat meal causes an increase in monocyte expression of CD11a and CD18, indicating that macronutrient meal composition effects monocyte adhesion and migration (Strohacker, 2012). Consistent with these reports, we demonstrated that consuming a high-fat meal increases expression of the CD11b/CD18 receptor complex in pro-inflammatory monocytes 457% 3 h postprandial. This data gives further support to the notion that pro-inflammatory monocytes have a key role in the development of atherosclerosis.

Scavenger receptors in monocytes and macrophages are important for regulating uptake of apoptotic cells and modified lipoproteins. CD36 is a scavenger receptor associated with atherosclerotic lesion development that expresses high affinity for
oxLDL (Glass, 2001; Pordez, 2002). Increased macrophage cell-surface expression of CD36 is positively correlated with increased foam cell formation and lesion development (Glass, 2001; Pordez, 2002). We observed that phagocytosis of oxLDL was associated with a 288% increase in pro-inflammatory monocyte fluorescent intensity of CD36; however, CD36 fluorescent intensity increased only 6% in classic monocytes when phagocytosis occurred. These findings indicate that pro-inflammatory monocytes have greater potential for oxLDL phagocytosis than classic monocytes.

These results indicate that eating a single high-fat meal increased the proportion of pro-inflammatory monocytes, which are pro-atherogenic, and their uptake of oxLDL. Furthermore, we discovered that this pro-inflammatory phenotype also presented a meaningful postprandial response in their adhesion molecule expression. Pro-inflammatory monocytes displayed a significant increase in expression of the CD11b/CD18 complex which plays a major role in monocyte adherence to endothelial cells and transmigration through the endothelial wall. The up-regulation of CD11b/CD18 supports the notion that oxLDL acts as an adhesion-promoting stimulant in inflammatory processes (Lehr, 1995). Pro-inflammatory monocytes also present a significant postprandial response in their expression of CD36. As CD36 is known to have a primary role in leukocyte recognition of many species of modified lipoproteins, our findings suggest that the consumption of a high-fat meal increases monocyte affinity for oxLDL. Overall, our data gives evidence that consuming a single high-fat meal increases susceptibility to foam cell formation for at least 5 h postprandial. More research is needed to investigate what happens beyond 5 h postprandial and if this process is enhanced by consuming consecutive meals.
REFERENCES


23
cell formation via the scavenger receptor CD36 and is enriched in atherosclerotic lesions. *The Journal of Biological Chemistry, 277*(41), 38517-38523.


