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Differential effect of 14 free fatty acids in the expression of inflammation biomarkers on human coronary arterial cells

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DIFFERENTIAL EFFECT OF 14 FREE FATTY ACIDS IN THE EXPRESSION OF INFLAMMATION BIOMARKERS ON HUMAN CORONARY ARTERIAL CELLS

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This thesis is dedicated to husband Franklin, who supported me every day and motivated me to never give up.

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TABLE OF CONTENTS

ABSTRACT

Cardiovascular disease is the leading cause of death in the US, and circulating free fatty acids (FFAs) are known risk factors associated with cardiovascular inflammation. The influence of 14 dietary FFAs (including saturated, mono-, poly-unsaturated and trans), on the expression of inflammatory markers in human coronary arterial smooth muscle (HACSM) and endothelial (HCAEC) cells using a cell culture model was investigated. HACSM and HCAEC cell cultures were incubated with 200 µM of each FFA for 8 or 24 h respectively at 37 °C in a 5% CO_2 humidified incubator. Inflammatory biomarkers were assessed by ELISA or Western Blot in the supernatant or cell lysates respectively. Results showed significant differences in the expression of inflammatory biomarkers among the fatty acid treatments and the control, with myristic and palmitic acids being identified as the most and linoleic acid as the least pro-inflammatory. This confirms that FFAs can induce low-grade inflammation in human coronary arterial cells and provides more information on mode of action.

CHAPTER 1
LITERATURE REVIEW

1.1 INTRODUCTION

Digestion and absorption of fat primarily involves emulsification and hydrolysis in the gastrointestinal tract, absorption as free fatty acids or monoglycerides and re-synthesis of new triglycerides in the liver that are carried as lipoproteins (mainly VLDL and chylomicrons) in the blood.[1] Free fatty acids (FFAs) are released into the bloodstream by the action of hormone-sensitive lipase on the adipose tissue or endothelium lipoprotein lipase on circulating lipoproteins. The FFAs are bound to serum albumin and transported to peripheral tissues.[2-5] Postprandial spikes in dietary fatty acids can occur from 4 to 8 hours after eating occasions. FFA levels and the time curves in serum are dependent on the fatty acid profile of the meal and the individual's daily diet.[6,7] FFAs in plasma in healthy adults (fasting-postprandial) range from 350-550 µmol/l, but are elevated due to several conditions including obesity (410-730 µmol/l), insulin resistance (560-570 µmol/l), and type 2 diabetes mellitus (690-770 µmol/l), and can increase up to 1 mmol/l after a high fat meal.[2,8-12] Plasma FFAs are elevated in obese individuals due to the release of FFAs by the enlarged adipose tissue and consequent reduction of FFA clearance from the blood; FFA elevation further results in the inhibition of the anti-lipolytic action of insulin, which increases the FFA release into the bloodstream from chylomicrons and the adipose tissue.[13] Elevation of circulating FFAs has been shown to induce proinflammatory changes and oxidative stress,[14] impair endothelial and vascular function,[12] inhibit immune response,[4] and even promote insulin resistance.[10] Elevated FFAs are thus now considered an

indicator of cardiometabolic stress and a risk factor for cardiovascular disease (CVD)[15] including ischemic heart disease[16] and sudden cardiovascular death.[17,18]

It has been suggested that fatty acid composition of the diet influences the overall inflammatory state, endothelial function, risk of type 2 diabetes and cardiovascular disease. Studies have mainly focused on *trans* fatty acids and indicate that they are directly related to increased health risks.[19-22] However, the mechanism by which not only *trans* but overall increased free fatty acids induce inflammation is not well understood. Furthermore, an assessment of inflammatory response to individual fatty acids has not yet been performed.

The purpose of this study was to evaluate the effect of 14 dietary relevant FFAs including saturated, mono-, poly-unsaturated and *trans*, on the expression of inflammation markers in human coronary arterial smooth muscle (HACSM) and endothelial (HCAEC) cells using a cell culture model. Evaluation of inflammatory biomarkers in cell culture supernatant as well as in the cell cytoplasm will allow for a better understanding of the mechanism by which FFAs promote inflammation and resulting undesirable cardiovascular risks.

1.2 REVIEW OF LITERATURE RELEVANT TO THIS APPLICATION

1.2.1 Atherosclerosis

Cardiovascular disease (CVD) is a group of disorders of the heart and blood vessels and the number one cause of death in the United States accounting for 25% of US deaths.[23-25] Atherosclerosis, a type of of CVD, is a process in which fat, cholesterol, cellular waste, and calcium build up in the inner lining of an artery forming smooth muscle cell-capped plaque. The rupture of plaque causes the release of tissue factor and formation blood clots that may block

blood flow to the heart, brain, or limbs.[26] It is now widely recognized that atherosclerosis is not a "plumbing" problem, where plaque eventually blocks blood flow by itself. Research has revealed that atherosclerosis is a process in which proinflammatory stimuli trigger the expression of adhesion molecules and chemoattractant proteins that allow the infiltration of leukocytes to the intima and the differentiation of monocytes into macrophages.[27,28] Macrophages transform into foam cells by engulfing modified lipoproteins, and the inflammatory response is amplified by other macrophages and T cells. Eventually, inflammatory proteins weaken the smooth muscle cell fibrous cap and make it prone to rupture, tissue factor is released, and a thrombus is formed.[27]

1.2.2 Free Fatty Acids, Inflammation, and CVD

Research shows that increased circulating free fatty acids (FFA) induce inflammation and are a risk factor for CVD.[12,14,15] Obesity results in elevated plasma FFAs due to the lipolysis of the enlarged adipose tissue, which results in reduction of FFA clearance from the blood; additionally, FFA elevation results in the inhibition of the anti-lipolytic action of insulin, which increases the FFA release into the bloodstream.[13] This phenomenon is usually accompanied by impaired insulin signaling due to increased gluconeogenesis in the liver and reduced glucose disposal in muscle, which also results in an impaired suppression of insulin-sensitive lipase in the adipose tissue and impaired removal of FFA from plasma.[29,30]

An increase in plasma FFA concentrations induces proinflammatory changes and oxidative stress including an increase in nuclear factor-κB (NF-κB) binding activity and p65 expression without changes in inhibitor κB (IκB) in circulating mono-nuclear cells (MNCs). Reactive oxygen species (ROS) are generated by MNCs and polymorphonuclear leukocytes

(PMNs) from subjects with elevated FFAs.[12] An increase in macrophage migration inhibitory factor (MIF), a proinflammatory cytokine, and an impairment of the endothelium-dependent flow-mediated vasodilation was also observed as a result of plasma FFA increase.[12] Elevation of FFA in healthy subjects to physiological levels similar to those found in obese individuals results in the expression of markers characteristic of vascular inflammation (myeloperoxidase), endothelial activation (ICAM-1, VCAM-1 and E-selectin), and thrombosis (total plasminogen inhibitor-1).[31]

In vitro, FFAs (palmitic, oleic, and linoleic) impair nitric oxide production in aortic endothelial cells by activating IκB kinase-β (IKK-β), which ultimately results in activation of NF-κB, as one of the underlying mechanisms of inflammation and endothelial dysfunction.[32] Additionally, treatment of RAW 264.7 macrophage-like cells with saturated fatty acids lauric and palmitic strongly induced COX-2, iNOS, and IL-1α through the activation of NF-κB through a possible upstream activation of TLR4. In a similar way, palmitic and stearic acid promote apoptosis of human coronary endothelial cells through NF-κB activation.[33]

Early animal studies carried out with dogs and swine showed that increased plasma FFA may extend the area of coronary ischemia and impair cardiac function due to increased oxygen requirements for FFA oxidation by the myocardium.[34,35] In non-ischemic dogs, myocardial function and oxygen consumption also increased with increasing FFA uptake although there were no changes in mechanical activity of the heart.[36]

Feeding studies have demonstrated that the ingestion of a single high-fat meal promotes inflammation and endothelial activation by increasing the circulating levels of inflammatory cytokines TNF-α, IL-6 and IL-17, plasma endotoxin, and adhesion molecules ICAM-

1 and VCAM-1.[37-39] A study based on the Nurse's Health Study also revealed that consumption of *trans* fatty acids (18:1 and 18:2) was positively related with plasma levels of soluble TNF-α receptors, and with levels of IL-6 and CRP in women with high BMI.[40]

Various authors have identified a relationship between FFA, inflammation and CVD risk. A study based on the Atherosclerosis Risk in Communities Study showed that FFA levels were positively associated with an inflammation score quantifying levels of six systemic inflammation markers (interleukin-6, C-reactive protein, orosomucoid, sialic acid, white cell count, and fibrinogen).[41] Additionally, a study carried out in Paris involving 5250 middle-aged men identified circulating FFA as an independent risk factor for sudden cardiovascular death (SCD) mainly attributed to proarrhythmic mechanisms.[18] A similar study performed in Germany with 3315 participants also identified elevated FFA as an independent risk factor for SCD.[17] A recent study carried out in the US has further demonstrated that FFAs positively correlate with insulin resistance in teens and young adults and with CVD risk factors in older adults, suggesting an effect of low-grade chronic inflammation.[42]

1.2.3 Selected Markers of Inflammation

1.2.3.1 Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is a multifunctional cytokine secreted mainly by monocytes but also produced by many other cells including endothelial and smooth muscle cells. Its functions include induction of antibody production, and B cell, T cell, and macrophage differentiation.[43] IL-6 production is induced by TNF-α and IL-1 through PKC activation or adenylate cyclase, while being a strong regulator of TNF-α. IL-6 can also regulate the hepatic synthesis of CRP and promotes atherogenesis by mechanisms including increasing macrophage uptake of lipids.[44] Its

regulation is controlled by NF-κB and its dysregulation plays a central role in chronic inflammation including diseases such as obesity and insulin resistance.[45]

1.2.3.2 Interleukin 8 (IL-8)

Interleukin 8 (IL-8) is a chemoattractant cytokine (chemokine) that is chemotactic for lymphocytes, particularly neutrophils, and that plays an important role in inflammation and angiogenesis.[46,47] IL-8 binds to receptors CXCR1 and CXCR2, and in atherogenesis it triggers the adhesion of monocytes to the vascular endothelium.[48] Mechanical stress, hypoxia, and ischemia may induce the production of IL-8 by cells including leukocytes, endothelial cells, and cardiac muscle through stimulation by LPS, IL-1, and TNFα.[49,50]

1.2.3.3 Monocyte chemoattractant protein 1 (MCP-1)

Also a chemokine, monocyte chemoattractant protein-1 (MCP-1) and its receptor (CCR2) mediate vascular inflammation by potently acting as chemotactic to monocytes.[51] MCP-1 is produced by various types of cells including activated monocytes, lymphocytes, and vascular endothelial and smooth muscle cells induced by oxidative stress, oxidized fat, and transcription of NF-κB, AP-1 and Angiotensin II.[52,53] MCP-1 is an important mediator in the progression and destabilization of established atherosclerotic lesions by also triggering the adhesion of monocytes to the vascular endothelium and promoting the production of tissue factor by arterial smooth muscle cells.[48,53-55]

1.2.3.4 Cyclooxygenase 2 (COX-2)

Cyclooxygenase 2 (COX-2) is a bifunctional heme-containing enzyme that catalyzes the production of PGH_2 from arachidonic acid to then form prostaglandins.[56] COX-2 expression is

exacerbated at sites of inflammation and its synthesis can be up-regulated by pro-inflammatory stimuli such as tumor promoters and cytokines such as IL-1β through the p38 MAPK pathway.[56,57] PGE_2, a prostaglandin derived from the action of COX-2 plays an important role including the development of the three major hallmarks of inflammation: swelling, pain, and fever.[56] Authors have also suggested a role of COX-2 in the resolution of inflammation through the production of prostanoids other than PGE_2 (such as PGD_2 and 15deoxy$\Delta^{12\text{-}14}PGJ_2$) which bind and activate peroxisome proliferator activated receptor (PPAR)-γ.[56-58]

CHAPTER 2
MATERIALS AND METHODS

2.1 MATERIALS

Fatty acids certified >99% purity (butyric, lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidonic, EPA, DHA, elaidic, *trans* vaccenic, and conjugated linoleic acid) were purchased from Nu-Chek Prep (Elysian, MN). Sterile Dulbecco's PBS was purchased from Invitrogen (Grand Island, NY). Fatty acid free bovine serum albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO). Human Primary Coronary Artery Endothelial (HCAE) and Smooth Muscle (HCASM) Cell cultures were purchased from ATCC (Manassas, VA). IL-6, IL-8, and MCP-1 ELISA kits were purchased from PeproTech (Rocky Hill, NJ). Goat anti-COX-2 polyclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Electrophoresis gels and Western Blot kits (WesternBreeze) were purchased from Invitrogen (Carlsbad, CA). All other reagents were of analytical grade and obtained from Fisher Scientific (Waltham, MA). Water was Milli Q (Millipore Corporation, MA) for all the studies.

2.2 PREPARATION OF FATTY ACID SOLUTIONS

Fatty acids were conjugated to BSA based on the methods developed by McIntosh, Toborek and Henning, and van Greevenbroek el al.[59-62] Briefly, for each 1ml of 10 mM solution, 10 µmoles of fatty acid were aseptically weighed in a sterile tube and dissolved in 90 µl hexane in a laminar flow hood. A 1N KOH solution was then added equimolar to the fatty acid and the mixture was vortexed for 10 seconds. The salt was dried under nitrogen until it had a white, chalky appearance. The salt was immediately diluted in 90 µl of warm (<50 °C) distilled

deionized water and mixed with 910 µl of a 25% BSA solution (25% BSA in DPBS+HEPES, pH 7.2, sterile filtered). The pH was checked and adjusted as necessary with 1N KOH. The conjugated fatty acid solutions were aliquoted, flushed with argon, and frozen at -80 °C. Aliquots of the BSA solution were stored similarly to be used as control. Conjugation of all mono- or polyunsaturated fatty acids was carried out under argon flow. For oxidized solutions the same procedure was followed except that fatty acid dilution in hexane was followed by a 30 minute incubation at 80 °C under oxygen.

2.3 CELL CULTURE

HCASM and HCAE cells were grown using Cascade Medium 231 or 200 (Gibco, Portland, OR), respectively. Cells were fed every other day until 80% confluence and split at 1 to 3 or 1 to 2. Trypsin solution from Cascade was applied to lift the cells; trypsin was then neutralized with CMF-PBS. Cells were spun down and resuspended at a density of 10,500 cells/cm^2 in their corresponding media using 6-well plates. For the different treatments, cells were incubated at 37 °C and 5% CO_2 in a humidified incubator (Sanyo Biomedical, Wood Dale, IL).

2.4 INDUCTION OF INFLAMMATION

Cells were treated with fatty acids at a final concentration of 200 µM in the media. A BSA control was included in each experiment. BSA never exceeded 0.5% in the media. Cells were incubated for 8 h (HCAE) or 20 h (HCASM). Following incubation, cell culture supernatant was removed and analyzed by ELISA for IL-6, IL-8, and MCP-1. Cells were washed once with PBS, scraped, and treated with RIPA lysis buffer [50 mM Tris-HCLHCl, ph 7.4, 150 mM NaCl, 0.1%

Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS] supplemented with protease inhibitor mixture, 1 mM PMSF, and 2mM sodium orthovanadate; followed by incubation at 4 °C for 30 minutes. The cell lysates were then centrifuged at 14,000 xg and 4 °C for 30 min to remove impurities. Protein concentration in the cytoplasmic extracts was determined with the Bio-Rad DC Protein Assay Kit (Hercules, CA). Supernatants and lysates were analyzed immediately or aliquoted and stored at -80 °C until further analyses. Cell viability was assayed using the MTT CellTiter 96® Non-Radioactive Cell Proliferation Assay from Promega (Madison, WI)

COX-2 was analyzed in selected treatments by Western Blot. Briefly, equal protein levels (50 µg) of the cytoplasmic extracts were separated using electrophoresis with 12% Bis-Tris gels and transferring to PVDF membranes. Membranes were blocked with 5% BSA in TPBS (PBS+Tween 20 at 0.5%), followed by an incubation with primary goat anti COX-2 antibody 1:200 with 5% BSA in TTBS. The bound antibody was visualized by probing with horseradish peroxidase-conjugated secondary antibody (rabbit anti-goat 1:2000) followed by exposure to chemiluminescent substrate (SuperSignal West Pico, Thermo Scientific, Rockford, IL) with X-ray film (Kodak X-omat 1000A processor). Densitometry of Western Blot bands was performed with Quantity One® 1-D Analysis Software version 4.6.5 (Bio-Rad Laboratories, USA).

2.5 STATISTICAL ANALYSIS

Biomarker levels were normalized based on the non-treated control and adjusted for cell viability, where biomarker levels for BSA control were set to 1.0. All results are presented as mean ± SD of triplicate analyses. Statistical analysis was performed using one-way analysis of

variance (ANOVA). Separation of means was performed by the Tuckey's Studentized Test (SAS®, version 9.2). Differences were considered statistically significant at $P<0.05$.

CHAPTER 3
RESULTS AND DISCUSSION

The effect of carbon chain length on levels of inflammatory markers was evident in both endothelial and smooth muscle cells. As seen in Figure 2.1, levels of IL-6 were directly related with carbon chain length for fully saturated fatty acids of 4-14 carbons on HCAE and all fatty acids on HCASM. The longest saturated fatty acids tested on SM, myristic (14:0), palmitic (16:0) and stearic (18:0), induced IL-6 levels significantly higher than the control. A similar chain length effect was observed in the levels of IL-8 for fatty acids of 4-14 carbons on HCAE and fatty acids of 12-16 carbons on HCASM. Palmitic acid (16:0) was the only fatty acid that increased IL-8 levels higher than control on both cell lines. MCP-1 levels also increased with increasing fatty acid chain length up to 16 carbons, however significant differences from control were only observed for palmitic acid on HCASM. Interestingly, MCP-1 levels decreased in cells treated with stearic (18:0) acid, and levels were significantly lower than control in SM cells treated with saturated fatty acids with 12, 14, and 18 carbons in the main chain. Overall, myristic and palmitic acid were the most pro-inflammatory of the fully saturated fatty acids.

The effect of *cis* double bonds on biomarker levels was evident in both cell lines (Figure 2.2). When treated with 18-carbon fatty acids, levels of IL-6 in both cell lines decreased as amount of double bonds increased from zero to two, and then slightly increased with 3 double bonds. This slight increase may have been due to oxidation during sample preparation. Levels of IL-6 decreased in cells treated with 20-carbon fatty acids. These longer unsaturated fatty acids also exhibited decreases in inflammatory markers with increasing unsaturation from 4 to 5 double bonds, but the effect was only evident in HCAE (Figure 2.2, see A). Similarly, IL-8 levels

in HCASM cells decreased with increasing unsaturation only for 20-carbon chain fatty acids (Figure 2.2, see B). MCP-1 levels in HCASM (Figure 2.2, see B) were equal or lower (18:0, 18:2) than control for all treatments, with no apparent relation to degree of unsaturation. IL-8 and MCP-1 levels remained unchanged for all treatments in HCAE (not shown).

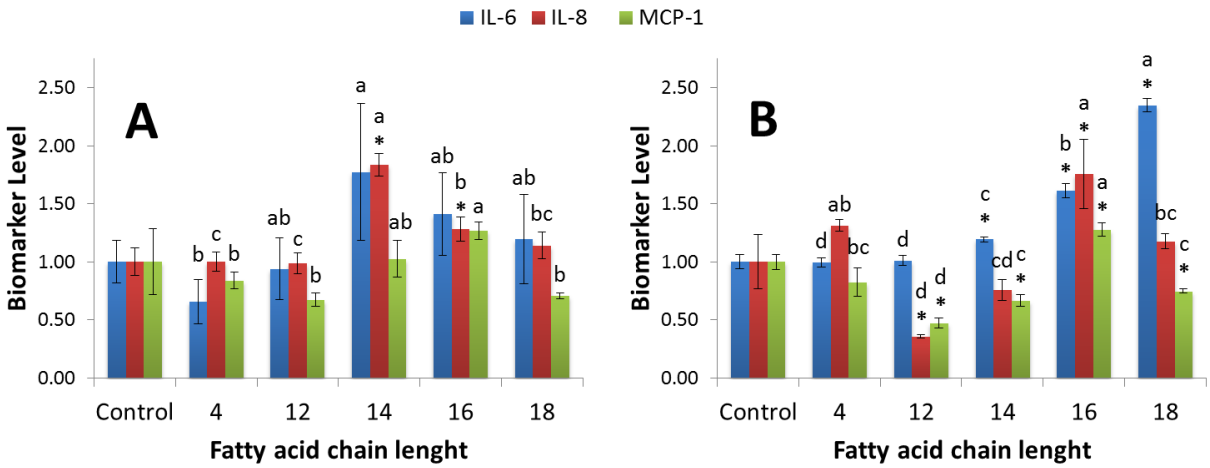

Figure 2.1. Effect of fatty acid chain length on levels of IL-6, IL-8, and MCP-1 in HCAE (A) and HCASM (B) cells. * Treatment is different from control. Different letters indicate differences between treatments.

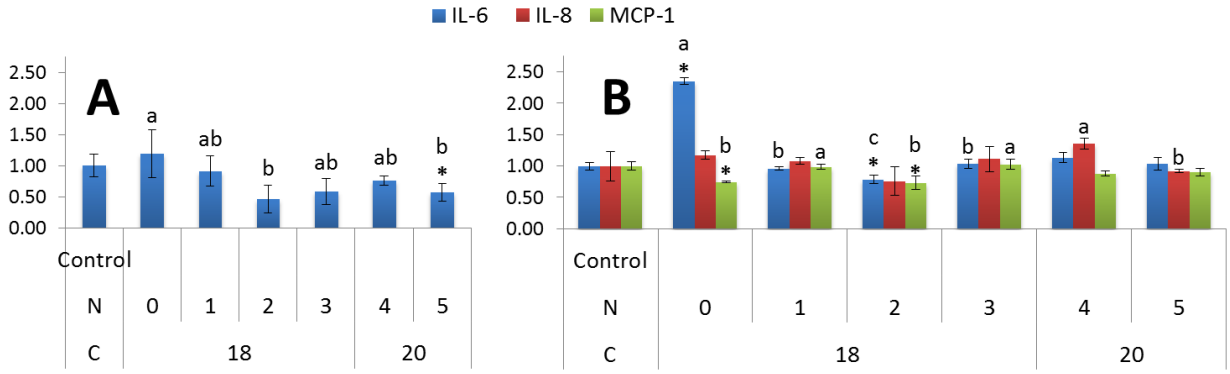

Figure 2.2. Effect of fatty acid unsaturation on levels of IL-6, IL-8, and MCP-1 in HCAE (A) and HCASM (B) cells. * Treatment is different from control. Different letters within treatments involving fatty acids with the same carbon chain length indicate differences between treatments.

The effect of *trans* double bonds was evaluated in the 18-carbon fatty acids oleic (18:1), elaidic (18:1 *trans* 9), and trans vaccenic (18:1 *trans* 11). Significant increases in IL-6 and MCP-1 levels were observed only in HCASM cells for *trans* vaccenic and elaidic acid, respectively (Figure 2.3, see B). All other treatments were not different from the control or from each other (not shown).

Linoleic acid (18:2) conjugation did not have a clear effect as compared to its non-conjugated counterpart (Figure 2.3). Levels of IL-6 did not differ amongst both forms of linoleic acid, but were equal or lower than control in both cell lines (Figure 2.3). MCP-1 levels marginally increased with conjugation in HCASM cells, where MCP-1 for linoleic acid was lower than control. IL-8 levels remained unchanged for both treatments and cell lines (not shown). Overall, linoleic acid proved to be the least pro-inflammatory fatty acid on both cell lines.

The effect of fatty acid oxidation on biomarker levels was evaluated using linolenic acid (18:3) and DHA (22:6), both omega-3 fatty acids (Figure 2.3). Oxidation of linolenic acid did not result in changes of inflammatory biomarkers on either cell line, as all levels did not differ from control or from the non-oxidized treatment. In contrast, oxidation of DHA resulted in a reduction of IL-6 for both cell lines to levels lower than control. Notably, IL-6 levels for HCASM treated with non-oxidized DHA were significantly higher than control. Levels of MCP-1 in HCASM did not differ for cells treated with oxidized DHA, as compared to its non-oxidized counterpart, however the levels were higher than control in both cases. IL-8 levels in both cell lines treated with DHA and oxidized DHA did not differ from control or from each other (not shown).

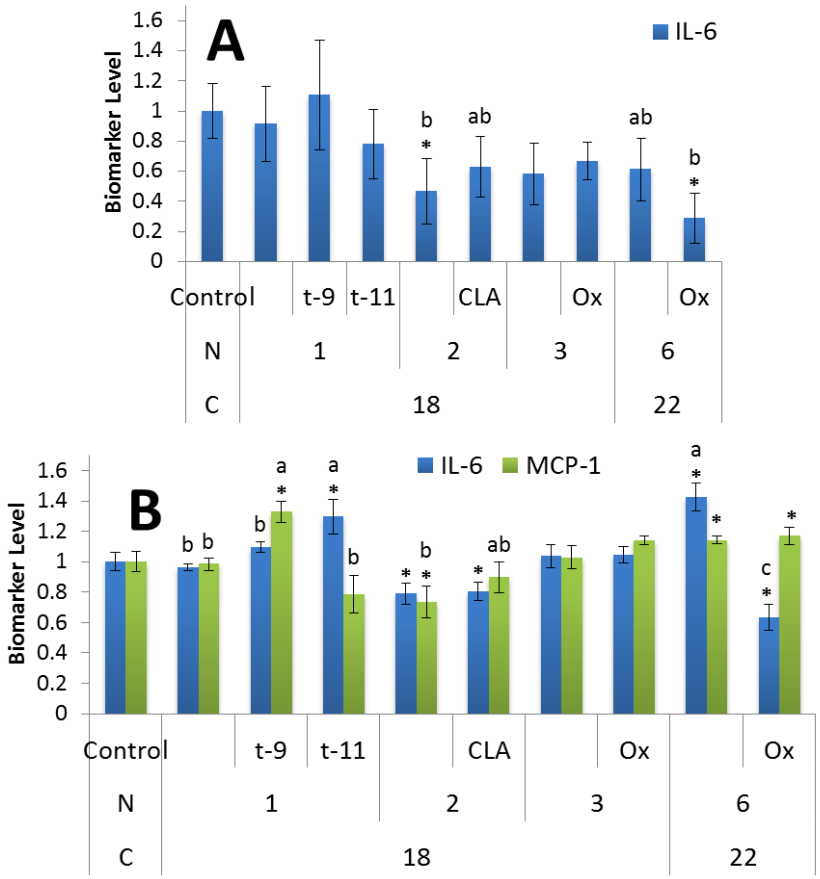

Figure 2.3. Effect of *trans* (t) fatty acids, conjugation (CLA), and oxidation (Ox) on levels of IL-6 and MCP-1 in HCAE (A) and HCASM (B) cells. * Treatment is different from control. Different letters indicate differences between the treatment and its corresponding non-*trans*, non-conjugated, or non-oxidized counterpart.

COX-2 analysis (Figure 2.4) revealed a stimulation of this biomarker by oxidized linolenic acid and DHA in HCAE and by palmitic acid in HCASM. No major differences were observed between oxidized and non-oxidized omega-3 fatty acid treatments, except for HCAE cells treated with non-oxidized DHA (22:6), which yielded COX-2 levels higher than control. In contrast, COX-2 levels in HCASM cells treated with DHA or oxidized DHA were consistently lower than control.

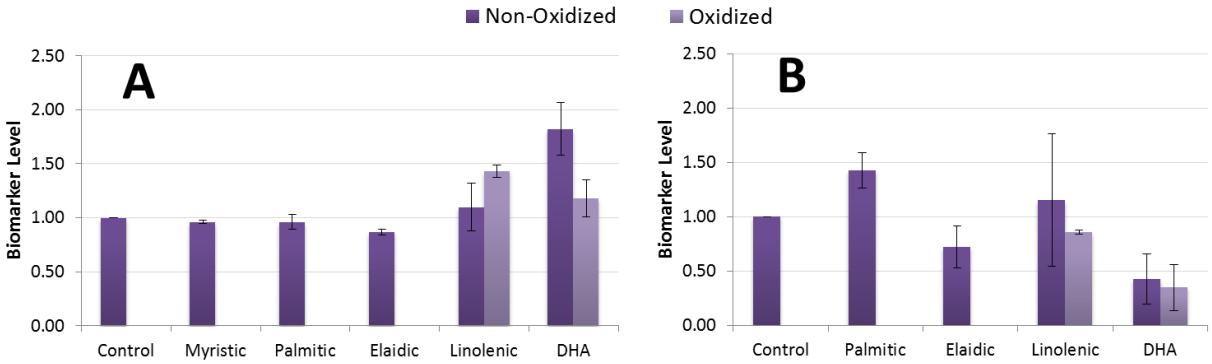

Figure 2.4. Changes in levels of COX-2 in the cytoplasm of HCAE (A) and HCASM (B) cells as influenced by treatment with selected fatty acids.

Increases in levels of secreted cytokines may be due to the production of mitochondrial reactive oxygen species (ROS) deriving from fatty acid β-oxidation in the cells, a phenomenon previously observed in kidney damage in diabetes.[63] Studies have also shown that increased oxidation of FFAs in aortic endothelial cells without added insulin results in an increase in production of superoxide in the mitochondria, which in turn activates the proinflammatory signal cascade and inactivates prostacyclin synthase and eNOS, important antiatherogenic enzymes.[64]

Reductions in IL-6 and MCP-1 observed with increasing double bonds in endothelial and smooth muscle cells (Figure 2.2, see A and B) may be due to reduction in cell activation, as previously observed in endothelial cells, where a greater number of double bonds (not necessarily the position of the last double bond) was critical for the greater activity of ω-3 as compared to ω-6 fatty acids in the inhibition of endothelial activation. This activation refers to the ability of the cell to promote monocyte migration, and both IL-6 and MCP-1 are important signaling proteins in this process.[65]

Table 2.1 illustrates the fatty acid composition of common fats and oils. Although a breakdown of *trans*- fatty acids is not shown, naturally occurring *trans*- fatty acids are present

in small amounts in ruminant fats and include *trans*-vaccenic acid and conjugated linoleic acid (CLA).[66] Partially hydrogenated vegetable oils may contain *trans*- fatty acids, mainly elaidic acid, at levels ranging 10-40%.[67] Major dietary sources of arachidonic acid (20:4) are meat, poultry, and game followed by fish and seafood. Dietary sources of EPA (20:5) and DHA (22:6) are mainly fish and seafood.[68] Shellfish fat may contain up to 4.5% arachidonic acid (sea scallops) 21.5% EPA (pacific oyster) and 22.6% DHA (sea scallops). Marine and fresh water fish oils may also contain up to 3.9% ARA, 13.5% EPA and 21.9% DHA.[69]

Table 2.1. Fatty acid composition of common fats and oils. Adapted from ISEO, 2006.[70]

Oil or Fat	**Fatty Acid %**								
	Butyric 4:0	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Trans FAs
Beef tallow			3	24	19	43	3	1	1-8
Butterfat	4	3	11	27	12	29	2	1	1-8
Canola				4	2	62	22	10	
Cocoa butter				26	34	34	3		
Coconut		47	18	9	3	6	2		
Corn				11	2	28	58	1	
Cottonseed			1	22	3	19	54	1	
Lard			2	26	14	44	10		
Olive				13	3	71	10	1	
Palm kernel		48	16	8	3	15	2		
Palm			1	45	4	40	10		
Peanut				11	2	48	32		
Safflower				7	2	13	78		
Soybean				11	4	24	54	7	
Sunflower				7	5	19	68	1	

Several studies have shown that plasma fatty acid composition is a good indicator of and can be modified by the fatty acid composition of the diet.[71-73] Based on the results of our study, the fatty acids with higher pro-inflammatory potential are myristic and palmitic acids. Important sources of these fatty acids include coconut and palm kernel oil (myristic) and palm oil (palmitic). In contrast, the least pro-inflammatory, and perhaps even anti-inflammatory fatty

acid was linoleic acid, an omega-3 fatty acid which can be found prominently in safflower, sunflower, corn, cottonseed, and soybean oil. Increased consumption of such oils could potentially result in reduced cardiovascular risk due to a reduction in inflammatory state. Studies have also identified hypercholesterolemic effects of myristic, palmitic and lauric acid and cholesterol lowering effects of linoleic acid.[74-76] Changes in plasma cholesterol in addition to inflammatory effects may further explain how increased FFAs in plasma contribute to an elevated risk of CVD. Although the results of our study do not suggest a clear anti-inflammatory effect of the omega-3 fatty acids EPA and DHA, consumption of foods and oils rich in EPA and DHA should not be discouraged due to other well documented beneficial actions that play an important role in cardiovascular health, such as cholesterol-lowering effects.[77]

CHAPTER 4
CONCLUSIONS AND FUTURE STUDIES

4.1 CONCLUSIONS

An *in vitro* model has been developed and utilized to identify the pro-inflammatory effect of free fatty acids in human coronary arterial cells. Careful literature review provides important background information justifying this study. Circulating free fatty acids have been identified as a risk factor for inflammation and resulting cardiovascular disease. Screening of various dietary relevant fatty acids in our model allowed for the identification of fatty acids that can be used to induce inflammation in human coronary arterial cells.

Saturated, unsaturated, trans, and oxidized fatty acids were conjugated to BSA to simulate physiological conditions. Cells were incubated with fatty acids and inflammation was assessed mainly through the expression of cytokines and chemokines in the supernatants. Inflammation increased with increased chain length in fully saturated fatty acids, and decreased with unsaturation regardless of the position of the last double bond. Trans double bonds or fatty acid oxidation were not strong predictors of inflammation in our study. The study allowed us to identify myristic and palmitic acids as pro-inflammatory, while the least pro-inflammatory and perhaps even anti-inflammatory fatty acid was linoleic acid, an omega-3 fatty.

4.2 FUTURE STUDIES

We propose that a further study is carried out to evaluate the effect of the combination of fatty acids on the inflammatory response. This experiment could be carried out in a dietary relevant way, such that the evaluated fatty acid combinations would represent the fatty acid

profiles of common fats and oils. Focusing on gene expression could also provide valuable information as to the mechanism of action of fatty acids in promoting or preventing inflammation.

The fatty acid-induced inflammation model than we have proposed in this dissertation could represent a time and cost-efficient approach to explore the anti-inflammatory potential of foods. Experiments can be carried out in the intervention or prevention approaches to evaluate the effect of food or their extracted bioactives in modulating fatty acid-induced inflammation. Due to the widespread availability of palmitic acid in foods, we suggest this fatty acid be used in the model for future studies.

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VITA

Adriana Soto Rodriguez Gil (Adriana Soto-Vaca) was born in Mexico City, Mexico and raised in Mexico City and San Pedro Sula, Honduras to Carlos Soto and Adriana Rodriguez Gil. The oldest of two siblings, she attended Instituto Experimental La Salle where she graduated second of her class of 100 students (December 2000). Adriana graduated from Zamorano University with a B.S. in Agroindustrial Engineering in 2004 at the top 3% of her class. Her thesis entitled *Development and evaluation of maize and low fat soy flour based dry masa flour* was completed under the guidance of Dr. Javier Bueso and received a mention of honor. This work was also the first to represent Zamorano University at an IFT meeting in 2005. Adriana has 3 years of experience in the food industry as Plant Manager at the Zamorano Meat Processing Plant, Head of Microbiology at Quimifar Laboratories, and Senior Quality Analyst of Raw Materials and Packaging for Carbonated Soft Drinks at Cerveceria Hondureña, a SAB-Miller subsidiary. After being granted an internship at the LSU AgCenter as a research scholar in the summer of 2008, Adriana joined the Food Science graduate program at LSU in the fall of the same year. She carried out her research under the mentorship of Dr. John W. Finley and currently has two peer-reviewed publications and numerous national and international presentations. Adriana will receive a dual degree of Master's and Doctor of Philosophy in Food Science during the August 2013 commencement ceremony. She is married to Franklin F. Vaca, a Ph.D. candidate in the Department of Agricultural Economics and Agribusiness at LSU.