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Effects of Fatty Acid Structure on Endothelial Cell Expression of Connexin43

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I would like to personally thank my committee for making this project possible. Dr. Dave Kurjiaka, my mentor, funded this research endeavor, provided the guidance in technical issues along the way, and lead the investigation towards connexin43 when problems arose in detecting the originally proposed protein, ICAM-1. Dr. Kurjiaka was especially helpful in revising any scientific writing along the way. Dr. Debra Burg generously donated stock LPS to be used in experiments. Additional thanks to Noah Zucker for the tutelage in Western blot techniques before he departed Grand Valley for the University of Michigan Medical School.

Abstract

The epidemic of obesity-related metabolic and cardiovascular diseases is linked to elevated fatty acids (FA). Epidemiological data argue that trans FA contribute to the development and progression of these disease processes. Previous work suggests the similarity in structure between saturated and trans FA may stimulate inflammation through trans FA interactions with toll-like receptor 4 (TLR4). To evaluate the impact of trans FA on cardiovascular health, the response of endothelial cells to trans FA was evaluated. Specifically, responses to the 18-carbon trans FA elaidic acid (EA) were compared to the same length cis FA oleic acid (OA). Endothelial cells were exposed to FA treatments for up to 48 hours and protein samples collected at specific times (1.5, 3, 6, 12, 24, and 48 hr). The expression of connexin43 (Cx43) was evaluated using Western blot analysis as a measure of their health (increasing in unhealthy endothelial cells). Lipopolysaccharide (LPS; 10 μg/mL), a TLR4 agonist, stimulated an increase in endothelial cell Cx43 expression at 12 hours. As the endothelial cells responded to a TLR4 agonist, they were then exposed to OA (30 μM), which decreased the expression of Cx43 at 3, 12, and 24 hours. In contrast, EA (30 μM) increased Cx43 expression at 12 hours. These results suggest that trans FA alter Cx43 expression in a similar pathway as LPS in endothelial cells (likely TLR4 signaling), whereas OA decreased Cx43, likely through a different mechanism.

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I. Literature Review

Introduction: The entrenchment of the Western diet, one high in fats and processed sugars, around the world and is frequently accompanied by limited physical activity. These factors have contributed substantially to the global obesity epidemic. Recent epidemiological studies indicate that 20% of the world population is overweight, with 300 million currently considered obese and with an especially high prevalence in the U.S. (Fig. 1).[4,9,43] The consequence of this epidemic is a marked increase in the incidence of cardiovascular pathologies caused by obesity, including atherosclerosis, hypertension, endothelial dysfunction, and insulin resistance (type II diabetes). A significant contributor to the pathology of cardiovascular disease (CVD) is the chronic state of low-grade inflammation that accompanies obesity and the resulting vascular dysfunction (a decreased response to vasodilatory stimuli pervasive in obese individuals).[16,27,36] This inflammation usually involves an increased release of cytokines from adipocytes, leukocytes, and endothelial cells in response to chemical cues from infected or damaged tissue to limit the extent of the damage.[38] Recent work has suggested that certain fatty acids (FA), typically elevated in obese individuals (dyslipidemia), may activate receptors that mediate the inflammatory response.[16] In particular, the trans FA, which are rare in nature but have become a regular part of the American diet, as they are generated via the heating of partially hydrogenated oils. These FA are deemed unhealthy as they have been shown to increase inflammation.[22,45] Thus, components of the Western diet could contribute to the chronic inflammatory response and thereby the development of CVD.

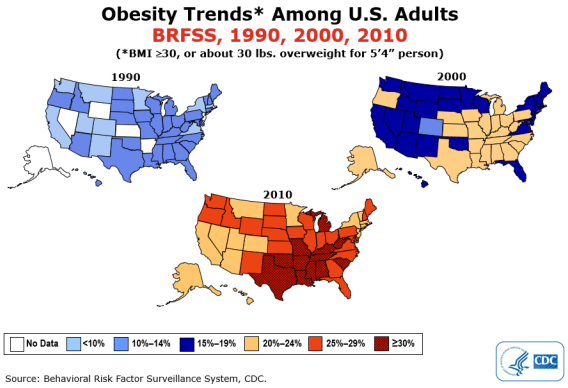


Figure 1: Obesity trends among U.S. Adults:[9] This image shows the increase in body mass index (BMI) in the U.S. Across the last three decades. BMI (mass in kg divided by the square of height in m) of 30 kg/m^2 or greater are considered obese.

FA and cardiovascular health implications: The dyslipidemia of obesity is associated with an increase in circulating triglycerides, free FA, and a decreased HDL: LDL ratio.[28] The free FA are synthesized by the enzyme FA synthase in the presence of excess acetyl-CoA (a glycolytic product) to store energy or to be used as a signaling lipid precursor (e.g., arachidonic acid). This energy storage involves binding of three FA to one glycerol to form a triglyceride molecule. FA consist of a carboxyl end, a long aliphatic tail, and a hydrocarbon backbone [$(CH_2)_nCH_3$]. These molecules can be synthesized in multiple conformations that vary by carbon backbone length (between 4 and 28 carbons long), number of double bonds (mono and polyunsaturated), and stereochemistry of those double bonds (cis and trans isomers; Fig. 2). While cis isomers (adjacent hydrogens on the same side of the alkene bond) are naturally produced by desaturating enzymes, trans FA are much less common in nature (found in less than 1% in the gut of ruminant animals as a by-product of fermentation).[33] The main source of trans FA in the diet is through consumption of partially hydrogenated oils, produced by heating vegetable oils in presence of a metal catalyst, as such is the case in deep-fryers, and they are estimated to account for 4-12% of American fat caloric intake.[22] In people, trans FA originate

solely from the diet since they are not synthesized as energy storage molecules in the body.[22,45]

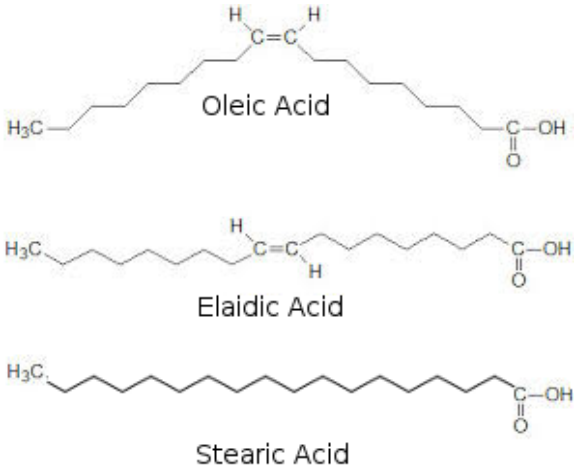


Figure 2: Structural comparison of cis and trans monounsaturated to saturated fatty acids: The structure of three 18-carbon FA are shown. Unsaturated FA may be cis (oleic acid) or trans (elaidic acid). Note that the linear structure of elaidic acid is similar to that of stearic acid, the saturated FA.

Epidemiological data argue that trans FA negatively impact human health,[33] with no shortage of public health studies linking diets high in trans and saturated FA with greater incidence in CVD, especially atherosclerosis,[22,45] whereas diets higher in cis monounsaturated and polyunsaturated FA are, to the contrary, atheroprotective.[6] The differences in the impact of the molecules on human health is based upon their structures. The linear carbon chains of trans FA have chemical properties analogous to saturated FA rather than cis isomers[45] and exhibit the same negative health outcomes[11,28] whereas the cis monounsaturated and omega-3 FA exhibit anti-inflammatory benefits, which likely explains their atheroprotective properties.[21,44] This anti-inflammatory quality of cis and omega-3 FA is largely attributed to a suppression of inflammatory cytokine production in the endothelium of blood vessels.[26,28,32] Conversely, trans and saturated FA increase

cytokine expression.[22] Whether these are through the same pathway has yet to be resolved. However, the mechanism linking FA to the inflammatory pathways is their ability to bind and act as pathogen-associated molecular patterns (PAMPs) and activate pattern recognition receptors (PRRs).[40,41] Recent studies have suggested that binding of saturated and trans FA to PRRs initiates and perpetuates an inflammatory response.[16]

FA and Toll-like recptors: The chronic low-grade inflammation that characterizes obesity results from a process intrinsic to innate immunity. Innate responses are initiated by the recognition of common molecular structures rather than by specific epitopes. PRRs expressed on endothelial and phagocytic cells initiate the non-adaptive innate response upon binding to PAMPs.[3,16] Of the PRRs utilized by cells of innate immunity, toll-like receptors (TLRs), membrane-bound PRRs integral to functional immunity, appear to provide the link between FA and inflammation.[40] Multiple TLR4 ligands have been identified, including lipopolysaccharide (LPS, an endotoxin and component of gram negative bacteria membranes) and alarmins (molecules released by injured or dying cells such as heat shock proteins, mitochondrial DNA, and histone H3).[19] Of particular interest, TLR4 has been shown to bind the saturated FA palmitate and stearate and thus these saturated FA act analogously to PAMPs.[27,38] Based on similarity in structure between saturated and trans FA,[45] we propose that TLR4 may be activated by trans FA. Thus, the Western diet promotes a chronic inflammatory response due to the presence of circulating saturated and trans FA, which potentially bind to the PRRs.[27]

Binding of a PAMP to TLR4 leads to activation of the transcription factor NF-κB.[16] Translocation of NF-κB into the nucleus initiates transcription of genes coding for

nitric oxide synthase (NOS), an enzyme that produces the vasodilator nitric oxide in blood vessels, and the cytokines TNF-α, IL-1β, and IL-6 (Fig. 3). These cytokines are known as the triad of inflammatory cytokines due to their role in producing vascular changes to facilitate leukocyte recruitment.[4,17] These changes in vascular permeability are the hallmarks of inflammation.[16] As a result of this inflammatory state, the extent of coupling between cells has been shifted[7] by alterations in the expression of connexin (Cx) proteins responsible for mediating cell-cell communication.[1,10,13]

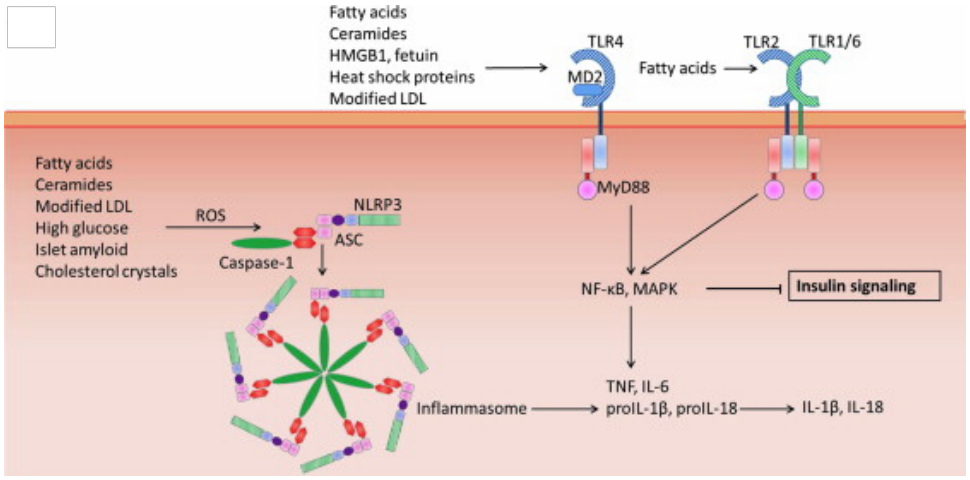


Figure 3: Activation of TLRs in obesity[16] FA interact with TLRs to stimulate the release of inflammatory cytokines in dyslipidemia.

Anatomy of gap junctions: Gap junctions allow electrical and chemical communication between adjacent cells. These gap junctions are aggregates of hemichannels (pores) formed by hexamers of the connexin (Cx) protein. Hemichannels dock between neighboring cells and, when gated-open, allow ions, second messengers, and molecules less than 1kD to diffuse between cells (Fig. 4).[7,23] Cx proteins, of which 21 are known, are identified by their apparent molecular weight, e.g., Cx43 being a 43 kD connexin.[7,8] The permeability, charge selectivity of permeants, and conductance are specific for each

connexin isoform; Cx40 is cation-selective (cations diffuse more freely than anions) whereas Cx43 is non-selective.[23] The permeation properties of channels are regulated by post-translational modifications, e.g., phosphorylation of the C-terminus of Cx43 which significantly reduces its electrophoretic mobility.[23,34]

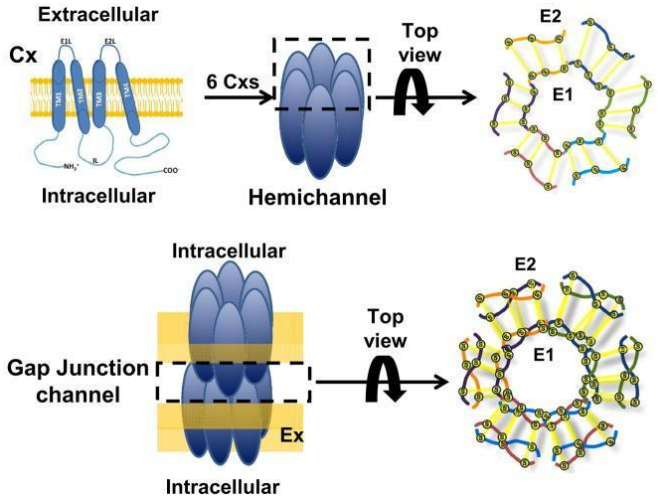


Figure 4: Membrane topology of a connexin protein, hemichannels, and gap junctions[37] A Cx protein contains four membrane-spanning regions, with two extracellular loops (which influence hemichannel docking), the carboxyl terminus, and the amino terminus located in the intracellular compartment. Six Cx proteins oligomerize to assemble a pore-containing hemichannel. Hemichannels of adjacent cells dock together to form a gap junction channel.

Gap junction coupling in vascular endothelium and in vascular smooth muscle cells is unevenly distributed, with the endothelial layer exhibiting extensive coupling compared to the smooth muscle cells and even more than the coupling between the muscular and endothelial layers.[20] Healthy endothelial cells are preferentially coupled by Cx37 and Cx40.[13] When the blood flow is disturbed, Cx43 expression increases and Cx37 decreases.[13] In addition, Cx43 expression is elevated in regions where atherosclerotic lesions are common, which supports their use as a marker of

cardiovascular pathology.[13,14,15] Vascular health, therefore, may be evaluated by assessing Cx43 expression within the endothelium.

In endothelial cells, engagement of TLR4 has been shown to reduce the coupling between neighboring endothelial cells.[7] The cytokines associated with inflammation (TNF-α, IL-1β, and IL-6) upregulate endothelial cell Cx43[1,30] and downregulate Cx40 functional expression.[5,18] Cx40 downregulation by LPS is associated with inhibition of endothelial responses in sepsis[7] and upregulation of endothelial Cx43 is associated with vascular dysfunction.[10,13,29] Thus, inflammation alters endothelial Cx43 in a manner that contributes to the endothelial dysfunction. The mechanisms of trans FA on cardiovascular pathology have yet to be completely understood. Since saturated FA activate the TLR4 pathway[40] and trans FA are similar in structure, endothelial Cx43 expression should be altered[30] by trans FA. To test the importance of the double bond isomerism, response of endothelial cells to trans FA must be compared to cis FA of the same length (18 carbons each). We hypothesize that elaidic acid (EA), an 18-carbon monounsaturated trans FA, will increase endothelial cell Cx43 expression whereas oleic acid (OA), the 18-carbon monounsaturated cis fatty acid, will not alter Cx43 expression. While the physiological ramifications of Cx43 upregulation are not completely understood, it is hypothesized that the shift in Cx43-containing hemichannels influences the nature of gap junction communication on the basis of altered permselectivity properties.

II. Experimental Manuscript

Introduction

Recent epidemiological studies have shown that 20% of the world is overweight.[4,43] The health risks associated with this obesity epidemic include the increase in the incidence of cardiovascular disease (CVD) and type II diabetes that is accompanied by a chronic state of low-grade inflammation due to elevated circulating cytokines.[16,27,36] Evidence suggests this inflammatory state may be a direct result of dietary fatty acids (FA).[39] Saturated FA, whose concentration is elevated in obese individuals, can bind to toll-like receptors (TLRs) found on the vascular endothelium and initiate the inflammatory response.[16,40] As trans and saturated FA are similar in structure, a common pathway would explain the similarity in development of an inflammatory response and thereby atherosclerotic lesions.[22,45]

The precise mechanism by which trans FA stimulate cardiovascular pathology has yet to be understood. The TLR4 pathway, a pattern recognition receptor (PRRs) on endothelial cells, could contribute by activation of the transcription factor NF-κB and thereby *de novo* synthesis of the cytokines TNF-α, IL-1β, and IL-6.[16,17,38,40] An important determinant of endothelial cell function is their ability to communicate directly with adjacent cells via gap junction.[7,12] Gap junctions are formed when hemichannels (composed of 6 connexin (Cx) proteins each) dock with another on a neighboring cell to allow chemical and electrical communication.[7,23] Cx expression on endothelial cells includes three isoforms: Cx37, Cx40, and Cx43.[20] While Cx40 and Cx37 are the principal connexins expressed in healthy endothelial cells,[20] Cx43 is upregulated in areas of disturbed flow as well as regions that are inflamed (as in atherosclerosis).[13,15,35]

Interestingly, the pathway stimulated by cytokines can increase Cx43 expression.[2] This same pathway is activated by lipopolysaccharide (LPS), a component of gram-negative bacteria and the natural ligand of TLR4, which leads to sepsis due to dysfunction of the endothelium.[7,17] Hemichannels consisting of Cx43 exhibit less permselectivity (increase gap junction permeability) than those without, which may contribute to associated endothelial dysfunction pathologies.[1,23] Thus, detection of endothelial Cx43 is likely indicative of poor vascular health.[12,35]

If trans FA upregulate expression of inflammatory cytokines via the TLR4 pathway (NF-κB), then Cx43 expression in endothelial cells should be elevated by inflammatory conditions.[22,27,30] As trans FA have been suggested to activate inflammation via this pathway, we evaluated the impact of FA isomerization on endothelial Cx43 expression. In particular, endothelial cell responses to the 18-carbon oleic acid (OA; cis) were compared to the trans isomer, elaidic acid (EA). We hypothesized that EA would increase Cx43 expression while OA would not affect Cx43 expression.

Materials and Methods

Solutions: Cell culture media was prepared using high-glucose (25 mM) Dulbecco's modified eagle's medium (HG-DMEM; Thermo Scientific, SH30243.02) supplemented with penicillin/streptomycin and 10% fetal bovine serum (FBS; Atlanta Biologicals; v/v). A 10 μg/mL LPS solution was prepared from stock LPS (5 mg/mL, Sigma L28880). A 30 mM stock FA solution was prepared for sodium oleate (TCI, O0057, in ddH_2O) and elaidic acid (TCI, O0010, in DMSO, Research Organics). The stock solution was added to HG-DMEM to make 30 μM treatment solutions. As water was the vehicle for LPS and OA, controls were unaltered HG-DMEM while EA vehicle was prepared by adding the same volume of sterile DMSO (Research Organics) to the media as the treatment. Running buffer was made from a stock 10X solution (BioRad, final concentrations: 25 mM Tris, 192 mM glycine, and 0.1% (w/v) SDS, pH 8.3). Transfer buffer was made from 100 mL of 10X transfer salt solution (final concentration: 25 mM Tris, and 192 mM glycine), 700 mL ddH_2O, and 200 mL methanol. Tris-buffered saline (TBS) was prepared from a 10X solution (final concentration: 20 mM Tris, 500 mM, pH 7.5). TBST was prepared by supplementing TBS with 0.05% Tween-20. Powdered nonfat dry milk (NFDM) was added to TBST to produce the 5% NFDM-TBST.

Cell Culture: The impact of FA structure on Cx43 expression in endothelial cells was evaluated utilizing a cell culture model. Mouse endothelial cells (bEnd.3, from ATCC) were grown in an incubator at 10% CO_2 and 37^{o}C. Cells were split using trypsin-EDTA and counted with a hemocytometer. Cells were plated in HG-DMEM at a final density of 800,000 cells per 100mm plate and placed into the incubator for 2-3 hours to adhere to plates. Once cells had adhered, the media was aspirated and 10 mL of treatment media

(LPS, OA, EA, or control) was pipetted onto each plate (time-zero). To establish the time course of Cx43 protein expression, each experiment involved producing 12 plates: half with treatment media and the other six with control (vehicle) media.

Western Blotting: At 1.5, 3, 6, 12, 24 and 48 hours, protein was isolated from two plates (treatment and control) using Laemmli sample buffer and sonication of cells. Protein concentrations were determined using a biophotometeric analysis of a bicinchoninic acid (BCA) assay. Equal amounts of total protein were loaded into BioRad Mini-PROTEAN TGX (4-20% gradient SDS-polyacrylamide gels) and run at 180V for 35 min. Protein was transferred to a polyvinyl-difluoride membrane using ice-cold transfer buffer at 100V for 2 hr. Transfer of protein to membranes was confirmed using Ponceau S and rinsed with TBST. Blotting was performed at room temperature. Following one hour of blocking with 5% NFDM and three subsequent rinses with TBST, the membranes were probed for expression of Cx43 using rabbit anti-mouse Cx43 polyclonal antibody (Sigma, #C6219) in 1% NFDM (1:2,000) for one hour. After three rinses, membranes were probed with biotinylated, anti-rabbit secondary (Sigma, #B8895) in 1% NFDM (1:3,300) for one hour. Following three more rinses, membranes were probed with HRP-conjugated streptavidin (Thermo, #21134) in 1% NFDM (1:330) for 30 minutes. The HRP signal was detected with chemiluminescence (Dura Supersignal) and documented with a SenTech STC-TB152USB-AS camera. Light intensities were quantified using ImageJ software (NIH).

Statistics: At each time point, Cx43 expression after treatment was normalized to vehicle control. Single-sample t-scores were used to determine whether Cx43 expression was changed. As the treatment was normalized to control, change in expression requires the

ratio to be different from 1 (1 would mean no change). Since LPS is reported to elevate Cx43, one-tailed t-tests were used for LPS samples. Two-tailed t-tests were implemented for EA , OA, and time controls to measure change in either direction. Significance between comparisons was set at the $p \leq 0.05$ level. To satisfy testing conditions, distribution was checked using a Q-Q plot and homogeneity of variance was confirmed with a Levene's test p-value greater than 0.05.

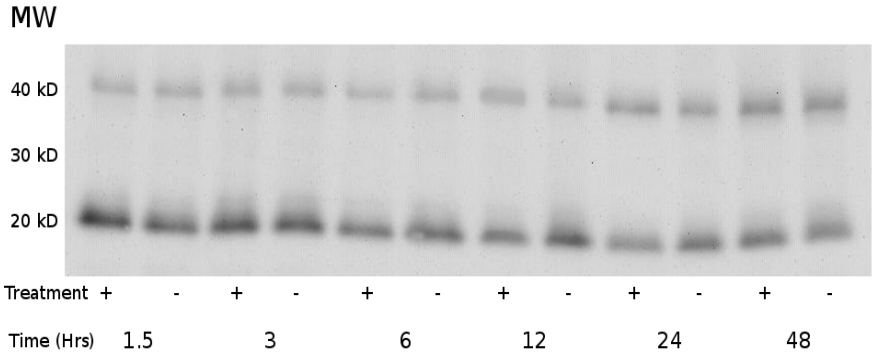
Results

Effect of LPS on Cx43 expression:

To ensure these endothelial cells expressed TLR4, the response to LPS was determined. Following treatment of 10 μg/mL LPS, Cx43 was upregulated after 12 hrs (p=0.0478; Fig. 5 and 6). To address whether there were any changes in the control over time, control intensities were normalized to the first protein isolation (1.5 hrs; Fig. 5 and 7). Control Cx43 expression did not change relative to the 1.5 hour time.

Effect of FA structure on Cx43 expression:

Since endothelial cells respond to LPS and therefore have the capacity to be altered by trans FA, the effect of FA unsaturation stereochemistry on endothelial Cx43 expression was investigated by comparing responses to 30 μM OA (cis) and 30 μM EA (trans). OA reduced Cx43 at 3 (p=0.028), 12 (p=0.0146), and 24 hrs (p=0.0048; Fig. 8 and 10). In contrast, EA increased Cx43 at 12 hrs (p=0.048, fig. 9 and 10) without changing expression at other times. As with LPS, normalizing controls to the 1.5 hr time showed



no changes in Cx43 in the controls over time (Fig. 8 and 11).

Figure 5: Western blot representative of the endothelial cell response to LPS. This membrane was probed for Cx43. The 10 μg/mL LPS-treated (+) samples were compared to the control (-) at each time (1.5 – 48 hrs). Since two bands were detected, molecular weights markers were utilized to ascertain which corresponded with Cx43 (43 kD).

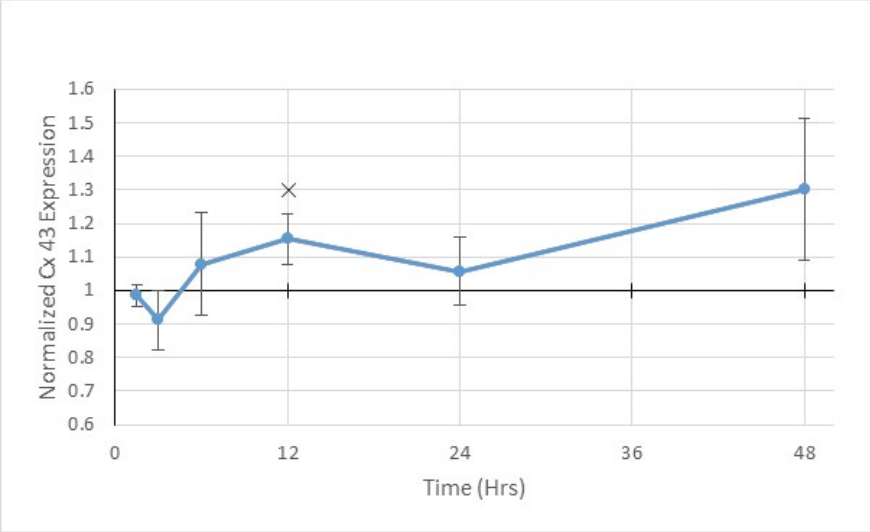


Figure 6: LPS alters connexin43 expression in endothelial cells under LPS treatment. The expression of Cx43was normalized to the control at each time point. Exposure to 10 µg/mL LPS increased Cx43 expression in endothelial cells at 12 hours (n=6) without affecting other times. × - indicates increase in Cx43 at time point ($p \leq 0.05$)

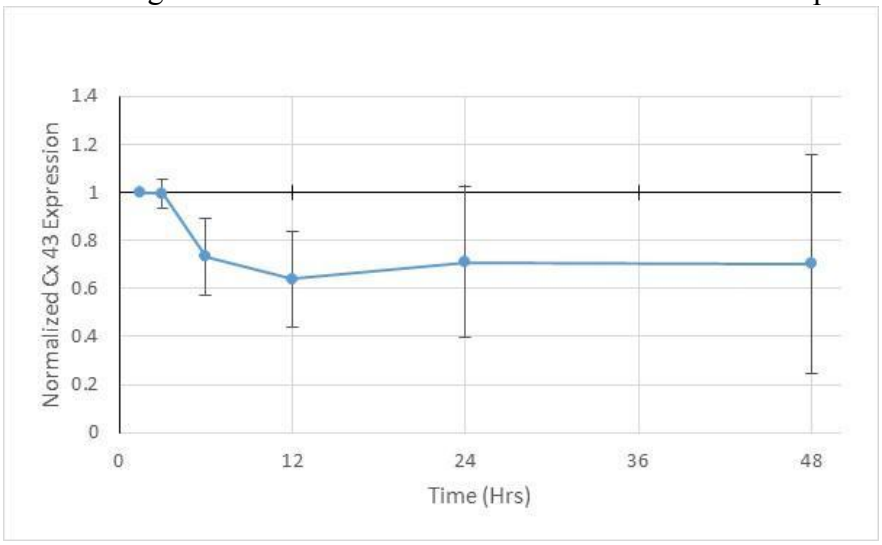


Figure 7: Cx43 expression in LPS endothelial cell controls for treatment. The expression of Cx43 was normalized to the 1.5 hour time. No significant changes in Cx43 expression were observed.

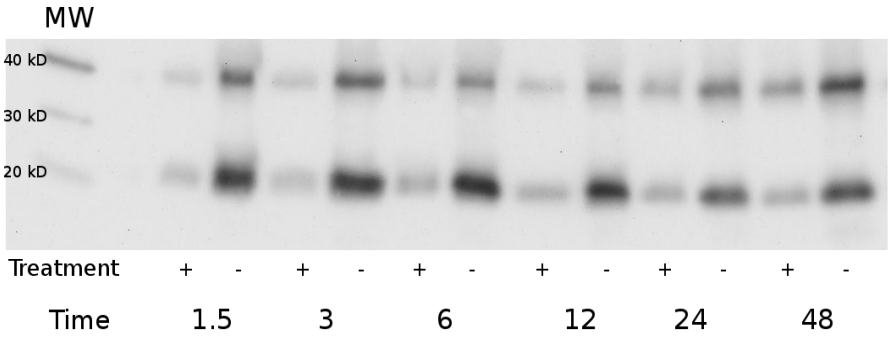


Figure 8: Western blot representative of endothelial cell response to OA. This membrane was probed for Cx43. The 30 μM OA-treated (+) samples were compared to the control (-) at each time (1.5 – 48 hrs).

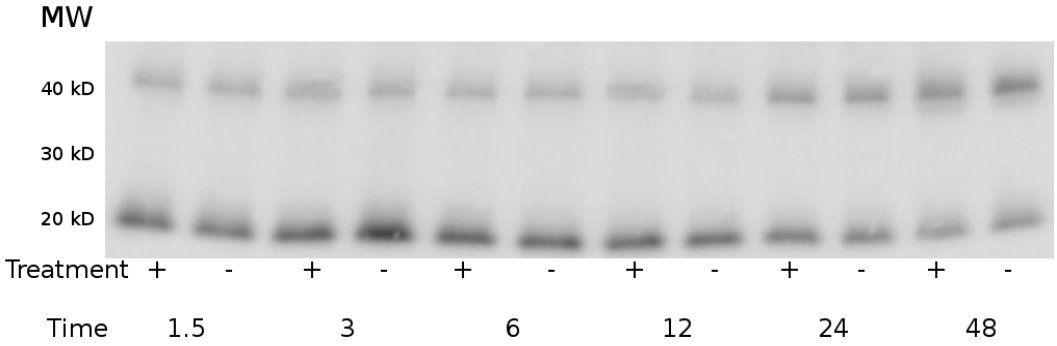


Figure 9: Western blot representative of endothelial cell response to EA. This membrane was probed for Cx43. The 30 μM EA-treated (+) samples were compared to the control (-) at each time (1.5 – 48 hrs).

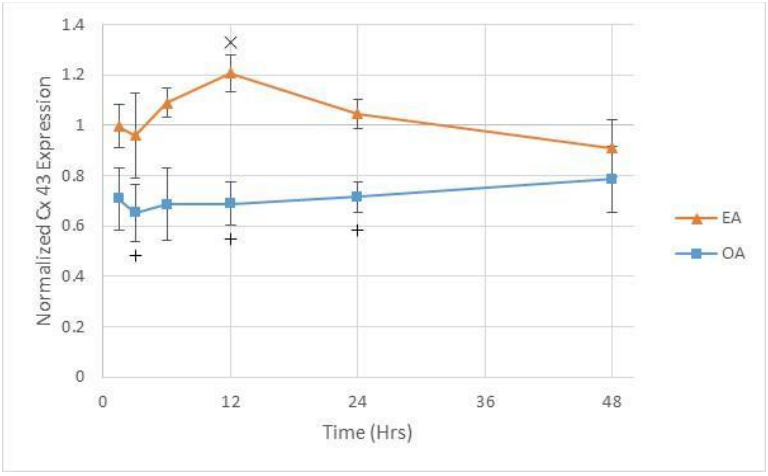


Figure 10: EA increases whereas OA decreases Cx43 expression in endothelial cells. Cx43 expression in OA or EA (30 μM) treated samples was normalized at each time to its vehicle control. OA significantly decreased Cx43 by about 25% at 3, 12, and 24 hours

(n=6). In contrast, EA increased Cx43 expression at 12 hrs (n=5), but was not altered at other times. × indicates increase in Cx43 ($p \leq 0.05$). * - indicates decrease in Cx43 ($p \leq 0.05$).

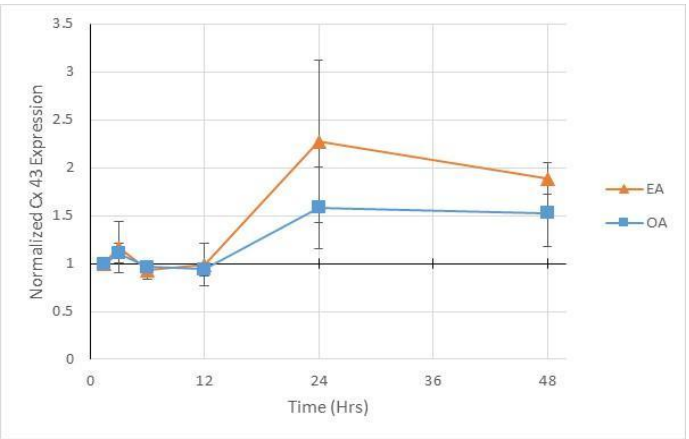


Figure 11: Cx43 expression in endothelial cell vehicle controls for FA treatment. The expression of Cx43 was normalized to the 1.5 hour time. No significant changes in Cx43 expression were observed.

Discussion

In this study, we examined the effects of monounsaturated FA double bond structure on Cx43 protein expression in endothelial cells. Since the endothelial cells responded to LPS, they should respond to EA as the TLR4 response was evident (Fig.2). The atheroprotective OA decreased Cx43 expression whereas the inflammatory EA increased Cx43 expression (Fig. 6). This increase in Cx43 expression and its time-course was consistent with the response to LPS, which suggests that these PAMPs may act through the same receptor and pathway. The implications of these results for endothelial dysfunction along with the limitations of this work will be addressed below.

The cell culture technique is well-suited for understanding cellular responses to particular treatments. By plating cells of a common origin and separating them into control and experimental treatment groups, any changes in Cx43 expression should result from the addition of FA to the media. This was a strength of the study, as it allowed more accurate assessment of time-dependent changes due to treatment. However, this type of work has limitations that impact our interpretation of the data (i.e., confounding factors). First, the confluency state of the cells prior to splitting them for each experiment was not well controlled. Often, the cells were super-confluent in order to ensure enough cells were available to complete an experiment, as each sample set required 9.6 million cells (12 plates of 800,000 cells each). If confluency affected the TLR receptor of its signaling pathway, it would contribute to the variability in our results. Furthermore, the level of endotoxins in the FBS was not minimized. While the treatment and control did not differ in FBS exposure, the basal level of endotoxin may have

influenced the response. Last, early issues with immunoblotting optimization resulted in weak signal detection and led to high levels of background noise.

The time course of the response to LPS was consistent with previous studies.[1,7,10] The peak of the Cx43 response to LPS was at 12 hours. As the half-life of Cx43 is 1-3 hours,[42] this suggests a secondary mediator is released by endothelial cells in response to LPS. As the response to TLR4 binding involves the production and release of cytokines TNF-α, IL-1β, and IL-6 and LPS is an established ligand for the receptor, the response to LPS is likely mediated through TLR4. LPS stimulation has also been shown to enhance hemichannel degradation by PKC, which phosphorylates Cx43.[31,42] As this enhanced degradation is more rapid, it may explain the trend towards a reduction in Cx43 at 1.5 hours (Fig. 2).

The similarity in the time course Cx43 response to EA suggests that a similar pathway may be involved. However, without employing receptor blockers or antisense nucleic acids to inhibit the TLR pathway (TLR4 or cytokine receptors), that assertion is speculative. Thus, the data collected herein on endothelial cell responses to EA are consistent with the involvement of TLR4, as it is similar to our LPS responses and data from others on saturated FA.

More importantly, the downregulation of Cx43 by OA is of greater interest, as it provides evidence that the conformation of FA double bonds determines endothelial cell responses to FA. Downregulation observed as soon as 3 hours suggests a different mechanism (from EA) may mediate endothelial responses to OA. The shorter time could be caused by the integration of OA into the membrane in a way that would affect gap junction communication and thereby expression. Previous work has inferred that OA

integrates into the membrane to bind directly to Cx43 and destabilize gap junctions to completely block communication at 30 μM.[24] Others have argued that this destabilization can also lead to Cx43 gap junction disassembly through increased phosphorylation by protein kinase C-ε and Src kinase.[25] It should be noted that the studies above were performed on freshly isolated cardiac myocytes (not exposed to growth factors) and not immortalized endothelial cells . As the nature of the response likely varies by cell type (cardiac vs endothelial) and by the conditions the cells were in when treated (primary isolate vs immortalized) future work will be necessary to determine the nature of the response in the endothelial cells specifically. Alternatively, the common aspects of the structure of OA and EA may lead OA to act as an antagonist blocking the TLR4 pathway. If that pathway was already activated by the endotoxins present in the media, then exposing these cells to an inhibitor of TLR4 would decrease Cx43 expression. This may account for the previously described anti-inflammatory properties of OA and other cis monounsaturated FAs.[21]

Alterations in specific Cx levels have implications on communication between endothelial cells.[12] As each connexin has very unique communication properties (unitary conductance, permselectivity, gating, etc), any changes in expression of a connexin can dramatically alter the message communicated between cells. In endothelial cells that usually express Cx37 and Cx40, which are permselective with higher unitary conductances, increasing Cx43 in the junctions would reduce their permselectivity thereby the message.[23] The challenge for the gap junction field is to understand the true importance of what is being communicated between cells. Once that is known, we can hypothesize about the impact of changes in communication on the function of these cells.

Summary: These data provide further evidence supporting the drive to reduce (eliminate) trans FA from our diet. Herein, we provide evidence trans FAs increase endothelial cell Cx43 expression that may contribute to the endothelial dysfunction that is observed in individuals with obesity and contribute to the resulting increase in CVDs.[6,13] The same was not the case for the cis FA that actually reduced the expression of Cx43 thereby supporting their anti-inflammatory function. While these results are intriguing, further work will be required to address the mechanisms that underlie the changes reported.

Implications: Trans FA pose a serious and unnecessary health risk for humans that we are only now trying to address. These FAs are not typically observed in nature. Rather, they are the product of a food manufacturing industry's attempt to meet the demands of the public for quality food (taste and texture especially as that is part of the reason partially hydrogenated oils are used in processed foods). These data provide supporting evidence for the assertion that trans FA may be contributing to the worldwide obesity epidemic. Thus, the current attempts to reduce the levels of trans FA in our diet are to be lauded. We are hopeful that these reductions may lead to a reduction in the incidence of obesity and more importantly the CVD associated with obesity and hyperlipidemia.

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