The Role of Fatty Acid Binding Protein as a Link Between Metabolic Switching and Immune Response in the Macrophage

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Abstract

Over the last years, there has been unprecedented growth of metabolically-related diseases yet the underlying cellular mechanisms by which each arise is still widely unknown. Recent evidence supports a strong link between the pro-inflammatory response and mechanisms regulating cellular metabolism. This project investigates the role of metabolic factors on the pro-inflammatory response of the macrophage. Macrophages are significant in their heterogeneous phases of activation. Activation change is triggered by cell environment, which is ultimately a product of cell metabolism. The macrophage's metabolic switching combined with its known role in the immune response indicates that a relationship may exist between these two functions. Fatty Acid Binding Protein 5 (FABP5) is exclusive to the macrophage. FABP5 has a known role in fatty acid metabolism but the pathways by which FABP5 connects to the inflammatory response, and thus to disease, is under investigation. We have found through observing changes in gene expression (i.e. Interleukin 12, IL-12; chemokine ligand 2, CCL-2; Acyl-CoA Oxidase 1, AOX; Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PGC-1α; and Cluster of differentiation 36, CD36) already known to be connected to metabolism and inflammatory response that FABP is a vital link between metabolic switching and immune response in the macrophage.

Abbreviations

LPS, lipopolysaccharide; IFN-γ, interferon gamma; IL-4, interleukin 4; M1, pro-inflammatory; M2, anti-inflammatory; CCl-2, chemokine (C-C motif) ligand 2; IL-12,

interleukin 12; CD36, fatty acid translocase; AOX, acyl-CoA oxidase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1-alpha; SBFI26, Fatty Acid Binding Protein inhibitor

1. Introduction

Through evolutionary history, the main threats to mankind's life revolved around "starvation, infection, and predation" (Odegaard, 2013). Advances in technology throughout the years have decreased these threats to human life; however, developed countries have seen an increase in "evolutionarily novel threats" such as cardiovascular disease, cancers, and diabetes (Odegaard, 2013). This is largely a result of changes in food behaviors. These often fatal diseases are exacerbated even further by obesity. From 1980 to 2008, the number of overweight people in the world doubled to surpass the number of underweight people for the first time in history. Overweight individuals have been found to have higher rates of all-cause mortality with this statistic increasing exponentially with extra pounds on the body (Odegaard, 2013). While the connection between pathology and obesity is clear, the cellular and molecular mechanisms behind this relationship are under continuous investigation and debate (Kasmi, 2015). This study focuses on the connection between metabolic diseases (e.g. obesity), the immune response and pathology.

The immune system and metabolism are closely linked processes. Metabolism is the series of pathways through which the body breaks down substrate for the creation of energy. Functions of the immune system and metabolic system are very much interrelated; Dr. Gökhan Hotamisligil, a leader in glucose and lipid metabolism, states

that, “immunity is a metabolically costly endeavor” (2008). An example of the immune response’s dependency on the metabolic system is perfectly framed by the metabolic costs of fever. Hotamisligil continues that, “fever… is approximately the same energy cost of a 70kg person walking 45km” (2008). This energy required to generate fever is derived from metabolic processes throughout the body. In this way, it can be seen that metabolic balance is vital to proper immune function. Thus, it is hypothesized that a metabolic imbalance, either through energy surplus or deficiency, can affect immune response (Hotamisligil, 2008).

Fatty liver disease (FLD) is a pathology that exemplifies the relationship between metabolism and immune response. In the common progression of FLD, liver first accumulates fat in steatosis. Then, the second step involves inflammation due to immune cell response termed non-alcoholic steatohepatitis (NASH). Scarring in NASH becomes permanent, progressing into fibrosis. From fibrosis, FLD can develop into liver cirrhosis or cancer, which then eventually leads to other related diseases or even death. This progression is depicted in Figure 1A.

This progression can be seen on the microscopic level in Figure 1B. A normal, healthy liver has a plethora of healthy, red liver cells. Progressing into steatosis, it can be observed that there is a dramatic increase in lipids, illustrated by the large, white lipid cells. Further progression into NASH is marked by the presence of punctate macrophage cells, which indicate the beginning of scarring. After some time, the scarring will become permanent, prompting liver cirrhosis.

Figure 1A. Common progression of FLD

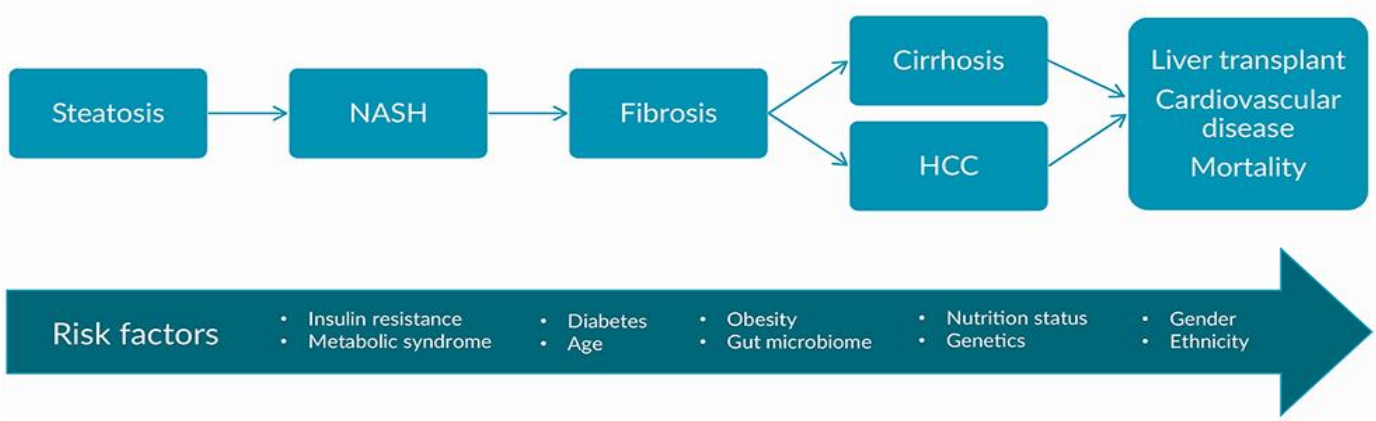
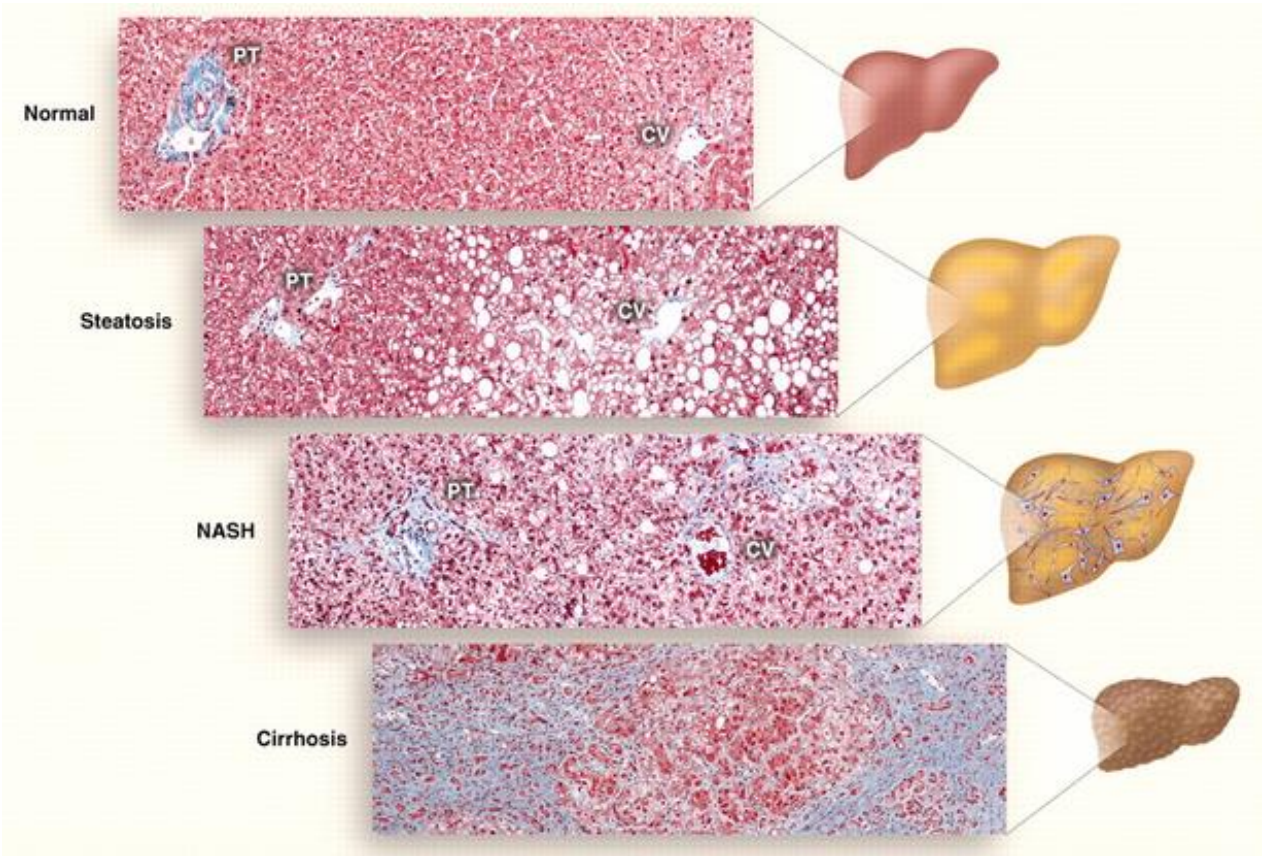
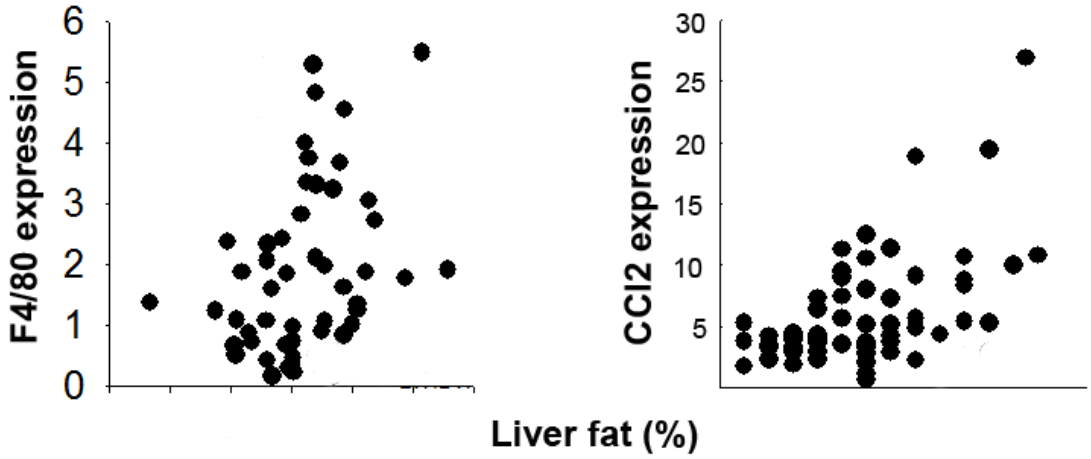


Figure 1B. Common progression of FLD on the microscopic level



While FLD can be exacerbated by a number of risk factors, seen in Figure 1A, of particular interest pertaining to the relationship between metabolism and immune response are those of obesity and nutrition status.

Figure 1C. Macrophage activation in FLD

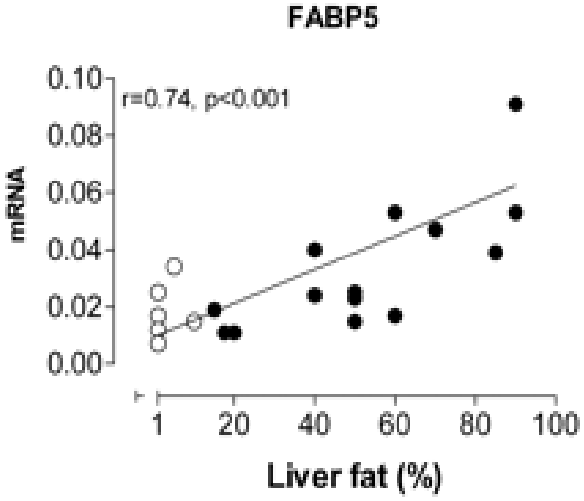


Macrophages are major player in immune response via phagocytosis and cytokine production. However, macrophages are now known to be heterogeneous in function expanding far beyond common knowledge. Macrophages have various phases of activation, known as phenotypes. Diverse macrophage phenotypes dictate how the macrophage will behave. The activation of these phenotypes is largely determined by the microenvironment in which the macrophage resides, ultimately directed by metabolism (Mosser, 2008). Figure 1C depicts that with increasing liver fat, there is an increase in F4/80 expression. F4/80 is used as an indicator for macrophage activation. Thus, increasing liver fat is correlated with an increase in macrophage activation. This is also accompanied by an increase in CCL-2 expression. CCL-2 is a hallmark pro-inflammatory response gene. As such, it can additionally be said that increasing liver fat

is correlated with an increase in inflammatory response. This strong correlation indicates that there may be a connection between fatty liver disease, macrophage activation, and the inflammatory response.

Lipids are involved in the activation of diverse macrophage phenotypes. An accumulation of lipid causes M1activation of macrophages. This phase of activation, or the pro-inflammatory response, is linked to metabolic disease such as atherosclerosis and liver disease. Reversely, the addition of unsaturated fatty acids to an M1 macrophage causes an M-2 shift in the macrophage, or the anti-inflammatory response, linked to protection of cells and wound healing (Kasmi, 2015). These finding suggest a mechanistic role of lipid metabolism in the regulation of the macrophage response. Thus, a critical factor in lipid metabolism such as FABP5 is studied.

Figure 1D. FABP5 mRNA expression vs. % liver fat



This study will specifically focus on Fatty Acid Binding Protein 5 (FABP5), a lipid chaperone exclusively expressed in liver macrophages. FABP5 has a known role in metabolism and pro-inflammatory response, but the mechanisms by which these occur

are still unknown (Hotamisligil, 2008). The chart above (Figure 1D) illustrates this significant correlation between increasing liver fat and FABP5 mRNA expression (Westerbacka, 2007). Given the strong relationship between activation of the macrophage and lipids, it is hypothesized that the lipid chaperone has a larger role than what is currently known in the literature. Knowledge of the mechanisms of FABP5 function is important to understand due to the fact that manipulation of the mechanism could reverse the harmful M1 activation and its pro-inflammatory effects (Kasmi, 2015).

The experiments outlined here identified genes related to FABP5 in activated macrophages in response to different metabolic environments. Macrophages were stimulated toward pro-inflammatory or anti-inflammatory activation in the presence or absence of FABP5 inhibitor SBFI26 or the presence or absence of lipid. Quantitative polymerase chain reaction (qPCR) was then used to quantify cytokine and metabolism genes' expressions. This study will be important to helping to establish the mechanistic link between FABP5 and macrophage response.

2. Materials and methods

2.1 Cell culture

Macrophages from blood, Raw 264.7 murine cell line, were cultured to confluency and subsequently plated. Raw cells were treated for 4 or 24 hours with LPS (0.25 mg/µL) / IFN-γ (20 µg/mL) to induce an M1 phenotype, or IL-4 (10 µg/mL) to induce an M2 phenotype. Cells were cultured in DMEM + 10% Fetal Bovine Serum (FBS) and 1% Penicillin Streptomycin G (PSG). Cells were also challenged with palmitic acid or a fatty acid binding protein inhibitor, SBFI26 (100 µM).

2.2 RNA isolation & CDNA preparation

RNA from RAW cells were isolated using Trizol (Invitrogen, Carlsbad, CA), chloroform and isopropanol. Isolated RNA was suspended in 40 µL H2O. CDNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's protocol.

2.3 Quantitative PCR

Gene expression was quantified using Maxima SYBR Green (ThermoFisher Scientific) according to manufacturer's protocols and normalized to the house keeping gene βactin of the untreated controls. Primer sequences for genes are shown in table 1.

Table 2.1 RT-PCR Primer sequences

β-actin	5'-TGAGAGGGAAATCGTGCGTG-3' 5'-TGCTTGCTGATCCACATCTGC-3'
PGC-1α	5'-TCACCCTCTGGCCTGACAAATCTT-3' 5'-TTTGATGGGCTACCCACAGTGCT-3'
CCL-2	5′-ATGCTTGGCTCAGCAC-3′ 5′-TCAATTTTTCATTTTGAGTGT-3
IL-12	5′-CTCACCTGTGACACGCCTGA-3′ 5′-CAGGACACTGAATACTTCTC-3′
CD36	5'-TCACCCTCTGGCCTGACAAATCTT-3' 5'-TTTGATGGGCTACCCACAGTGCT-3'
AOX	5'-GACTGGTTCCAATTGACAAGC-3' 5'-GCAAATGGCATTCTGACATCC-3'

3. Results

3.1 The effect of excess lipids on macrophage response (Figure 3A)

Relative CD-36 mRNA expression increased in Raw cells when treated with palmitic acid treatment in both control and LPS/IFN-γ treated cells. Relative AOX

mRNA expression similarly increased in both control and LPS/IFN-γ treated cells, although overall response was mitigated slightly in the LPS/IFN-γ activation. Relative PGC-1α expression proliferated significantly when treated with palmitate. This increase was even more exaggerated in the LPS/IFN-γ activation. Relative CCL-2 mRNA was scarcely expressed in control but significantly increased when treated with LPS/IFN-γ. Interestingly, this effect was stunted when treated with palmitic acid with LPS/IFN-γ activation. Finally, relative IL-12 mRNA was expressed in similar levels between control and LPS/IFN-γ activation and treatment with palmitic acid.

3.2 The effect of FABP5 inhibition on macrophage response (Figure 3B)

Relative CD-36 mRNA showed no change in expression between control and LPS/IFNγ treatment. Increase did occur when activated with LPS/IFN-γ and treated with SBFI26. Relative AOX mRNA expression increased with treatment of SBFI26, although the overall response was mitigated slightly when treated when LPS/IFN-γ activated. Relative PGC1-α mRNA expression showed some increase with SBFI26 treatment, the effects of which were exaggerated by LPS/IFN-γ activation. Relative CCL-2 mRNA expression was scarce in control, but proliferated massively with LPS/IFN-γ activation. Treatment with SBFI26 of LPS/IFN-γ activation further multiplied the expression. Relative IL-12 mRNA expression remained level in control, but massively increased in LPS/IFN-γ activation with treatment of SBFI26.

3.3 Overall effects

CD-36, AOX, and PGC-1α are involved in fatty acid metabolism. CD-36 is a membrane protein that binds and transports fatty acids. AOX is an enzyme involved in

the TCA cycle. PGC-1α is a key regulator in shifts toward oxidative metabolism. Relative CD-36 expression increased with palmitic acid treatment. However, this response was significantly blunted when compared with treatment with FABP inhibition. Most significantly, CD-36 expression is mitigated in LPS/IFN-γ activation with FABP inhibition, whereas comparatively it was increased in LPS/IFN-γ activation with palmitic acid treatment. AOX had no significant change between treatments. Relative PGC-1α expression with FABP inhibition decreased massively in comparison to the huge increase when treated with palmitic acid. This trend is observed across both control and LPS/IFN-γ activation.

CCL-2 and IL12 are pro-inflammatory cytokines. CCL-2 expression, which noticeably increased just with LPS/IFN-γ activation even in the presence of palmitic acid, was even further proliferated with FABP inhibition. IL12 showed no significant change in palmitic acid treatment, but increased significantly with FABP inhibition and LPS/IFN-γ activation.

Figure 3A. Expression of fatty acid metabolism-related genes

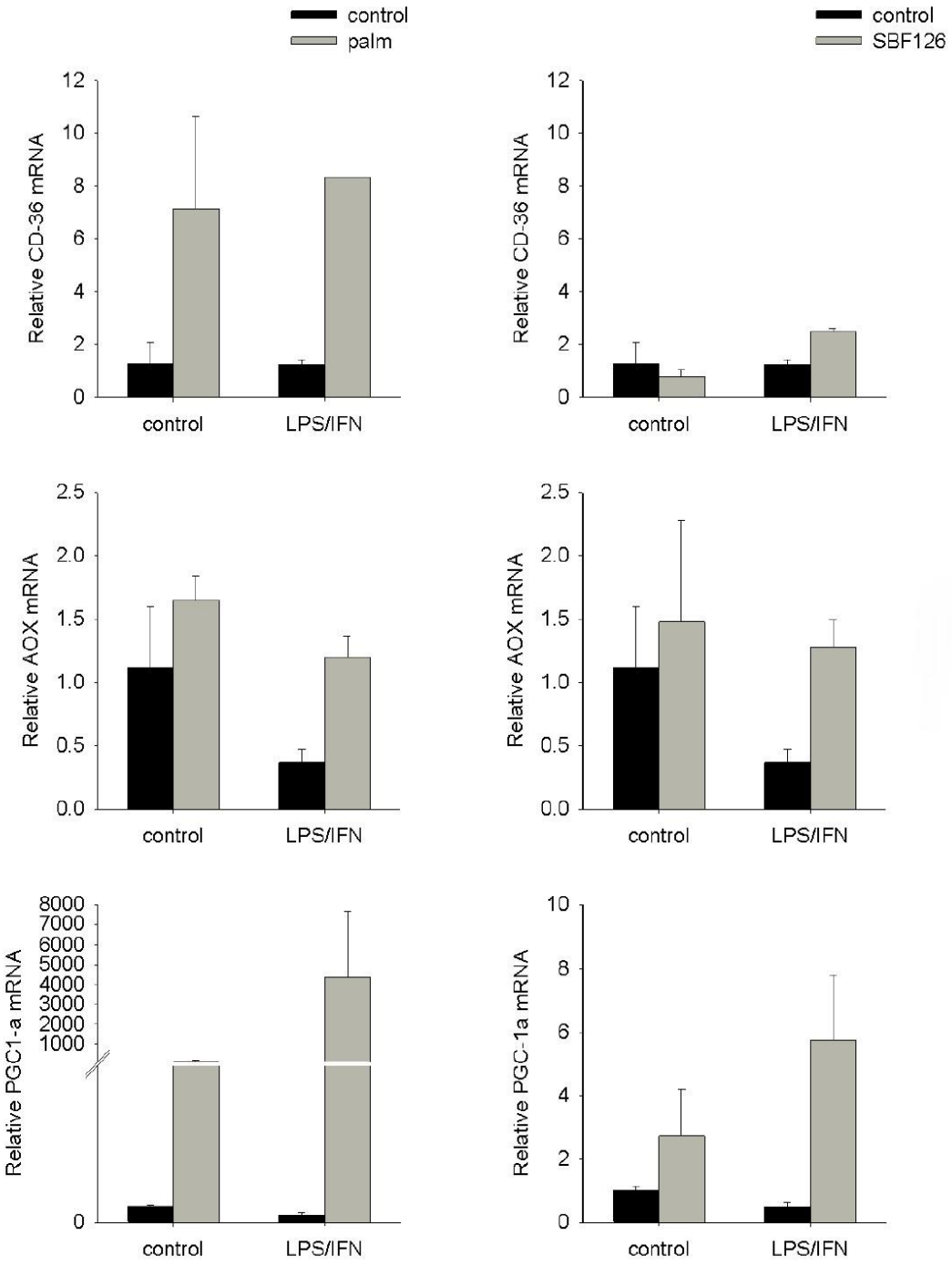
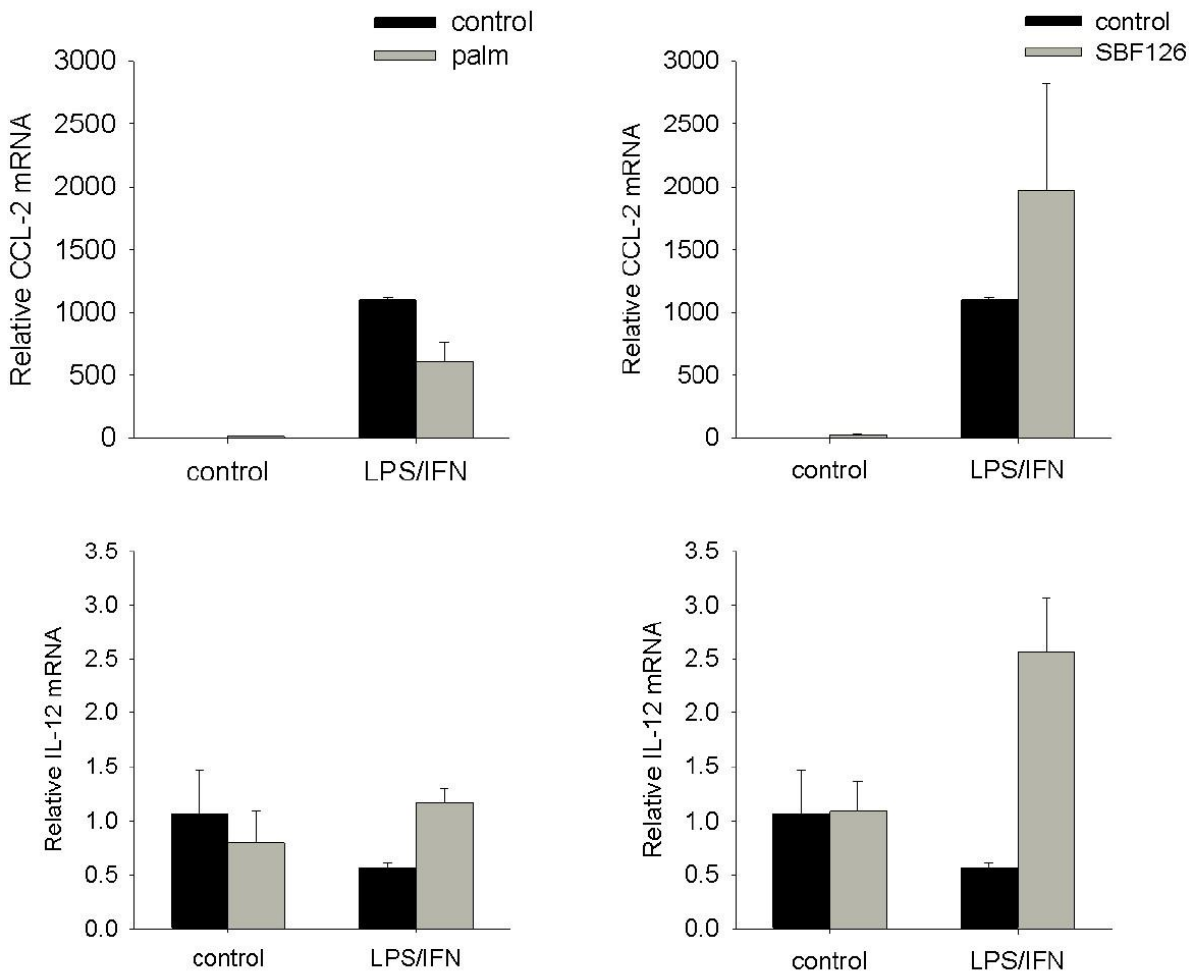


Figure 3B. Expression of pro-inflammatory response genes



4. Discussion

Fatty acid binding protein 5 (FABP5) is thought to be vital to the uptake, transport, and utilization of fatty acid in metabolism. SBFI26 is an FABP inhibitor while palmitic acid is a fatty acid that would be used as a substrate for fatty acid oxidation. Since fatty acid oxidation is linked with a shift from a pro-inflammatory M1 response, the treatment with palmitate should mitigate the M1 response by LPS/IFN but increase the M2 response by IL4. Theoretically, SBFI26 should decrease expression genes associated with FABP and fat oxidation, while palmitic acid should have the opposite effect and cause an increase of genes downstream of FABP. Inhibition of FABP should increase LPS/IFN-γ activation, which causes the macrophage to shift toward its M1, or glycolytic, phase.

The overall decrease in expression of fatty acid metabolism-related genes and increase in pro-inflammatory genes with FABP5 inhibition asserts fatty acid binding protein as a vital link between metabolic switching and immune response in macrophages. This data suggests that FABP5 is vital for the transport of fatty acids into the macrophage for fatty acid metabolism. Thus, in the absence of fatty acid metabolism, there should be an increase in glycolysis and thus the M1 pro-inflammatory response. Further research to confirm this relationship could be to quantify glycolysis-related metabolism genes in the presence and absence of FABP5 inhibitor. Additionally, recent literature suggests that palmitate may be an inhibitor of FABP5. If palmitate is both a substrate and an inhibitor in the fatty acid metabolism process, then further exploration must be done into the exact role of palmitate in the inhibition or activation of FABP5. This also raises the question whether fatty acid saturation affects

lipid interaction with FABP5. This could be further explored by comparing treatments with saturated and unsaturated fatty acids. This process could underlie the common notion that unsaturated versus saturated fatty acids have different health implications.

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