이학석사 학위논문

The mechanism of macrophage activation by down-regulation of fatty acid synthase in obesity

비만에서 지방산합성효소에 의한

대식세포 활성화 기전에 관한 연구

2016년 2월

서울대학교 융합과학기술대학원

분자의학 및 바이오제약전공

홍 은 별

Abstract

The mechanism of macrophage activation by down-regulation of fatty acid synthase in obesity

Eunbyeol Hong

Molecular Medicine and Biopharmaceutical Sciences

WCU Graduate School of Convergence Science and Technology

The Graduate School Seoul National University

It is well known that obesity induces inflammation through *in vivo* studies. However, the mechanistic studies of how an event of inflammation links to obesity are still under investigation. Here, we found that in macrophages of an obese mouse, both mRNA and protein expression of Fatty Acid Synthase (FASN) is down-regulated. They also show a significant accumulation of intra-cellular acetyl coenzyme A, a precursor of

fatty acid synthase, as well as lipid droplet in cytoplasm. Acetyl coenzyme A accumulation resulted in an increase of histone acetylation which ultimately caused the transcriptional up-regulation of various inflammatory genes such as IL-1β and iNOS. The FASN knock-down macrophages produced more IL-1β and iNOS compared to control macrophages whereas their level decreased back to normal when FASN is rescued by over expression. In addition, the expression of CD36, an external fatty acid transporter, was increased in FASN knock-down macrophages. Thus, there were much more external fatty acid accumulation occurred in cytoplasm. When CD36 is blocked by its inhibitor (SSO), IL-1β and iNOS decreased correspondingly which suggests that FASN knock-down macrophages may facilitate the uptake of external fatty acids through CD36 and that the accumulation of those fatty acids induces inflammation. Together, these findings suggest that obesity-induced down-regulation of FASN causes inflammation through promoting histone acetylation and an accumulation of external free fatty acids up-taken by CD36.

Keywords: obesity, fatty-acid synthase, inflammation, macrophages

Student ID: 2012-22858

Table of Contents

Introduction

Obesity is a chronic inflammatory condition and is a key risk factor for the development of insulin resistance causing type II diabetes and other metabolic diseases (1) (Fig1a). Macrophages reside throughout the body as resident elements of most organizations (2). In obese condition, however, adipocytes produce monocyte chemotactic protein 1 (MCP-1), which allows monocyte infiltration into adipose tissue (3). Such infiltrated monocytes become activated and accumulated within adipose tissue, resulting in inflammatory condition accompanied by obesity. Mobilization of proinflammatory macrophages to adipose tissue is the center of inflammatory response (4). Thus, they increase production of various proinflammatory cytokines and gene, such as interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNFα), IL-6, and iNOS (3). It has been known that these inflammatory cytokines provoke serious problems related to obesity such as insulin resistance and atherosclerosis.

During inflammation, additional macrophage will be recruited from the proliferation of resident cells or the bone marrow by monocyte developments (5, 6). Lipids contribute to obesity and atherosclerosis. Furthermore, oxidized LDL and free fatty acids can induce inflammation (7). Importantly, reducing the accumulation of the lipid in macrophages reduces inflammation and improves whole body insulin sensitivity (8, 9).

Macrophages can polarize into M1 (also known as classically activated) and M2 (also known as alternatively activated) macrophages that have distinct functional phenotypes (10). M1 macrophages are induced by proinflammatory mediators such as LPS and IFN-γ. M1 macrophages have enhanced proinflammatory cytokine production (TNF-α, IL-6, IL-12) and generate reactive oxygen species such as NO via activation of iNOS (Nos2) (11). In contrast, M2 macrophages are generated by exposure to IL-4 and IL-13 (12) and they are believed to participate in the blockade of inflammatory responses and in the promotion of tissue repair (13).

Such macrophage polarization is a critical event in terms of explaining inflammatory condition in obesity. Healthy lean adipose tissue contains resident M2-polarized macrophages that help to maintain tissue homeostasis (14). In an early stage of obesity, adipocytes also become obese due to excess of nutrition and immunes cells such as neutrophil, T lymphocytes, mast cell, and M1 macrophages are infiltrated into adipose tissue. Furthermore, hypertrophic adipocytes become inflammatory and/or necrotic, thereby attracting M1 macrophages organized in crown-like structures. Obesity is associated with a switch in the M1/M2 macrophage balance, with M1 macrophages overwhelming the number of M2 macrophages (14). However, the mechanism of monocyte/macrophage activation in metabolic disorders or obesity remains elusive (Fig1b).

Fatty Acid Synthase (FASN) is a multi-subunit enzyme which participates in fatty acid synthesis. FASN is composed of 7 subunits and utilizes acetyl-CoA and malonyl-CoA as substrates to synthesize 16-carbon fatty acid, palmitic acid.

In vivo studies revealed that upon high fat diet, the protein level of FASN is reduced in liver and adipose tissue (15). Furthermore, up-regulation of FASN results in an activation of PPAR delta, which leads to activation of M2 macrophage (16). Thus, we were able to hypothesize that FASN would be down-regulated in obese condition, which leads to M1 macrophage activation.

This paper aims to understand the mechanism by which fatty acid synthase is related to macrophage activation in obesity.

Material and Method

Cells and reagents

Raw264.7 cells were grown in RPMI medium containing 10% fetal bovine serum with 1% antibiotics at 37℃ in a 5% CO_2 incubator.

Palmitic acid conjugation with BSA

Palmitic acids were conjugated to fatty acid-free and endotoxin-free bovine serum albumin (BSA, Sigma-aldrich A8806). We prepared 200μM of Palmitic acid (Sigma-aldrich P0500) in ethanol and mixed it with an aqueous 10% BSA solution (BSA in RPMI1640 culture medium) for 4μM by dissolving them at room temperature until homogeneous.

Experimental agents

100mg/ml Lipopolysaccharide (LPS, Sigma-aldrich) was dissolved in PBS. A final concentration of 100mM Sulfo-N-succinimidyl oleate (SSO, santacruz biotechnology, sc-208408) was dissolved in dimethyl sulfoxide.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Expression levels of genes were analyzed with real-time, quantitative PCR

using a SYBR Green-based method. RNA was harvested using TRIZOL (invitrogen), mRNA was converted into cDNA using RTase (Fermentas). The primer sequences are summarized in below. Average fold changes were calculated by differences in threshold cycles (Ct) between pairs of samples. L32 were used as endogenous controls in Raw 264.7.

Primer (5'-3')

L32 Forward GGCATTGACAACAGGGTGCGGA

L32 Reverse AACTTGCGGAAGCCGCTGGG

FASN Forward AGGTGGCAGAGGTGCTGGCT

FASN Reverse GCGCAGGGTCGGAAGGGTTC

CD36 Forward TGGCCTTGCACTCTCTCATCGG

CD36 Reverse TTCTACGTGGCCCGGTTCTACTAA

iNOS Forward CAAGCACCTTGGAAGAGGAG

iNOS Reverse AAGGCCAAACACAGCATACC

IL-1β Forward TTGACGGACCCCAAAAGATG

IL-1β Reverse AGAAGGTGCTCATGTCCTCA

IL-10 Forward CTGAGGCGCTGTCATCGATT

IL-10 Reverse AGGTCCTGGAGTCCAGCAGA

TNFα Forward TCAGCCGATTTGCTATCTCA

TNFα Reverse CGGACTCCGCAAAGTCTAAG

Plasmid

shControl and shFASN model using pLL3.7 shRNA systems

shControl and shCD36 model using pKLO.1 shRNA systems

FASN over expression plasmid (pCMV-SPORT6) openbiosystems

Western blot analysis

Whole cell were isolated using RIPA buffer (Sigma-aldrich) and nuclear were extracted using digitonin buffer (20mM Tris-Cl pH7.4, 0.3% digitonin, protease inhibitor). Antibody lists are summarized in below.

FASN Cell signaling #3180

CD36 Thermo scientific PA1-46480, PA5-27236

iNOS Santacruz biotechnology SC-650

Actin Thermo scientific MA5-15739

Acetyl-lysine Santacruz biotechnology sc-32268

H3K9,15 Merck Millipore 06-599

H3K27 Cell signaling #8173

H4K5 Cell signaling #9672

H4K8 Cell signaling #2594

H4K12 Cell signaling #2591

H4K16 Active motif 39930

H4(PAN) Active motif 39926

total H4 Cell signaling #2935

Lamin A/C Cell signaling #2032

Isolation of stromal vascular fraction from mice

Adipose tissue was harvested from the gonadal fat in normal diet or high fat diet fed male mice. Adipose tissue was washed with phosphate-buffered saline (PBS). Then tissue was chopped and digested enzymatically with 0.1% Collagenase A in PBS for 1 hour at 37°C with orbital shaking. Enzyme activity was then neutralized by adding Dulbecco's modified
Eagle's medium (DMEM, invitrogen) supplemented with 10% fetal bovine serum (FBS). The digested adipose tissue was centrifuged for 10 min at 1800rpm. The cell-containing pellet was re-suspended in PBS and repeat centrifugation. Then, the cell pellet was lysed with TRIZOL or RIPA buffer.

Click-iT assay

Exogenous palmitic acid uptake was detected by click reaction between palmitic acid-azide and Alkyne-647. Raw264.7 cells were treated with palmitic aicd-azide for 6hours, then with Alkyne-647. After the reaction we observed exogenous palmitic acids with confocal microscopy.

FASN activity assay

FASN activity was measured by monitoring oxidation of NADPH at 340 nm using spectrophotometry. Cells were harvested by scrapping, pelleted by centrifugation, washed and re-suspended in ice-cold PBS. Cells were sonicated for 30sec 3 times and centrifuged for 10 min at 4˚C. We transfered the supernatant to a new tube and protein was quantified with BCA system. The protein lysate containing FASN, K3PO4, malonyl-CoA and NADPH mixture were monitored at 340 nm in a heated chamber spectrophotometer at 37°C for 3 min to measure background NADPH oxidation.

Intracellular Acetyl-CoA

Acetyl-CoA level was measured using PicoProbe™ Acetyl-CoA Assay Kit (ab87546). In the assay, free CoA is quenched then Acetyl-CoA is converted to CoA. The CoA is reacted to form NADH which interacts with PicoProbe to generate fluorescence (Ex=535/Em=587 nm).

Results

High fat diet reduces the level of fatty acid synthase (FASN) mRNA and protein expression in stromal vascular fraction (SVF)

It has been previously reported that FASN is down regulated in liver and adipose tissue of obese mouse model (17). However, the causal relationship between obesity and FASN expression needed a further confirmation. In order to do so, we tested the mRNA and protein level of fatty acid synthase in a normal diet (NLD) and in a high fat diet (HFD) mice. After they have been fed approximately for 6 months, we extracted stromal vascular fraction (SVF) from gonadal fat of individuals and measured mRNA and protein expression of FASN. SVF includes preadipocyte, mesenchymal stem cells, T cell, B cell, and macrophage. In normal diet, 15% of SVF is macrophages; in high fat diet (HFD), its percentage increases up to 60% (18).

The high fat diet group showed a significant decrease of FASN expression both in mRNA (Fig2a) and protein (Fig2b). In order to test whether it is macrophage specific, immunofluorescence (IF) assay was conducted using macrophage specific marker, CD11b, merged with FASN (Fig2c). The data confirmed that FASN is indeed down-regulated in CD11b-

positive macrophages in HFD.

To mimic high fat diet effect *in vitro*, exogenous free fatty acid, palmitic acid, was treated in Raw264.7 cells. The mRNA level of FASN was reduced upon pamitic acid treatment (Fig2d). Thus, these data confirm that FASN is indeed down regulated *in vivo* and *in vitro* in obese condition.

Based on the above data, we prepared a FASN knockdown cell line using shFASN plasmid on top of mouse monocytic cell line, Raw264.7 (shFASN Raw264.7). The selection process was done using GFP-tag where only GFP expressing cells were selected and single-cell cultured. The shFASN Raw264.7 indeed had a reduced amount of FASN mRNA (Fig2e) and protein (Fig2f). In addition, FASN activity was tested by measuring consumption of malonyl-CoA, a substrate of FASN, and the result indicates a complete shutdown of FASN activity in shFASN Raw264.7 (Fig2g).

Reduced level of fatty acid synthase induces transcription and translation of inflammatory genes

To examine whether provoked inflammation in obesity is related with through FASN down-regulation in macrophages of adipose tissue, we checked profiles of pro-inflammatory markers in shFASN Raw264.7. The mRNA levels of inflammatory markers such as iNOS and IL-1β were increased in shFASN Raw264.7 compared to the control group. (Fig3a).

Similarly, the protein level of iNOS was also increased in FASN-short condition (Fig3b). Since IL-1β is a secretory cytokine, we performed ELISA assay, which demonstrated that IL-1β expression is dramatically increased in shFASN Raw264.7 after LPS stimulation.

In order to confirm these data, rescue experiments were conducted. We transiently transfected FASN overexpression plasmid into shControl and shFASN Raw264.7 cell line and checked the expression of iNOS, IL-6, and IL-1β. Quantitative RT-PCR and western blot results revealed that when FASN is recovered, iNOS, IL-6 and IL-1β mRNA expressions are reduced back to their normal state (Fig3d) as well as the protein expression of iNOS (Fig3e). These data demonstrate that a reduced level of FASN induces transcriptional and translational up-regulation of inflammatory genes. Also, when FASN is rescued, inflammatory gene expression is reversed back to its normal condition, which demonstrates that such inflammatory effect is FASN-dependent.

Knockdown of fatty acid synthase results in the accumulation of intracellular acetyl coenzyme A and increased histone acetylation

Then, we were interested in finding the link between inflammatory state in obesity and the role of down-regulated FASN expression. Since

FASN utilizes malonyl-CoA and acetyl-CoA as its substrates, we hypothesized that down regulation of FASN will result in the accumulation of palmitic acid precursor molecules such as acetyl-CoA and malonyl-CoA (Fig4a).

Indeed, when FASN is down regulated, the consumption of acetyl-CoA decreased which is demonstrated by measuring the level of accumulated intracellular acetyl-CoA (Fig4b). Since histone acetylation process uses acetyl-CoA as a substrate, we then hypothesized that elevated levels of intracellular acetyl-CoA will cause general increase of histone acetylation. Thus, we performed a nuclear fractionation for western blot analysis using shControl and shFASN Raw264.7. Using various antibodies that capture various acetylated lysine residues of histone, acetylation level of histone was tested by western blot. The result illustrates that H3 and H4 acetylation is increased in shFASN Raw264.7. Specifically, H3K9,15, H3K27, H4K5, and H4K8 showed most dramatic increase in acetylation level (Fig4c). Importantly, in HFD mouse macrophages, total level of histone acetylation also increased both in total lysate (Fig4d) and nuclear fraction (Fig4e).

Furthermore, we aimed to investigate whether the event of histone acetylation occurs specifically in the promoter regions of inflammatory genes. Chromatin immunoprecipitation analysis result demonstrated that

iNOS and IL-1β gene transcription levels were increased upon acetylation of H3 (K9, 15). However, the level of endogenous control, L32, remained constant. Therefore, these results illustrate that when FASN is down-regulated, macrophages are using accumulated intracellular acetyl-CoA in stimulating histone acetylation and that increased level of histone acetylation specifically affects the gene expression of iNOS and IL-1β.

Knockdown of fatty acid synthase increases the level of CD36 and induces exogenous fatty acid uptake

We assumed that in the absence of properly functioning FASN, its end product, palmitic acid, will be depleted within the cytoplasm as well as its derivatives such as stearate or palmitoleate (Fig5a). However, the level of palmitic acid, stearate, and palmitoleate were not significantly altered in shFASN Raw264.7 (Fig5b). Thus, we hypothesized that if FASN is down-regulated, macrophages will urge to compensate the loss of free fatty acid within the cytoplasm. In order to do so, fatty acid scavenger receptor CD36 may increase in number to uptake external free fatty acid. Consistent with our hypothesis, the protein expression of CD36 was significantly increased in shFASN Raw264.7 compared to shControl (Fig5c,d and e). When FASN is transiently overexpressed, CD36 protein level decreases again (Fig5d and e), confirming that the amount alteration of CD36 is surely due to FASN.

This phenomenon was also observable *in vivo*. We extracted SVF from fat tissues of either normal diet- or high fat diet- mice and checked the mRNA and protein expression levels of CD36. The result demonstrates that CD36 mRNA (Fig5f) and protein (Fig5g) level is indeed increased in high fat diet group.

Induced inflammatory genes in shFASN is through scavenger receptor CD36

In order to confirm that the external fatty acid influx is due to increased CD36 surface expression, we performed click assay. Click assay utilizes a commercially available palmitic acid conjugated with azide which reacts with alkyne conjugated with fluorescent protein upon copper catalyst addition. After 6 hour pretreatment of palmitic acid, we are able to visualize exogenous palmitic acid up-taken by the cells. Figure 6a illustrate that while there is no difference in the total lipid amount between shControl and shFASN (BODIPY, green), exogenous palmitic acid (Click assay, red) has been increased in quantity in shFASN Raw264.7. Furthermore, shCD36 and shFASN double knock-down Raw264.7 cells reduced the uptake of exogenous palmitic acid (Fig6a), illustrating that the uptake of exogenous palmitic acid is indeed through CD36.

In order to verify if induced inflammatory gene activation in

shFASN Raw264.7 is through scavenger receptor CD36, CD36 inhibitor sulfo-N-succinimidyl oleate (SSO) was used to test mRNA fold induction of iNOS, IL-1β, TNFα and IL-6. Also, exogenous palmitic acid was treated in order to induce inflammation in shContol and shFASN Raw264.7 and the result clearly indicates that all four inflammatory markers respond well to it. Upon SSO treatment, mRNA levels of iNOS, IL-1β, TNFα and IL-6 dramatically decreased in a dose dependent manner, suggesting that FASN down-regulation up-regulates CD36, which ultimately results in facilitated exogenous fatty acid uptake and inflammatory gene expression (Fig6b).

Consistent with *in vitro* data, bone marrow macrophages from HFD mouse showed increased gene expression of iNOS and IL-1β. This result becomes more dramatic upon exogenous palmitic acid treatment. Furthermore, the increased inflammatory gene expression is inhibited via SSO treatment (Fig6c).

Therefore, we concluded that down regulation of FASN induces inflammatory state through upregulated CD36 allowing more exogenous free fatty acids uptake.

Figures

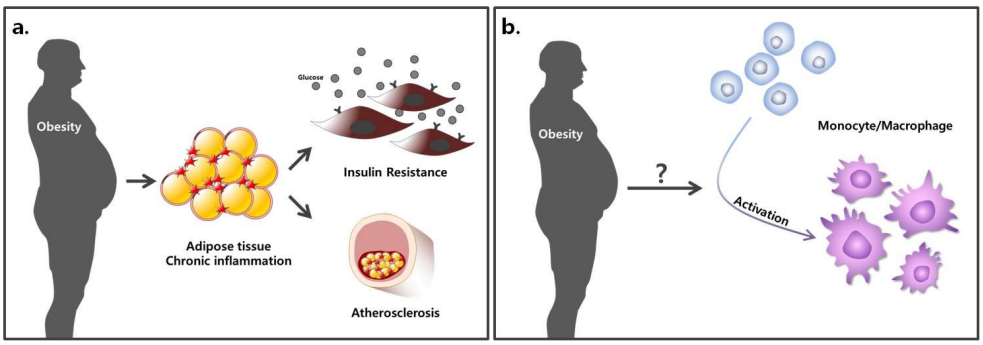

Figure 1. **Obesity is a chronic inflammatory condition**

A) Obesity induces chronic inflammatory state in adipose tissue, which is a key factor for the development of insulin resistance and atherosclerosis. B) the mechanism of monocyte/macrophage activation in metabolic disorders or obesity remains elusive.

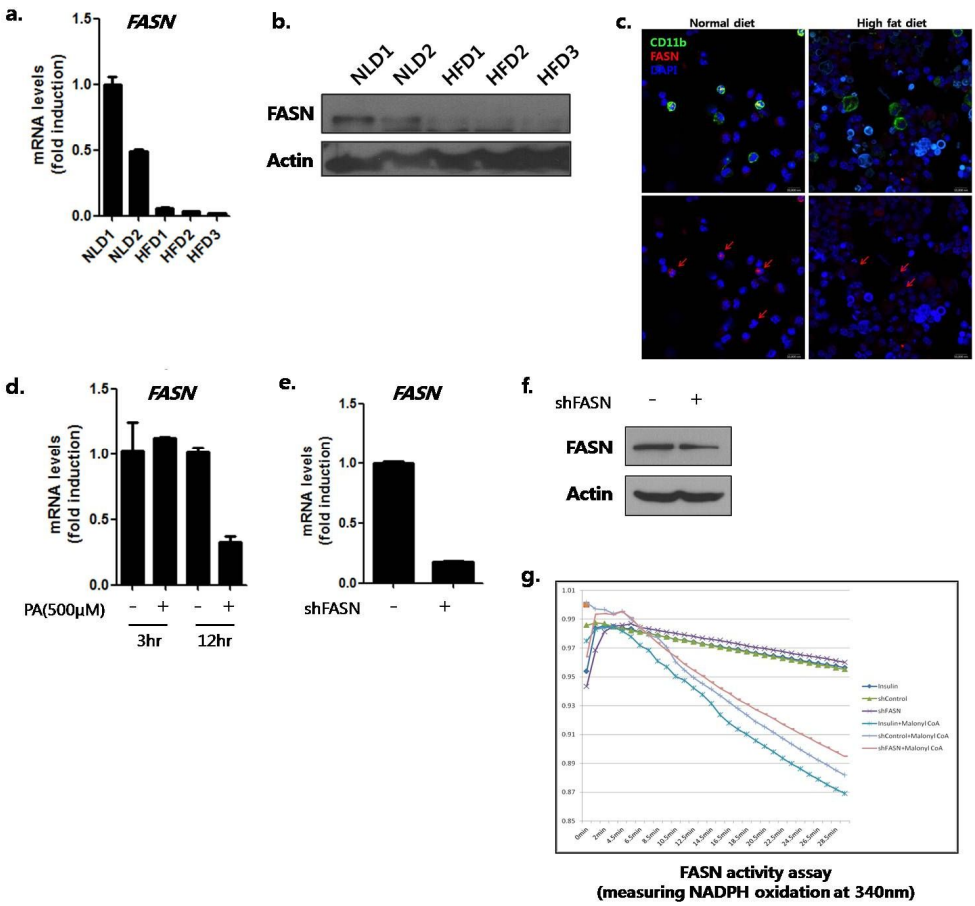

Figure 2. **High fat diet reduces both mRNA and protein level of fatty acid synthase (FASN) expression in stromal vascular fraction (SVF)**

a-b) The SVF harvest from adipose tissue of normal diet (NLD) or high fat diet (HFD) fed mice. The SVF was digested with TRIZOL reagent and extracted mRNA for qPCR (a) or lysed with RIPA buffer and extracted protein for western blot. NLD1-2, HFD1-3 is independent individual.

c) Immunofluorescence (IF) assay was conducted using macrophage

specific marker, CD11b, merged with FASN

d) We mimicked high fat diet environment *in vitro*. Palmitic acid 500uM was treated in Raw264.7 cells. The mRNA level of FASN was reduced upon pamitic acid treatment.

e-f) Prepare a FASN knockdown cell line using shFASN plasmid. Transfection of plasmid into Raw264.7 cell line, we select single-cell by GFP expression. mRNA (e) and protein level(f) of FASN.

g) FASN activity was measured by spectrophotometrically monitoring oxidation of NADPH at 340nm. NADPH oxidation is not altered in non-substrate group (insulin, shControl, shFASN). A group of Malonyl-CoA treated (insulin+Malonyl CoA, shControl+Malonyl-CoA, shFASN+Malonyl-CoA) showed that NADPH was consumed by FASN. The most active NADPH oxidation occurred in insulin+Malonyl-CoA-treated positive control group. shControl+Malonyl-coA group showed second most active NADPH oxidation while shFASN+Malonyl-CoA group showed least NADPH oxidation activity.

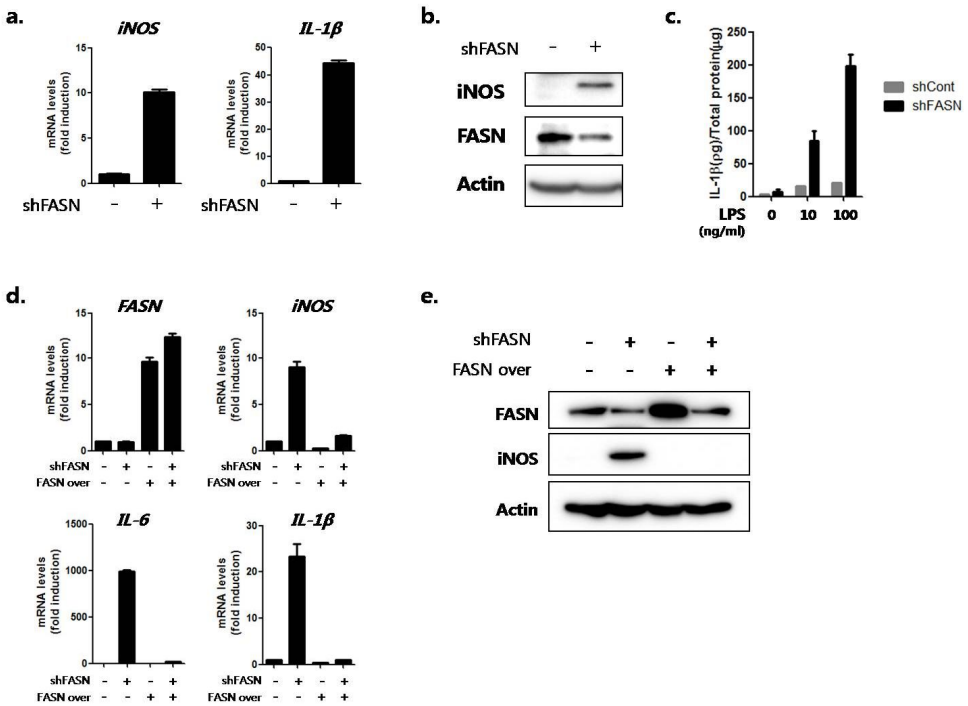

Figure 3. **Reduced level of fatty acid synthase induces transcription and translation of inflammatory genes**

a-b) The mRNA levels of inflammatory markers such as iNOS and IL-1β were increased in shFASN Raw264.7 compared to the control group (a). Also protein expression levels of iNOS is dramatically increased in shFASN in western blot analysis (b).

c) ELISA result demonstrate that IL-1β expression is dramatically increased in shFASN Raw264.7 after LPS stimulation. Cells was seeded (7x10^5cells/well in 6well plate) in complete medium. After overnight, medium was changed to serum-free and LPS treated 0,10,100ng/ml. After

48 hour, supernatant harvest and concentrated using centricon (millipore). d-e) . Quantitative RT-PCR and western blot results revealed that when FASN is recovered, iNOS, IL-6 and IL-1β mRNA expressions are reduced back to their normal state (Fig3d) as well as the protein expression of iNOS

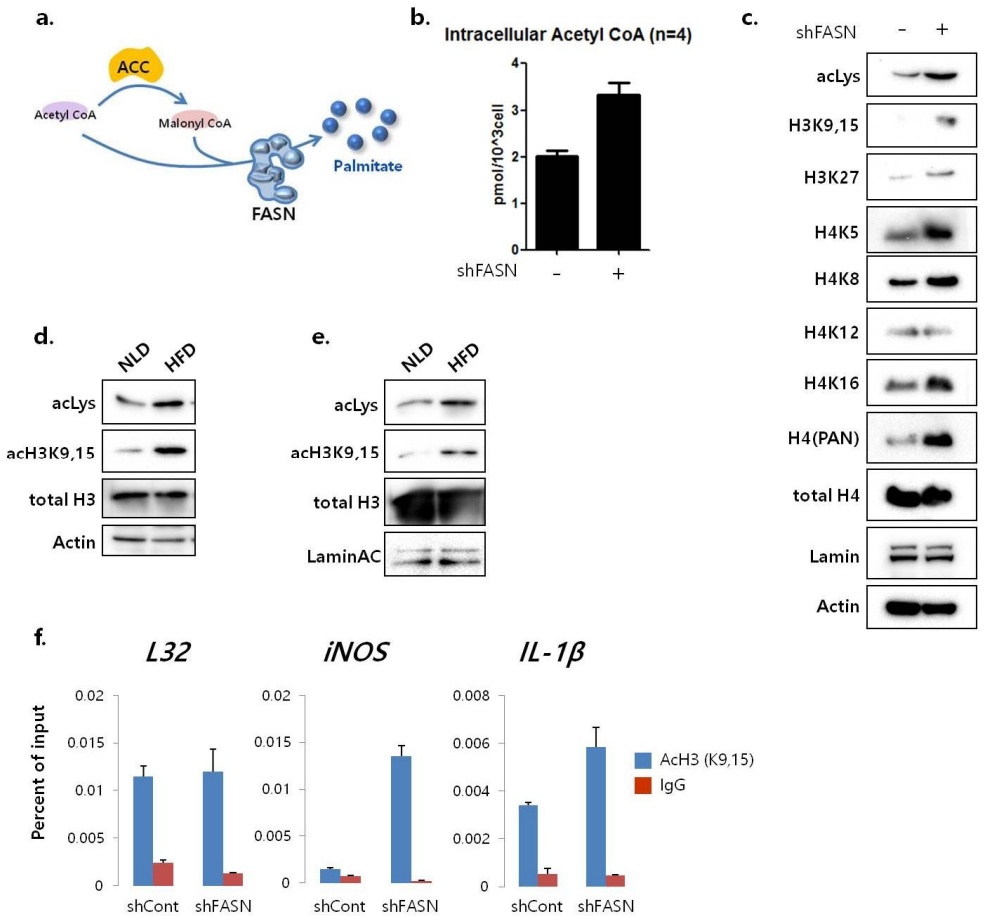

Figure4. **Knockdown of fatty acid synthase results in the accumulation of intracellular acetyl coenzyme A and increased histone acetylation**

a) Mechanism of fatty acid synthesis. Acetyl-CoA or malonyl-CoA is used to substrate of fatty acid synthesis.

b) When FASN is down regulated, the level of intracellular acetyl-CoA was accumulated. The digested cell lysate was measured using acetyl-CoA assay kit (PicoProbe™, ab87546) according to manufacturer's protocol.

c) Performed a nuclear fractionation for analysis histone aceylation level for western blot.

d-e) NLD vs HFD total lysate (d) nuclear fractionation (e)

f) Chromatin immunprecipitation analysis. These result showed that iNOS and IL-1β gene transcription levels were increased upon acetylation of H3 (K9, 15).

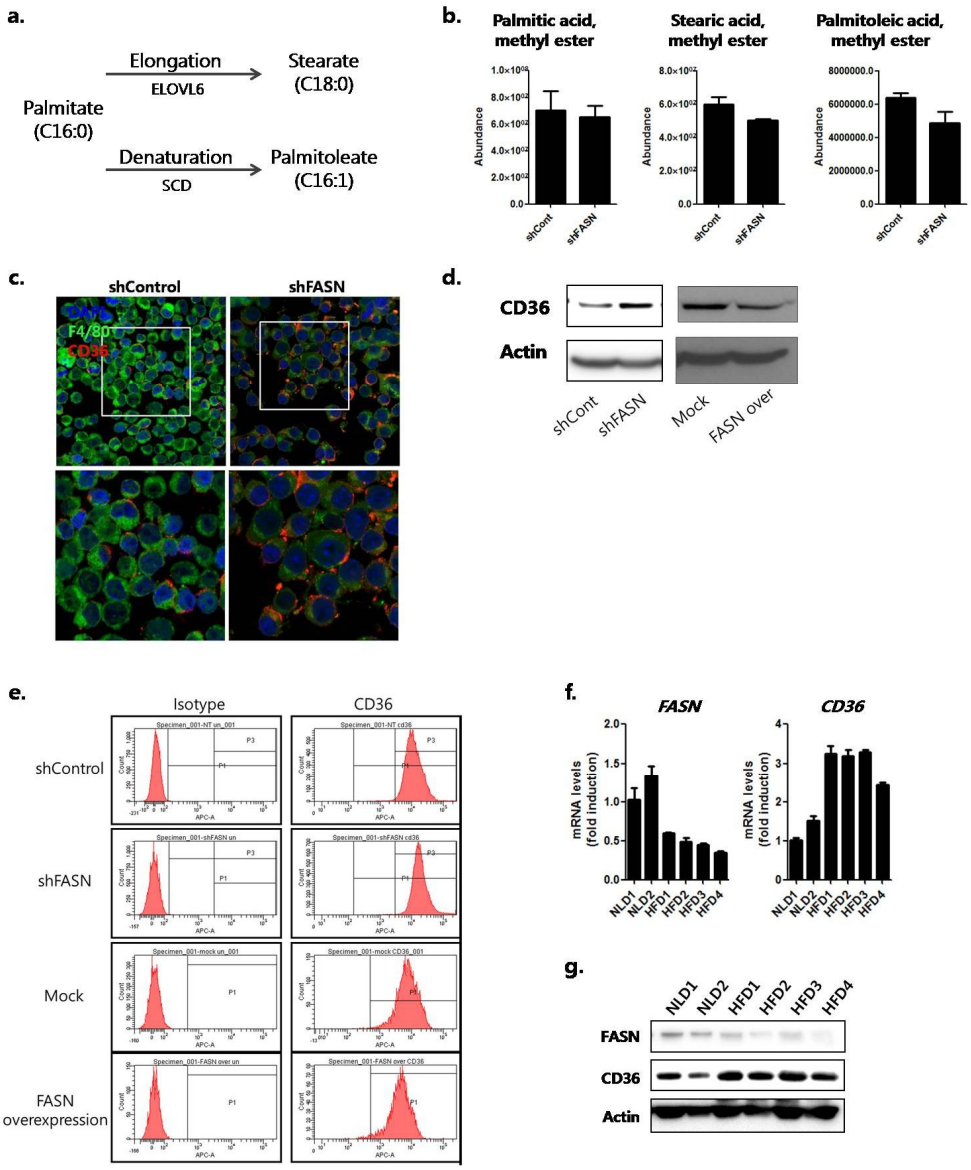

Figure 5. **Knockdown of fatty acid synthase increases the level of CD36 and induces exogenous fatty acid uptake**

a) Palmitic acid derivatives such as stearate or palmitoleate by Long chain fatty acid elongase 6 (ELOVL6) or Stearoyl-CoA desaturase (SCD).

b) The amount of palmitic acid, stearate, and palmitoleate were not significantly difference in shFASN Raw264.7

c-e) The protein expression of CD36 was significantly increased in shFASN Raw264.7 compared to shControl in immunofluorescence staining (c). In western blot (d) and FACS analysis (e), increased CD36 expression is reversed back to normal level in FASN rescue cell line.

f-g) SVF from fat tissues of either normal diet-or high fat diet- mice for confirm that the mRNA and protein expression levels of CD36. The result demonstrates that CD36 mRNA (f) and protein (g) level is indeed increased in high fat diet group.

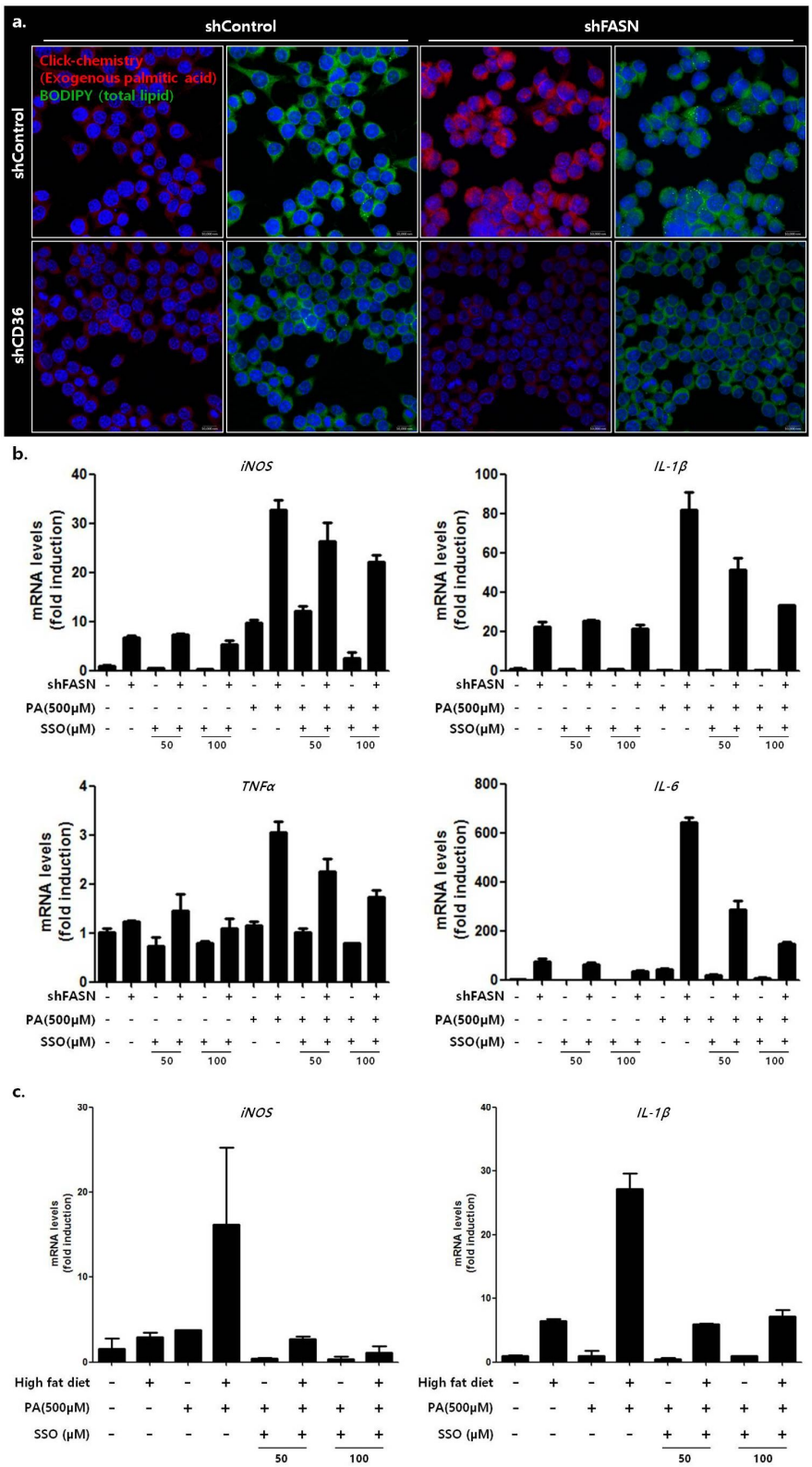
a.
shControl
shFASN
Click-chemistry
(Exogenous palmitic acid)
BODIPY (total lipid)
shControl
shCD36
b.
iNOS
IL-1β
TNFα
IL-6
mRNA levels
(fold induction)
shFASN
PA(500μM)
SSO(μM)
50
100
c.
iNOS
IL-1β
mRNA levels
(fold induction)
High fat diet
PA(500μM)
SSO (μM)
50
100

Figure 6. **Induced inflammatory genes in shFASN is through scavenger receptor CD36**

a) Exogenous fatty acid uptake through up-regulated CD36. Click assay perform a commercially available palmitic acid conjugated with azide (invitrogen, C10265) which reacts with alkyne conjugated with fluorescent protein (invitrogen, A10278). shFASN Raw264.7 promotes rigorous uptake of exogenous palmitic acid (red) and that uptake is suppressed by knocking down CD36 on top of shFASN Raw264.7.

b-c) Pamitic acid induce inflammatory gene expression through CD36. And SSO was used to inhibitor of CD36. Upon SSO treatment, mRNA levels of iNOS, IL-1β, IL-6, and TNFα dramatically decreased in a dose dependent manner, suggesting that those inflammatory gene upregulation is due to CD36 upregulation (b). Compared to normal diet mouse bone marrow macrophages, high fat diet mouse bone marrow macrophages showed decreased iNOS and IL-1β mRNA levels upon SSO treatment (c).

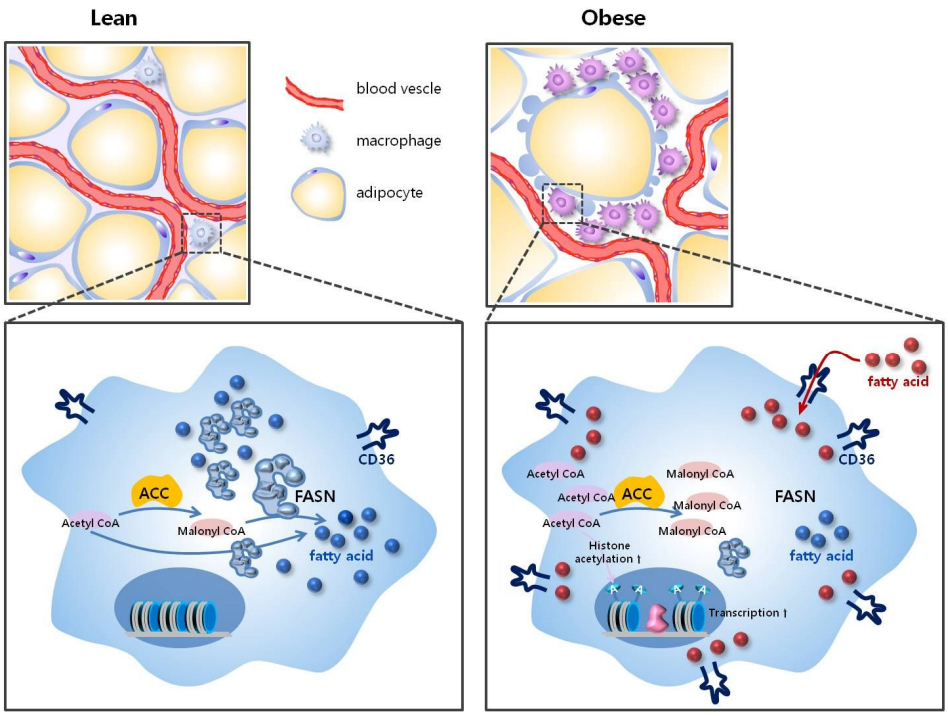

Figure 7. **Schematic figures of inflammatory gene expression in macrophage**

A schematic figures illustrating the event of inflammatory gene transcription regulated by FASN expression in lean or obese mouse macrophages.

Discussion

Various in vivo studies revealed that FASN is down-regulated in HFD mouse compared to NLD mouse. Our findings suggest that FASN is indeed down-regulated in obese model and that this event is tightly correlated with mediating inflammation. In mediating inflammation due to FASN down-regulation, we proposed two possible mechanisms: accumulation of acetyl-CoA provokes histone acetylation which ultimately turns on inflammatory gene expression such as iNOS and IL-1β, and compensatory mechanism in which CD36-mediated uptake of exogenous free fatty acid results in inflammatory signaling via yet unknown mechanism. Since acetyl-CoA is a major source of global histone acetylation (19), we were able to assume that histone acetylation would globally increase in amount. Also because histone modifications such as lysine acetylation and methylation, serine phosphorylation and arginine methylation play major regulatory roles in transcriptional position (20,21,22), we were able to hypothesize that histone acetylation event specifically on inflammatory genes could possibly mediate transcriptional activation of inflammatory genes. Since we found that histone3 (H3) lysine residue 9 and 15 (K9,15) is majorly acetylated in shFASN Raw264.7 compared to shControl cell line, we only performed ChIP assay based on H3 K9,15. Further ChIP experiments are needed using other acetylation sites.

However, it would be true that global acetylation may affect many other genes besides inflammatory genes; detailed mechanistic studies are needed to unveil how acetyl-CoA specifically regulates inflammatory gene promoters. Although we proved that inflammatory gene expression is surely altered by a global increase in histone acetylation, it would be true that it may affect many other genes besides inflammatory genes; detailed mechanistic studies are needed to unveil how acetyl-CoA specifically regulates inflammatory gene promoters. In addition, there are not much known regarding the amount of intracellular acetyl-CoA or the degree of histone acetylation occurs in obese condition; there are much more to explore finding the exact mechanism of which inflammation is mediated due to FASN down regulation.

Besides, the effect of exogenous palmitic acid on macrophage activation is still under careful examination. Although, it is not clear that exogenous palmitic acids do harm in general, we were only able to conclude that exogenous palmitic acid uptake result in increase of inflammatory gene expression in shFASN Raw264.7. In addition, the cause and effect relationship of CD36 and FASN level needs to be clarified.

This paper aims to unveil the possible mechanism by which FASN down regulation mediates inflammatory gene regulation. It is possible that two hypotheses are tightly correlated; it is also possible that increase in

global acetylation and increase in palmitic acid uptake are two independent events leading to inflammation. Although there are in large part under investigation, it is of note that obesity can induce inflammation through histone acetylation and/or exogenous palmitic acid uptake.

Reference

(1) Nature. 2006 Dec 14;444(7121):860-7. Inflammation and metabolic disorders. Hotamisligil GS.

(2) Nat Immunol. 2014 Sep;15(9):846-55. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, Beatty W, Love-Gregory L, Lam WY, O'Neill CM, Yan C, Du H, Abumrad NA, Urban JF Jr, Artyomov MN, Pearce EL, Pearce EJ.

(3) Front Endocrinol (Lausanne). 2013 Aug 8;4:93. Recent advances in obesity-induced inflammation and insulin resistance. Tateya S, Kim F, Tamori Y.

(4) Diabetes. 2011 Apr;60(4):1100-10. Hematopoietic cell-restricted deletion of CD36 reduces high-fat diet-induced macrophage infiltration and improves insulin signaling in adipose tissue. Nicholls HT, Kowalski G, Kennedy DJ, Risis S, Zaffino LA, Watson N, Kanellakis P, Watt MJ, Bobik A, Bonen A, Febbraio M, Lancaster GI, Febbraio MA.

(5) Science. 2010 Feb 5;327(5966):656-61. Development of monocytes, macrophages, and dendritic cells. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K.

(6) Nat Rev Immunol. 2013 Aug;13(8):607-14. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Gause

WC, Wynn TA, Allen JE.

(7) Nat Rev Cardiol. 2009 Jun;6(6):399-409. Obesity, inflammation, and atherosclerosis. Rocha VZ , Libby P.

(8) J Clin Invest. 2010 Mar;120(3):756-67. DGAT1-dependent triacylglycerol storage by macrophages protects mice from diet-induced insulin resistance and inflammation. Koliwad SK, Streeper RS, Monetti M, Cornelissen I, Chan L, Terayama K, Naylor S, Rao M, Hubbard B, Farese RV Jr.

(9) J Clin Invest. 2008 Jul;118(7):2640-50. Adipocyte/macrophage fatty acid-binding proteins contribute to metabolic deterioration through actions in both macrophages and adipocytes in mice. Furuhashi M, Fucho R, Görgün CZ, Tuncman G, Cao H, Hotamisligil GS.

(10) Nat Rev Immunol. 2013 Sep;13(9):621-34. Scavenger receptors in homeostasis and immunity. Canton J, Neculai D, Grinstein S.

(11) J Clin Invest. 2007 Jan;117(1):175-84. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. Lumeng CN, Bodzin JL, Saltiel AR.

(12) Nat Rev Immunol. 2003 Jan;3(1):23-35. Alternative activation of macrophages. Gordon S.

(13) Nat Rev Immunol. 2005 Dec;5(12):953-64. Monocyte and macrophage heterogeneity. Gordon S, Taylor PR.

(14) Trends Immunol. 2011 Jul;32(7):307-14. Defining macrophage phenotype and function in adipose tissue. Dalmas E, Clément K, Guerre-Millo M.

(15) PLoS One. 2009 Sep 3;4(9):e6884. Leptin contributes to the adaptive responses of mice to high-fat diet intake through suppressing the lipogenic pathway. Jiang L, Wang Q, Yu Y, Zhao F, Huang P, Zeng R, Qi RZ, Li W, Liu Y.

(16) Cell Rep. 2015 Mar 4. pii: S2211-1247(15)00170-9. M-CSF from Cancer Cells Induces Fatty Acid Synthase and PPARβ/δ Activation in Tumor Myeloid Cells, Leading to Tumor Progression. Park J, Lee SE, Hur J, Hong EB, Choi JI, Yang JM, Kim JY, Kim YC, Cho HJ, Peters JM, Ryoo SB, Kim YT, Kim HS.

(17) PLoS One. 2009 Sep 3;4(9):e6884. Leptin contributes to the adaptive responses of mice to high-fat diet intake through suppressing the lipogenic pathway. Jiang L, Wang Q, Yu Y, Zhao F, Huang P, Zeng R, Qi RZ, Li W, Liu Y.

(18) Physiol Rep. 2015 Sep;3(9). pii: e12534. Exercise training attenuates neutrophil infiltration and elastase expression in adipose tissue of high-fat-diet-induced obese mice. Kawanishi N, Niihara H, Mizokami T, Yada K, Suzuki K.

(19) Mol Cell. 2006 Jul 21;23(2):207-17. Nucleocytosolic acetyl-coenzyme

a synthetase is required for histone acetylation and global transcription. Takahashi H, McCaffery JM, Irizarry RA, Boeke JD.

(20) Nature. 2000 Jan 6;403(6765):41-5. The language of covalent histone modifications. Strahl BD, Allis CD.

(21) Curr Opin Genet Dev. 2002 Apr;12(2):142-8. Histone modifications in transcriptional regulation. Berger SL.

(22) Cell. 2002 Nov 1;111(3):285-91. Cellular memory and the histone code. Turner BM.

(23) J Clin Invest. 2003 Dec;112(12):1821-30. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H.

(24) J Nutr. 2005 Jan;135(1):33-41. Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. Kim S, Sohn I, Lee YS, Lee YS.

(25) J of Proteomics & Bioinformatics. February 13, 2012; High Fat Diet-Induced Changes in Hepatic Protein Abundance in Mice. Moulun Luo, April E. Mengos, Tianna M. Stubblefield and Lawrence J. Mandarino.