The protective effect of fat specific protein 27 on tumor necrosis factor-alpha induced lipolysis and insulin resistance in human adipocytes

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Thesis

THE PROTECTIVE EFFECT OF FAT SPECIFIC PROTEIN 27 ON TUMOR NECROSIS FACTOR-ALPHA INDUCED LIPOLYSIS AND INSULIN RESISTANCE IN HUMAN ADIPOCYTES

by

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B.S., Georgia Institute of Technology, 2013

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AMBER HYESUK LIM

ABSTRACT

Adipose tissue is a key regulator of energy metabolism and glucose homeostasis by promoting triglyceride storage and breakdown in various physiological states. Obesity, however, alters adipose tissue metabolism, inducing chronic inflammation, followed by excessive lipolysis. This results in higher systemic free fatty acid (FFA) levels, leading to desensitization of insulin signaling and ultimately to insulin resistance. Although the link between obesity and progression of insulin resistance and type 2 diabetes mellitus (T2DM) remains unclear, tumor necrosis factor-α (TNF-α) has been proposed to be a key player in promoting obesity-related development of T2DM through chronic inflammation of adipose tissue. TNF-α has direct and indirect mechanisms by which it elicits insulin resistance in adipocytes. TNF-α attenuates insulin signaling by directly inhibiting insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1). Indirectly, TNF-α activates signaling pathways to increase lipolysis and FFA release into circulation, leading to insulin resistance. Lipid droplet-associated fat specific protein 27 (FSP27) protects adipocytes from lipolysis by regulating the lipolytic capacity as well as transcription of adipose triglyceride lipase (ATGL). It has been observed that
TNF-α promotes lipolysis by reducing the expression of FSP27 in murine adipocytes. The effect of TNF-α on lipolysis human adipocytes has also been studied; yet its effect on promoting insulin resistance in human adipocytes still remains elusive. In the present study, we examined the effect of FSP27 on TNF-α induced lipolysis and insulin resistance in human adipocytes. TNF-α enhanced lipolysis in cultured human adipocytes. In addition, TNF-α reduced the expression of endogenous FSP7 and the phosphorylation of AKT, inhibiting the activation of insulin signaling pathway in cultured human adipocytes. FSP27 overexpression, however, attenuated TNF-α induced lipolysis and restored activation of insulin signaling through phosphorylation of AKT in cultured human adipocytes. Taken together, these data suggest that FSP27 has a protective effect against TNF-α induced lipolysis and insulin resistance through regulating lipolysis and insulin signaling in human adipocytes.
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>DMEM</td>
<td>Dulbecco’s modified Eagle’s medium</td>
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<td>FSP27</td>
<td>Fat specific protein 27</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
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<tr>
<td>HSL</td>
<td>Hormone sensitive lipase</td>
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<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>IKK-β</td>
<td>Inhibitor of κβ kinase-β</td>
</tr>
<tr>
<td>IBMX</td>
<td>3-isobutyl-1-methylxanthine</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
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<td>IR</td>
<td>Insulin resistance</td>
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<td>KRH</td>
<td>Krebs Ringer HEPES</td>
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<td>Lipoprotein lipase</td>
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<td>MCP-1</td>
<td>Macrophage chemoattractant protein-1</td>
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<tr>
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<td>Mitogen-activated protein kinase kinase kinase kinase-4</td>
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<td>Nuclear factor-κβ</td>
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<td>Perilipin</td>
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<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
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<tr>
<td>TG</td>
<td>Triglyceride</td>
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<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
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<td>T2DM</td>
<td>Type II diabetes mellitus</td>
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INTRODUCTION

**Obesity is a modern-day epidemic**

The prevalence of obesity is a globally recognized problem as a result of urbanization with more availability of food, surplus of energy intake, and sedentary lifestyle. In the United States, two-thirds of the population is obese, defined as having a BMI of greater than 30 kg/m², and with it comes pathological consequences comprised of various metabolic diseases, including cardiovascular disease, atherosclerosis, and in particular, type 2 diabetes mellitus (T2DM) [2]. Statistics reveal that T2DM is projected to double world-wide to 350 million cases by 2030. In the U.S., $132 billion yearly expenditure is devoted to prevention and treatment of T2DM [3, 4].

**Obesity, Insulin resistance, and T2DM**

Although the mechanism is not fully understood, obesity is associated with insulin resistance, defined as the body's inability to respond to insulin properly, which plays a major role in the onset of T2DM [5]. Obesity-related insulin resistance that leads to the development of T2DM exhibits two common features. First, development of T2DM starts with a precondition where insulin-responsive peripheral tissues, such as the liver, skeletal muscle, and adipose tissue, fail to respond to insulin [1]. Further progression of insulin resistance ultimately gives rise to T2DM when the function of pancreatic beta cells is impaired and they fail to secrete the necessary levels of insulin to maintain normal glucose levels [6-8].
Metabolic consequences of insulin resistance

Because insulin plays a critical role in energy and metabolic homeostasis, development of insulin resistance in major insulin-responsive tissues brings detrimental consequences and leads to fundamental defects in function [9, 10]. Insulin resistance leads to hyperglycemia most notably, which result from various causes such as failure of insulin to suppress hepatic glucose production and to promote glucose uptake and metabolism by peripheral tissues. Hyperglycemia may also arise from dysfunctional pancreatic beta cells and their failure to respond to excess plasma glucose and to secrete insulin effectively. With progression of insulin resistance, pancreatic beta cells produce excess insulin in order to maintain normal plasma glucose concentration. However, beta cells ultimately fail to maintain adequate levels of insulin secretion and thus plasma glucose level increases and T2DM develops [9, 10].

The development of insulin resistance is often attributed to the failure of insulin signaling because of excess free fatty acids (FFAs) in circulation as well. Increased plasma FFA impairs insulin signaling and promotes FFA oxidation in insulin-responsive tissues [11-13]. High plasma FFA provides substrate for hepatic TG synthesis (results in fatty liver), provides substrate for gluconeogenesis for hepatic glucose production, and leads to more VLDL secretion in the liver to reduce overall insulin sensitivity [14, 15]. Increased plasma FFA leads to the impairment of insulin signaling and decreased glucose uptake and glycogen synthesis, which all contribute to reduce insulin sensitivity in
the skeletal muscle [13]. High plasma FFA levels are toxic to pancreatic beta cells, accelerating dysfunction and apoptosis of the beta cells [16]. High plasma FFA levels affect adipose tissue by decreasing clearance of plasma TG rich lipoproteins by inhibiting LPL activity, reducing insulin sensitivity overall [15, 17].

Taken together, excess plasma FFA interferes with insulin signaling in major insulin-responsive tissues to contribute to the development of metabolic diseases which ultimately progresses into T2DM [18].

**Adipose tissue plays a critical role in energy metabolism**

Adipose tissue is a master regulator in maintaining whole-body glucose and energy homeostasis by regulating storage and breakdown of triglyceride [1]. Adipose tissue is also the gatekeeper for fatty acids in circulation where in postprandial state, secretion of insulin promotes glucose uptake, synthesis and accumulation of TG into lipid droplets in adipocytes while suppressing lipolysis and FFA release [1]. During fasting, adipocytes undergo lipolysis to break down TG stores to release FFA into circulation to serve as a major source of energy. On the other hand, adipocytes store excess nutrient into TG and then utilize TG stores to release FFA to the rest of the body in starvation to power glucose production to maintain whole-body glucose homeostasis [18].

**Obesity causes chronic inflammation in adipose tissue leading to insulin resistance**

In a normal, non-obese condition, adipose tissue regulates the release of a number of adipose tissue-derived factors, such as FFA, glycerol, hormones,
and adipokines in an optimal manner depending on physiological and metabolic needs [18]. But in obese conditions, FFA and other adipose tissue-associated factors are released uncontrollably into circulation [1]. In addition, nutrient excess in obesity leads to adipocyte enlargement, hypertrophy, and such progressive adipocyte enlargement leads to reduced blood supply to adipose tissue to result in hypoxia [19]. Hypoxic environment of adipose tissue elicits necrosis and release of macrophage chemoattractants, most notably MCP-1 [1]. Release of MCP-1 causes macrophage recruitment and infiltration into adipose tissue, causing local inflammation of the adipose tissue [20]. Such localized inflammation induces lipolysis, secreting more FFAs to propagate an overall systemic inflammation in a feed-forward process leading to a chronic inflammation (Figure 1d) [1, 20, 21]. MCP-1 also encourages the release of a number of pro-inflammatory cytokines, most notably tumor necrosis factor α (TNF-α), to elicit lipolysis and FFA release, contributing to the development of chronic inflammation (Figure 1) [1].
Figure 1: Obesity causes chronic inflammation in adipose tissue leading to a systemic insulin resistance [1]. (a) Small adipocytes store TG efficiently in lean state, which can be mobilized during starvation to peripheral tissues such as the skeletal muscle. (b) Small adipocytes become enlarged, hypertrophied, due to increased TG storage during excess caloric intake. (c) With further caloric intake, TG overloading continues; hypertrophy of adipocytes leads to hypoxia; macrophage chemoattractant secretion occurs, most notably MCP-1, recruiting additional macrophages to the site. (d) Macrophage recruitment and infiltration into adipose tissue results in more inflammation causing a pro-inflammatory state in obese, hypertrophied adipose tissue; infiltration of macrophages promotes the release of tumor necrosis factor-α (TNF-α), which results in a chronic inflammation and impaired adipocyte function; excessive lipolysis and ineffective TG storage disrupts insulin signaling in peripheral tissues, such as the skeletal muscle.

**TNF-α is a modulator of obesity-induced insulin resistance**

...
mechanisms affecting adipose tissue metabolism [18]. A plethora of studies examined the role of TNF-α in insulin signaling and insulin resistance. TNF-α has been proposed to be a major factor stimulating lipolysis and triggering FFA release from the adipose tissue [18]. TNF-α inhibits LPL activity in 3T3-L1 adipocytes to yield high plasma FFA and reduce insulin signaling [22, 23]. Long term exposure to TNF-α induces insulin resistance both in vivo and in vitro, whereas neutralization of TNF-α in Zucker rats increases insulin sensitivity [22-24]. Absence of TNF-α or TNF-α receptors in knock-out mice improves insulin sensitivity in obese rodents [25, 26]. However, neutralizing TNF-α in obese T2DM patients failed to restore insulin sensitivity [27]. Although supporting evidence suggests a potential role of TNF-α as a modulator of obesity-induced insulin resistance, TNF-α’s mechanism of action leading to pathogenesis of insulin resistance and T2DM in humans remains to be determined [18].

**TNF-α attenuates insulin signaling directly**

TNF-α attenuates insulin signaling through various proposed mechanisms, ultimately leading to insulin resistance. TNF-α has a direct effect on insulin signaling by inhibiting insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1), in part by induction of serine phosphorylation of IRS-1 through inhibitor of κβ kinase-β (IKK-β) [28]. Reduced insulin signaling over time impairs insulin signaling and leads to insulin resistance.
**TNF-α indirectly promotes insulin resistance by enhancing lipolysis**

TNF-α leads to insulin resistance indirectly by enhancing lipolysis through activation of numerous signaling pathways. First, TNF-α reduces the expression of various adipogenic genes at multiple levels, including the transcription, translation, and turnover of mRNA and protein (Figure 2). TNF-α activates nuclear factor-κB (NF-κB) to reduce the expression of peroxisome proliferator-activated receptor γ (PPARγ), the master regulator of adipogenesis required for maintenance of mature adipocyte function (Figure 2) [29-31]. TNF-α promotes PPARγ mRNA turnover and degradation through NF-κB activation and PPARγ protein degradation by activation of caspases (Figure 2) [1]. TNF-α upregulates mitogen-activated protein kinase kinase kinase kinase-4 (MAP4K4) to inhibit PPARγ translation (Figure 2). Taken together, TNF-α activates various players at multiple levels of expression to decrease PPARγ activity [1]. In addition, a previous study has also demonstrated that TNF-α induced NF-κβ activation inhibits expression of many adipocyte-associated genes responsible for uptake and storage of FFA in 3T3-L1 adipocytes [18]. Downregulation of PPARγ expression along with other adipocyte-associated genes impairs adipogenesis and alters adipose tissue function as TG storage, hydrolysis, and deposition in the lipid droplet, enhancing the release of plasma FFA in circulation that impairs insulin signaling and leads to insulin resistance.
Figure 2: TNF-α attenuates adipogenesis by downregulating PPARγ [1]. (1) Peroxisome proliferator-activated receptor-γ (PPARγ) expression is downregulated at the transcriptional level by TNF-α through NF-κB activation. (2) TNF-α enhances PPARγ mRNA turnover and degradation. (3) TNF-α upregulates mitogen-activated protein kinase kinase kinase kinase-4 (MAP4K4) to inhibit PPARγ translation. (4) TNF-α activates caspases to promote degradation of PPARγ protein.

Another mechanism by TNF-α leads to insulin resistance is through chronic stimulation of lipolysis [1]. TNF-α suppresses perilipin (PLIN1) function by enhancing cAMP levels (Figure 3). Lack of PLIN1 increases the association of hormone sensitive lipase (HSL) with TG in the lipid droplet to increase basal lipolytic rate and circulating FFA (Figure 3) [1].
FSP27 regulates fat metabolism by protecting the lipid droplet from lipolysis and restore insulin sensitivity

Synthesis, deposition, storage, and hydrolysis of adipocyte triglyceride require PPARγ-mediated regulation of adipocyte-regulatory proteins [31-33]. PPARγ regulates lipid droplet associated proteins like PLIN, S3-12, and myocardial lipid-droplet protein, which all serve important roles in association with the lipid droplets [34-36]. A novel lipid droplet associated protein, fat specific...
protein 27 (FSP27, also known as CIDEC), has been found to protect the deposition and storage of TG in lipid droplets in adipocytes [37]. Experimental evidence solidifies the role of FSP27 in regulating the formation and destruction of lipid droplets in mice and humans. Homozygous nonsense mutations in FSP27 are associated with lipodystrophy in white adipose tissue and depletion or mutation of FSP27 decreases lipid droplet size associated with hyperlipidemia in adipocytes [38, 39]. FSP27 protects adipocytes from lipolysis by regulating the lipolytic capacity as well as the transcription of ATGL [40].

FSP27 is co-localized with lipid droplets in adipocytes and is critical in enhancing lipid-droplet size in non-adipose cells as well [37, 41, 42]. In accordance with other lipid droplet associated proteins, FSP27 is under the influence of PPARγ, where its expression in mouse adipocytes increases 50-fold during adipogenesis [37].

FSP27 has been proposed to play a potential role in protection against insulin resistance and the development of T2DM. FSP27, along with other lipid droplet associated proteins, promotes formation and deposition of TG into lipid droplets and decreases the release of plasma FFA through inhibition of lipolysis [1, 37, 43]. Decrease in circulating FFA in plasma attenuates the deleterious effect of excess FFA to maintain a normal insulin signaling and sensitivity in insulin-responsive tissues.
FSP27 protects against TNF-a mediated lipolysis in murine adipocytes

Our lab has performed extensive research to confirm the regulatory role of FSP27 in lipid droplet morphology and adipocyte metabolism. We have shown that FSP27 is highly expressed in mouse adipocyte and is associated with lipid droplets [43]. Depletion of FSP27, mediated by short interfering RNA (siRNA), increases lipolysis of TG storage in adipocytes [44]. Overexpression of FSP27, on the other hand, enhanced TG deposition and accumulation in adipocytes [44]. To investigate the protective role of FSP27 in adipocyte metabolism during obesity-induced inflammation, our lab investigated the role of FSP27 in regulating TNF-α and catecholamine induced lipolysis in murine adipocytes. TNF-α treatment of mouse adipocytes increased lipolysis and depleted FSP27 levels and lipid droplet size. On the contrary, FSP27 overexpression protected against TNF-α induced lipolysis and reduction of lipid droplet in mouse adipocytes. Taken together, FSP27 serves an essential role in protecting lipid droplet as a key target of TNF-α induced lipolysis in mouse adipocytes. In human adipocytes, however, the role of TNF-α in lipolysis and insulin resistance remains elusive. The aim of the present study is to identify the potential role of FSP27 in regulating lipolysis and insulin resistance in human adipocytes. We hypothesize that FSP27 protects against TNF-α induced lipolysis and FFA-induced impaired insulin signaling to restore impaired insulin sensitivity and insulin resistance in human adipocytes.
METHODS

Stromal vascular cells (adipose stem cells) handling

Isolated stromal vascular cells (adipose stem cells) from human adipose tissue were received from the Boston Nutrition Obesity Research Center adipocyte core. The cells were subcultured up to five passages, grown to 70-80% confluency, and frozen down in 10% dimethyl sulfoxide supplemented growth medium upon receiving. Frozen cells (5000 cells/ml/well) were seeded in a 12-well plate in growth medium at 37°C composed of 13.5 g of alpha minimum essential medium power (Gibco), 10% fetal bovine serum (Gibco), 100 units/ml of penicillin and streptomycin (Gibco) and 25 mM sodium bicarbonate (Fisher Scientific) reconstituted in double distilled water (ddH2O) to 1 liter, pH 7.2-7.3.

Cell culture

Two days after the cells reached confluency, the medium was switched to adipogenic differentiation medium for induction [13.5 g of Dulbecco's Modified Eagle's medium (DMEM/F12) (Gibco) with 17.5 mM glucose, 100 units/ml of penicillin and streptomycin (Gibco) with 15 mM HEPES (Sigma), 25 mM sodium bicarbonate (Fischer Scientific), with 500 μM IBMX (Sigma), 100 nM insulin (Humulin), 100 nM Dexamethasone (Sigma), 1 μM Rosiglitazone (BioMol), 2 nM triiodothyronine (Sigma), 10 μg/mL transferrin (Sigma), 33 μM d-riboflavin (Sigma), and 17 μM pantothenic acid (Sigma) reconstituted in ddH2O to 1 liter, pH 7.4 ] [45]. D0 was indicated as the first day of the induction of differentiation. After induction in the differential media for 7 days, cells were kept in maintenance
medium (13.5 g of DMEM/F12 (Gibco), 25 mM sodium bicarbonate (Fischer Scientific), 100 units/ml of penicillin and streptomycin (Gibco), 33 μM d-biotin (Sigma), 17 μM pantothenic acid (Sigma) with 10 μM insulin and 10 nM dexamethasone in ddH2O to 1L in pH 7.4). Combination of insulin and dexamethasone in the medium has been shown to maintain adipocyte gene signatures [45]. Despite our labs incessant efforts to differentiate preadipocytes into mature adipocytes as much as possible, the degree of differentiation varied among the samples, with the majority of the cultures differentiated 40% to 60%.

**Generation of adenovirus and overexpression of FSP27 in adipocytes with viral infection**

The adenoviral constructs containing FSP27 with CFP and HA tags were generated as indicated by previous studies [40]. For infection of adipocytes, 75 μL of FSP27 adenovirus was added directly to 500 μL of the well containing maintenance media for 48 hours on day 9 of differentiation. After 3 hours of administration of the virus, fresh maintenance medium was added and cells were incubated at 37°C for the remaining 45 hours. All experiments were designed such that the cells were exposed to virus 48 hours prior to the treatment with TNF-α.

**Lipolysis**

Glycerol release served as an indicator of lipolysis in culture media during TNF-α treatments. The cells were incubated in maintenance medium with 10 ng/mL of TNF-α for 14 hours at 37°C. For the assessment of lipolysis, the
medium was changed from the maintenance medium to 500μL of Krebs- Ringer biocarbonate buffer [24.6 mM NaHCO₃, 1.11mM KH₂PO₄, 130 mM NaCl, 4.7 mM KCl, 1.24 mM MgSO₄, 3.3 mM CaCl₂, 4% w/v bovine serum albumin (BSA), 5 mM glucose in pH 7.4] still containing the indicated concentration of TNF-α 2 hours prior to harvesting the cells. The buffer was then collected and analyzed for glycerol release under basal conditions. 12.5 μL KRB or consecutive dilutions of glycerol standard solution were combined with 50 μL of free glycerol reagent (Sigma) and incubated at 37°C for 15 minutes. Spectrophotometer (Bio-Rad) was used to quantify the absorbance at the wavelength of 540 nm. Using the glycerol standards prepared, a standard curve was generated to extract values for concentrations of glycerol in each sample.

**Western Blotting**

After collecting KRB from cell culture, we washed the cells three times with ice-cold PBS. Then the cells were scraped off in 100 μL of cell lysis buffer with 5% sodium dodecyl sulfate, protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche). Cell lysates were sonicated to rupture the cell and centrifuged at 14,000 g for 10 min to obtain clear lysates. Clear layer with protein was transferred and 10 μg of protein was resolved in 12% bis-acrylamide gels (BioRad) and transferred to polyvinylidene difluoride membranes. After blocking, blots were probed for endogenous FSP27 and FSP27-CFP (different molecular weights), phospho-AKT, and loading controls (β actin and total AKT). Primary antibodies used were diluted in 2% BSA in 200 mM
Tris(hydroxymethyl)aminomethane, 1.5 M NaCl, and 1% Tween20 with pH 7.5. Antibodies used were mouse anti-phospho-AKT in 1:1000 dilution (Invitrogen), and mouse anti-β-actin in 1:3000 dilution (Invitrogen). Secondary antibodies were purchased from Santa Cruz Biotechnology, administered in 1:5000 dilution. Chemiluminescence images were developed using Pierce CL-Xposure Film (Thermo Scientific) and band intensities were quantified using Adobe Photoshop and ImageJ.

**Insulin Signaling**

After the overnight treatment with 10 ng/mL of TNF-α, media was collected and the cells were washed with phosphate buffered saline (PBS) three times. Then Krebs Ringer HEPES (KRH) buffer with glucose was added without cytokine as a control, 10 ng/mL TNF-α for serum starvation. After 2.5 hours at 37°C, the KRH was aspirated off and the cells were washed with PBS three times. After, KRH without glucose containing no cytokine as a control, TNF-α with or without insulin (10 nM) was added. Cells were incubated for 15 minutes at 37°C. After 15 minutes, cells were washed twice with ice-cold PBS and harvested for analyzing AKT phosphorylation as a measure for insulin signaling. Phosphorylated AKT was normalized to total AKT.

In order to evaluate the protective effect of FSP27 on TNF-α mediated insulin resistance, the appropriate wells were treated with FSP27-CFP adenovirus on day 9 of differentiation. Cells were assessed for CFP expression, 48 hours post infection. At 48 hours, differentiated adipocytes were incubated
with maintenance media as a control, 10 ng/mL for 16 hours at 37°C, and AKT phosphorylation was measured as described above.

**Statistics**

Results are expressed as means +/- SE, and the significance of the treatments was assessed using paired t-test (P<0.05)
RESULTS

**TNF-α increases lipolysis in differentiated human adipocytes**

TNF-α is an inflammatory cytokine that stimulates lipolysis in adipocytes [18]. To explore the impact of TNF-α on the rate of lipolysis on fully differentiated human adipocytes in culture, we first determined the optimal rate of TNF-α stimulated lipolysis by treating varying concentrations of TNF-α following different time periods and measured the rates of glycerol release. Based on the experimental designs from our lab's previous studies, TNF-α was added to human adipocytes in increasing dosages of 10, 20, and 50 ng/mL over 4, 8, and 16 hours [44]. 10 ng/mL over 16 hours was the smallest concentration and shortest duration of TNF-α treatment that led to the strongest effect on lipolysis in human adipocytes (Figure 4). Although the effects of both 20 and 50 ng/mL of TNF-α treatments also induced lipolysis, the lower levels of glycerol release compared with 10 ng/mL TNF-α may be due to a toxic effect on human adipocytes (Figure 4). These data are consistent with results from other studies,
confirming that TNF-α treatment increases lipolysis in differentiated human adipocytes [44, 46].

![Dose Dependent Time Course](image)

**Figure 4:** TNF-α time course and dose dependent experiment reveals that 10ng/mL over 16 hours gives the greatest increase in lipolysis rate in human adipocytes.

**TNF-α decreases FSP27 levels in differentiated human adipocytes**

Previous studies from our lab showed that TNF-α stimulates lipolysis and downregulates FSP27 levels and depletion of FSP27 leads to a greater rate of lipolysis in murine adipocytes [44]. To explore the impact of TNF-α on the expression of endogenous FSP27 in adipocytes, we determined the protein expression levels of FSP27 after 16 hour treatment with 10 ng/mL TNF-α on differentiated human adipocytes in culture. TNF-α treatment decreased the
expression of endogenous FSP27 protein levels compared to the control by more than 50% (Figure 5a, 5c). Overexpression of FSP27 rescued endogenous FSP27 protein levels from TNF-α induced repression, but only partially to 50% of that of the control level (Figure 5a, 5c). β-actin served as a loading control, where equal loading was evident as expected (Figure 5b). These data confirm that TNF-α decreases the expression of endogenous FSP27 and overexpression of FSP27 is able to restore TNF-α induced repression of endogenous FSP27 to a certain degree.

Figure 5: TNF-α decreases endogenous FSP27 protein expression when overexpression of FSP27 attenuates the inhibitory effect of TNF-α on endogenous FSP27 protein expression. (a) TNF-α administration decreases endogenous FSP27 protein expression; overexpression of FSP27 in TNF-α treatment increases endogenous FSP27 protein expression compared to that of
TNF-α alone, but is not effective in rescuing the endogenous FSP27 expression completely to that of the control. (b) β-actin expression levels are equal in all of the wells which serves as the loading control. (c) The intensities of the bands were collected and normalized; experiment done once in triplicate.

**Expression of adenoviral FSP27 protects against TNF-α mediated lipolysis**

A previous study by our lab confirmed the protective effect of FSP27 against TNF-α modulated lipolysis in murine adipocytes [44]. In order to test whether FSP27 regulates TNF-α induced lipolysis in differentiated human adipocytes, we first maintained the level of FSP27 in the presence of TNF-α by generating CFP-tagged FSP27 construct into an adenovirus expression system in differentiated human adipocytes in culture (Figure 6a) [40]. The rate of lipolysis increased to 20% following the treatment with TNF-α overnight (Figure 6b, 6c). Overexpression of FSP27-CFP reduced the rate of lipolysis by 70% compared to the basal level (Figure 6b, 6c). Overexpression of FSP27-CFP followed by TNF-α treatment overnight reduced the rate of lipolysis to slightly lower than the control level by 10% (Figure 6b, 6c). Overexpression of FSP27 not only reduced the rate of basal lipolysis, but also was able to reduce TNF-α induced lipolysis to the basal level (Figure 6b, 6c).
(a) FSP27-CFP overexpression

(b) FSP27 protects against TNF-α induced lipolysis (fold change)

![Image of cell cultures with green fluorescence]
Figure 6: Overexpression of FSP27 protects against TNF-α induced lipolysis in human adipocytes. (a) Fluorescence of overexpression of FSP27-CFP in human adipocytes. (b)-(c): Treatment of TNF-α alone increases the rate of lipolysis; Overexpression of FSP27 reduced the basal lipolysis rate; overexpression of FSP27 attenuates TNF-α induced lipolysis to that of the control or lower; experiment done in triplicate; *Statistically significant compared to control p<0.05; **statistically significant compared to each other p<0.05.

TNF-α inhibits insulin signaling by interfering with activation of AKT in human adipocytes

TNF-α inhibits insulin signaling through various pathways. Studies have confirmed its role in rodent models where insulin signaling is reduced with TNF-α treatment in adipocytes [22, 24, 25, 28, 47, 48]. To test whether TNF-α inhibits insulin signaling in human adipocytes, we maintained the level of FSP27 in the presence of TNF-α with FSP27-CFP overexpression in differentiated human adipocytes in culture. We treated the cells with 10 ng/mL of TNF-α overnight,
then starved the cells, and subsequently stimulated the cells with glucose and 10 nM insulin. TNF-α treatment decreased insulin stimulated AKT phosphorylation compared to that of the insulin stimulated treatment down to about 75% (Figure 7a, 7c). Although the loading control shows TNF-α treated proteins are loaded more than others, there is still a remarkable reduction in phosphorylated AKT levels when normalized (Figure 7b, 7c). Taken together, these data suggest that TNF-α reduces phosphorylation of AKT and thus inhibits its activation in insulin signaling pathway in human adipocytes.

**Expression of adenoviral FSP27 protects against TNF-α induced insulin resistance by activating AKT in human adipocytes**

Given our lab’s finding that FSP27 protects against TNF-α induced lipolysis in murine adipocytes and FFA induced impairment of insulin signaling in human adipocytes, we examined whether FSP27 expression attenuates TNF-α induced impairment of insulin signaling in human adipocytes [40, 44]. Following the expression of FSP27-CFP, we treated the cells with 10 ng/mL TNF-α overnight. Then we starved and subsequently stimulated the cells with glucose and 10 nM insulin. FSP27-CFP overexpression partially rescued phosphorylation of AKT from inhibitory effect of TNF-α (Figure 7a, 7c). FSP27 overexpression with TNF-α treatment appeared to activate AKT more than the insulin-stimulated control (Figure 7a). This may be attributed to the unequal loading of the protein, but the effect of FSP27 in attenuating TNF-α induced inhibition of AKT phosphorylation and restoring AKT activation is clearly demonstrated even after
normalization with total AKT (Figure 7c). FSP27 overexpression restored AKT phosphorylation to more than 60% of insulin stimulated levels of AKT phosphorylation (Figure 7c). In conclusion, these data suggest that FSP27 protects against TNF-α induced inhibition of insulin signaling in human adipocytes. More studies are needed to confirm the protective effect of FSP27 against TNF-α induced insulin resistance.
Figure 7: TNF-α decreases phosphorylation of AKT but overexpression of FSP27 attenuates the inhibitory effect of TNF-α on AKT phosphorylation and rescues AKT activation. (a) TNF-α administration decreases p-AKT protein levels; overexpression of FSP27 in TNF-α treatment rescues the inhibitory effect of TNF-α and restores the AKT activation to more than the insulin-stimulated control level. (b) Total AKT expression levels are unequal in the controls and the treatments but shows protective effect of FSP27 on rescuing the phosphorylation of AKT in presence of TNF-α. (c) The intensities of the bands were collected and normalized; experiment done once in triplicate.
DISCUSSION

In obesity, adipocytes become hypertrophied from nutrient excess and experience hypoxic environment from increased cell size [1]. Hypertrophy and hypoxia leads to local inflammation in the adipose tissue to stimulate release of pro-inflammatory cytokines, such as TNF-α, to further induce chronic inflammation and enhanced lipolysis in adipocytes [20]. Although the mechanism remains unclear, TNF-α has been proposed to be the major player linking obesity to insulin resistance and T2DM through inhibition of insulin signaling and reduction of insulin sensitivity [18]. TNF-α most commonly acts through indirect mechanisms by activating various signaling pathways to enhance lipolysis in adipocytes [18]. Both Zhang et al. and Lee and Fried propose a mechanism in which TNF-α promotes insulin resistance by stimulating lipolysis through activation of PKA signaling pathway in human adipocytes [5, 49]. Other studies also propose a possible mechanism through which TNF-α reduces overall insulin sensitivity by stimulating lipolysis by downregulating PLIN expression at the mRNA and protein levels in 3T3-L1 adipocytes through PKA signaling pathway [50]. Thus, in the present study, we examined whether the regulation and protection of lipolysis could restore insulin signaling and sensitivity from TNF-α induced lipolysis and insulin resistance in human adipocytes. We utilized FSP27 to control lipolysis considering its role in protecting the lipid droplet from lipolysis in mice and human adipocytes in addition to its role in regulating ATGL’s lipolytic capacity as well as its transcription [40]. Also, FSP27 has been shown to interact
directly with other lipid droplet-associated proteins and to regulate their mRNA levels to encourage TG synthesis and deposition and attenuate lipolysis [40].

In agreement with Lee and Fried, our data confirm that TNF-α stimulates lipolysis in differentiated human adipocytes [5]. Moreover, the present study showed that TNF-α causes a dramatic depletion of endogenous FSP27 in cultured human adipocytes. We also demonstrated that restoration of FSP27 protein levels by adenovirus-mediated overexpression attenuates TNF-α induced lipolysis. These results suggest that depletion of FSP27 is a possible mechanism acting in parallel with other mechanisms by which TNF-α triggers lipolysis in human adipocytes. Downregulation of transcription of FSP27 by TNF-α could also be attributed to the decrease in C/EBPα levels and decrease in PPARγ protein levels which all take part in regulating FSP27 activity and producing FSP27 proteins in adipocytes [29, 31, 42, 51]. Ranjit et al. has shown that TNF-α treatment downregulates FSP27 at a transcriptional level in 3T3-L1 adipocytes, but further studies are encouraged to examine the effect of TNF-α at translational level to see how TNF-α affect the protein itself in both animal and human adipocytes to enhance lipolysis [44].

We confirmed that FSP27 serves as a modulator to control lipolysis in TNF-α induced lipolysis in cultured human adipocytes. FSP27 regulation of lipolysis is not confined to actions of TNF-α specifically, but rather extends to a variety of proinflammatory cytokines such as with IL-1β and IL-6 to inhibit their
actions from triggering lipolysis in human adipocytes by reducing FSP27 protein levels (unpublished).

Prolonged exposure to high FFA from enhanced lipolysis may desensitize insulin signaling in adipocytes and develop into insulin resistance. To define the role of FSP27 in insulin signaling in human adipocytes, we showed that inhibition of insulin-stimulated AKT phosphorylation by TNF-α was countered by FSP27 overexpression in cultured human adipocytes. TNF-α treatments reduced the insulin-stimulated AKT activation and insulin signaling. However, overexpression of FSP27 restored inhibition of insulin-stimulated AKT activation by TNF-α in human adipocytes [40]. FSP27 overexpression protected against FFA-induced insulin resistance in human adipocytes. These results suggest that FSP27 may protect from FFA-induced insulin resistance through suppression of lipolysis in human adipocytes.

Although we only examined the indirect effect of TNF-α in promoting insulin resistance through mechanism enhancing lipolysis, TNF-α has been proposed to act directly to block insulin signaling through inhibition of tyrosine phosphorylation of the IRS-1 partly by induction of serine phosphorylation of IRS-1 by the actions of IKK-β [28, 52]. FSP27 may interact directly with insulin signaling pathways to inhibit the actions of TNF-α. Further studies need to be performed to find additional roles of FSP27 in protecting against TNF-α induced insulin resistance and development of T2DM.
Optimal fat storage in adipocytes is essential to maintain a healthy metabolic phenotype and whole body glucose homeostasis [1, 53-56]. In obese individuals, where production of proinflammatory cytokines, such as TNF-α, are at maxima, increased lipolysis in adipocytes produce uncontrolled, excessive FFA release into circulation. Prolonged exposure to high FFA in circulation may desensitize insulin signaling and develop into insulin resistance and T2DM. The present study suggests that FSP27 may protect from TNF-α induced insulin resistance through suppression of lipolysis in human adipocytes. Potential role of FSP27 in regulating TG accumulation and reducing lipolysis in human adipocytes opens up new windows for therapeutic remedies to combat obesity-related insulin resistance and development of T2DM.
# LIST OF JOURNAL ABBREVIATIONS

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<tr>
<th>Journal Abbreviation</th>
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<tr>
<td>Am J Med</td>
<td>The American Journal of Medicine</td>
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<td>Am J Physiol Endocrinol Metab</td>
<td>American Journal of Physiology Endocrinology and Metabolism</td>
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Proc Natl Acad Sci U S A  Proceedings of the National Academy of Sciences of the United States of America
REFERENCES


VITA

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Year of Birth
1990

Graduate Education
Institution: Boston University
Date of Attendance: January 2014- Current
Expected Date of Graduation: May 2015
Degree/School: Candidate for Masters of Science, Nutrition and Metabolism

Undergraduate Education
Institution: Georgia Institute of Technology
Date of Attendance: August 2008- May 2013
Degree/School: Bachelors of Science, Biology

Health Care Volunteer or Volunteer Experience 1
Dates: October 2011- October 2012, Fall 2013
Site: Vistacare Hospice at Emory Hospital Midtown (Now: Gentiva Hospice)
Supervisor: Barbara Kruger, the Volunteer Coordinator (at the time of volunteering)
Stacey Coachman, the Volunteer Coordinator (present)
Duties and describe the Experience: worked as an In-Patient Unit Volunteer; visited patients and their families to socialize, converse, and to comfort them; holding hands, reading books aloud, and praying for the patients were the main duties for patients who were unconscious.

Health Care Volunteer or Volunteer Experience 2

Dates: February 2012 - April 2013

Site: New Hope Medical Group
Supervisor: Dr. Young W. Jun
Name: Dr. Levitt
Duties and describe the Experience: worked as the doctor’s translator for under-represented Korean group for proper gynecologic care, but also worked as a helper to medical assistant and physician’s assistant.

Health Care Volunteer or Volunteer Experience 3

Dates: 2011-2012

Site: Park Renewal Day, Hands-on-Atlanta soap recycling day
Supervisor: N/A
Name: N/A
Duties and Describe the Experience: volunteered at city reforming events, helping with shaping local parks by removing invasive, introduced species of plants, and by shaving used-soap for recycling and sending them to countries in need.

Health Care Volunteer or Volunteer Experience 4
Dates: September 2010- March 2011
Site: Grady Memorial Hospital Emergency Triage
Supervisor: Brooke Eldridge
Duties and describe the Experience: worked as a volunteer at the Emergency Triage, caring for patients, following up with patients’ needs, and assisting patients in stretchers to appropriate areas for examination.

Health Care Practitioner Job Shadowing 1
Dates: April 2\textsuperscript{nd}, 2014
Site: Boston Medical
Supervisor: N/A
Name: Dr. Donald Hess
Title: Bariatric Surgeon
Duties and Describe Experience: shadowed Dr. Hess to observe laparoscopic, bariatric surgeries on obese patients.

Health Care Practitioner Job Shadowing 2
Dates: October 16\textsuperscript{th} 2013- December 2014
Site: Sandy Springs Internal Medicine
Supervisor: N/A
Name: Dr. Kelly J. Ahn
Title: Internal Medicine Physician
Duties and describe the Experience: shadowed Dr. Ahn and observed numerous cases an internalist encounters in his clinical practice; experienced first-hand the
assumed duties of a doctor, as well as building patient-doctor relationships; provided the exposure to clinical setting with a business point of view of a clinical practice.

**Health Care Practitioner Job Shadowing 3**

Dates: February 2012- June 2013

Site: New Hope Medical Group

Supervisor: Dr. Young W. Jun

Name: Dr. Levitt

Title: OB GYN

Duties and describe the Experience: shadowed Dr. Levitt under Dr. Jun’s supervision and observed various situations and complications that an OB GYN faces in a clinical setting; observed various surgical operations, including labor, C-section, mammoplasty, and various cases of hysterectomy and robotics surgery.

**Health Care Practitioner Job Shadowing 4**

Dates: October 1st – November 19th, 2011

Site: Vistacare Hospice at Emory University Hospital and Vistacare Hospice at Emory Midtown Hospital (Now: Gentiva Hospice)

Supervisor: N/A

Name: Dr. Asid Syed

Title: Internalist
Duties and describe the Experience: shadowed Dr. Syed who worked in palliative care department; experienced basic patient-doctor interactions to watching over any pain or uneasiness felt by patients.

Health Care Practitioner Job Shadowing 5

Dates: August 5th- 27th, 2011

Site: New Hope Medical Group

Supervisor: Dr. Young W. Jun

Name: Dr. Gladstone

Title: OBGYN

Duties and describe the Experience: shadowed Dr. Gladstone under Dr. Jun’s supervision to experience general patient-doctor interactions to light to grave surgeries.

Employment or Work Experience

Employer: Roma Academy of Music and Arts

Job Title: Head tutor

Employment Type: Part Time

Paid Position: Yes

Dates: 09/01/2007 - 08/01/2008

Address: 3256 Buford Highway Duluth, GA 30096

Phone: (770) 623-5955

Type of Work or Activity: tutored K12 students with school-related academics and taught mathematics at the institution's summer camp.
Research Experience 1

Dates: January 2014- May 2014

Site: Boston University School of Medicine

Supervisor: Susan K. Fried, Ph.D.

Name: Susan K. Fried, Ph.D., Mi-Jeong. Lee, Ph.D.

Title: Professor

Duties and describe the Experience: worked as a graduate student under Dr. Fried and Dr. Lee’s supervision, studying the effect of different hormones on the rate of lipolysis in adipocytes of obese individuals, depot specifically.

Research Experience 2

Dates: November 2011- August 2012, August 2013- April 2013

Site: Georgia Institute of Technology School of Environmental Engineering

Supervisor: Jim Spain, Ph.D.

Name: Jim Spain, Ph.D.

Title: Professor

Duties and describe the Experience: volunteered at the lab as a research undergraduate student under post doctorate’s and under Dr. Spain’s supervision, studying novel microorganisms’ utility of naturally occurring nitroaromatic compounds as carbon and nitrogen source.

Awards/Extracurricular/ Leadership Activities

1. Host of Take-a –Doctor-out-for-Dinner in Korean Pre-health Society

2. Vice President (‘11-‘12) and an Active Member of Korean Pre-health Society
3. Georgia Tech Community Emergency Response Team (CERT) graduate
4. Treasurer of Korean Undergraduate Student Association (KUSA)
5. Women in Engineering Mentor-Mentee program former member
6. Bankcard Services Scholarship Award ('08-'09)