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Growth hormone controls lipolysis by regulation of FSP27 expression

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Abstract

Growth hormone (GH) has long been known to stimulate lipolysis and insulin resistance; however, the molecular mechanisms underlying these effects are unknown. In the present study, we demonstrate that GH acutely induces lipolysis in cultured adipocytes. This effect is secondary to the reduced expression of a negative regulator of lipolysis, fat-specific protein 27 (FSP27; aka Cidec) at both the mRNA and protein levels. These effects are mimicked *in vivo* as transgenic overexpression of GH leads to a reduction of FSP27 expression. Mechanistically, we show GH modulation of FSP27 expression is mediated through activation of both MEK/ERK- and STAT5-dependent intracellular signaling. These two molecular pathways interact to differentially manipulate peroxisome proliferator-activated receptor gamma activity (PPAR γ) on the FSP27 promoter. Furthermore, overexpression of FSP27 is sufficient to fully suppress GH-induced lipolysis and insulin resistance in cultured adipocytes. Taken together, these data decipher a molecular mechanism by which GH acutely regulates lipolysis and insulin resistance in adipocytes.

Key Words

- ▶ PPAR-gamma
- ▶ metabolism
- ▶ acromegaly
- ▶ adipose tissue
- ▶ obesity
- ▶ diabetes

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Introduction

Although growth hormone (GH) has been primarily studied for its effects on linear growth, pronounced stimulation of lipolysis was among the first metabolic effects reported in human subjects following the introduction of pituitary-derived human GH (Raben & Hollenberg 1959). Studies in mice have indicated that ablation of the GH receptor or Jak2 in adipose tissue

reduces GH-induced lipolysis (List *et al.* 2013, Nordstrom *et al.* 2013, Shi *et al.* 2014). In addition, pharmacological blockade of the hormone-sensitive lipase (HSL) abrogates the lipolytic effects of GH in human subjects (Nielsen *et al.* 2001). However, the direct molecular mechanisms by which GH induces lipolysis have not been fully elucidated.

The importance of deciphering this mechanism is clear, as several lines of clinical evidence demonstrate that GH regulates insulin sensitivity in humans as a direct result of its lipolytic action. In both healthy volunteers and patients with GH deficiency, the insulin resistance caused by acute GH treatment is reversed by a pharmacological blockade of lipolysis (Segerlantz *et al.* 2003, Moller *et al.* 2009, Salgin *et al.* 2009, Cornford *et al.* 2012). Additionally, the 'dawn phenomenon', which describes an early morning increase in insulin resistance in diabetic patients, has been directly attributed to the diurnal peak of GH levels (Schmidt *et al.* 1981, Bolli & Gerich 1984, Monnier *et al.* 2012, 2013, Bouchonville *et al.* 2014). Strikingly, the dawn phenomenon can be almost entirely corrected by reducing GH levels (Campbell *et al.* 1985, Davidson *et al.* 1988) or by blocking the lipolytic action of GH (Salgin *et al.* 2009). Taken together, these studies demonstrate that GH-mediated lipolysis is a critical regulator of insulin sensitivity in both healthy and diabetic patients.

Lipolysis in adipose tissue requires the sequential activation of several enzymes including the rate-limiting enzyme Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (Zimmermann *et al.* 2004). The cell death-inducing DNA fragmentation factor alpha-like effector (CIDE) family proteins associate with lipid droplets and regulate fatty acid homeostasis in adipocytes (Puri *et al.* 2008a,b). CIDEC, also known as FSP27, regulates lipid droplet dynamics and lipolysis in adipocytes through suppression of the catalytic capacity as well as transcription of ATGL (Grahn *et al.* 2014, Singh *et al.* 2014). Consistent with these studies, mutation of FSP27 in humans leads to increased lipolysis (Rubio-Cabezas *et al.* 2009). In addition, adipose-specific disruption of FSP27 causes insulin resistance in high-fat-fed mice (Tanaka *et al.* 2015).

In the present study, we demonstrate that GH-induced lipolysis is associated with an acute reduction in FSP27 mRNA and protein expression. Mechanistically, we show GH-induced reduction of FSP27 is mediated through GH activation of both MEK/ERK- and STAT5-dependent signaling, which coordinately regulate peroxisome proliferator-activated receptor gamma (PPAR γ) transcriptional activity. Finally, we demonstrate that overexpression of FSP27 is alone sufficient to fully repress GH-induced lipolysis and insulin resistance in adipocytes. Taken together, these data clearly demonstrate a transcriptional mechanism by which GH acutely regulates lipolysis and insulin action in adipocytes.

Materials and methods

Mice

bGH and *STAT5^{ΔN/ΔN}* mice were housed at 22°C under a 14-h light, 10-h darkness cycle, 3–4 mice per cage and *ad libitum* access to water and standard laboratory chow (ProLab RMH 3000). All experiments were approved by the Ohio University Institutional Animal Care and Use Committee.

Cell culture

3T3-L1 (from ATCC: CL-173; passages 4–12) were grown in DMEM, high glucose (4.5 g/L) supplemented with Glutamax, Pen-Strep and 10% FBS. For differentiation into adipocytes, cells were seeded at 200,000 cells/well in six-well plates. After cells reached confluence, the medium was replaced by differentiation medium (growth medium with 1 μ M dexamethasone, 0.5 μ M isobutylmethylxanthine, 100 nM insulin and 1 μ M rosiglitazone. After 2 days, the medium was replaced with growth medium containing 100 nM insulin and 1 μ M rosiglitazone for two more days, then for 2–3 days with growth medium alone.

Lipolysis

Differentiated adipocytes were serum deprived in DMEM, high glucose for 2 hours then treated in KRH buffer supplemented with 2 mM sodium pyruvate in the presence or absence of U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio) butadiene), rosiglitazone or STAT5 inhibitor and GH for 2 h. The cells were washed carefully and incubated in Krebs Hepes bicarbonate (KRH) buffer supplemented with 2 mM sodium pyruvate in the presence or absence of U0126, rosiglitazone or STAT5 inhibitor, and GH for an additional hour. Glycerol release was measured by colorimetric reaction as previously described (Lee *et al.* 2013).

Real-time RT-PCR

Analysis of gene expression was conducted using real-time reverse transcriptase quantitative PCR (qPCR). TRIzol Reagent (Life Technologies) was used to extract total RNA from adipose tissue samples, and RNA was quantified by measuring absorbance at 260 and 280 nm with a ratio ≥ 1.8 . For a list of real-time primers and sequences see Supplementary Table 1 (see section on [supplementary data](#) given at the end of this article).

Western blot analysis and nuclear fractionation

Proteins were extracted from adipose tissue and cells, subjected to SDS-PAGE, transferred to polyvinylidene fluoride membranes and blots were blocked and probed with antibodies as specified in Supplementary Table 2. Protein were quantified as a ratio to the content of β -actin except for Ppar γ in the nuclear fractionation studies in which proliferating cell nuclear antigen (PCNA) and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were utilized as loading controls. Nuclear fractionation was performed utilizing the Rapid, Efficient And Practical (REAP) method as previously described (Suzuki *et al.* 2010).

Reporter assays

293T cells (from ATCC: CRL-3216; passages 4–8) were transfected with polyethylenimine (PEI) transfection reagent while still in suspension in 96-well plates. Each well was transfected with 50 ng of a previously described FSP27 luciferase construct (Kim *et al.* 2008), along with a total of 50 ng of expression plasmids containing STAT5A, STAT5B or empty vector controls and 10 ng of Renilla-TK plasmid. The cells were harvested 24 h later and luciferase activity was measured using a Dual Renilla Luciferase II Assay Kit and normalized to Renilla luciferase measurements (Promega). Site-directed mutagenesis of the FSP27 constructs by PCR was performed using the primers listed in Supplementary Table 1.

FSP27 overexpression

Fully differentiated 3T3-L1 adipocytes (day 6) (from ATCC: CRL-173; passages 2–6) were either infected with control adenovirus or adenovirus expressing FSP27 (1–2 moi/cell). Cells were treated 24 h later.

Statistics

Normality was tested with the Shapiro–Wilk test and by using the normal probability plot on raw data. Non-normal distributed data were ln-transformed. Differences between groups were tested using a Student's *t*-test or a two-way repeated measurements analysis of variance as appropriate. Statistical significance was assumed for $P < 0.05$. Data are presented as arithmetic means \pm s.e. unless otherwise stated.

Results

GH acutely induces lipolysis and reduces FSP27 expression

In order to determine the effects of GH on lipolysis, we examined the expression of known regulators of lipolysis in the perigonadal adipose tissue of bovine (b) GH mice transgenic mice. bGH mice have serum GH levels of approximately 2 μ g/mL, leading to a two-fold increase in serum IGF-1 levels (Chen *et al.* 1990, 1991). In the perigonadal adipose tissue, mRNA levels of *ATGL* and perilipin were increased 2- and 3-fold, respectively, while levels of FSP27 were reduced by 40% compared to levels in WT controls (Fig. 1A). In order to determine which of these regulators was directly and acutely regulated by GH action, differentiated 3T3-L1 adipocytes were treated with 500 ng/ μ L of recombinant bGH for 2 h. qPCR analysis indicates expression of FSP27 was acutely reduced by ~35% by GH treatment, with no changes in mRNA levels of other regulators of lipolysis (Fig. 1B). This GH-mediated decrease in FSP27 mRNA and protein expression was transitory, as qPCR and Western blot analysis indicates that FSP27 mRNA and protein levels were reduced by 35–40% at 2–4 h after GH treatment with levels returning to baseline by 12 h (Fig. 1C and Supplementary Fig. 1A). On the other hand, the known target gene of GH, insulin-like growth factor I (*Igf1*) was significantly increased at 4, 12 and 24 h after GH treatment (Chia *et al.* 2010) (Supplementary Fig. 1B). Furthermore, treatment of differentiated 3T3-L1 adipocytes with GH for 2 h reveals that lipolysis, as measured by glycerol release, is increased in a dose-dependent manner (Fig. 1D). This increased lipolytic rate is accompanied by a rate dependent decrease in FSP27 mRNA and protein levels at 2 h (Fig. 1E and Supplementary Fig. 1C). Thus, in cultured adipocytes, GH treatment directly increases lipolysis that is associated with a rapid and transient decrease in FSP27 expression.

GH regulates lipolysis in a MEK- and PPAR γ -dependent manner

Since FSP27 is transcriptionally regulated by PPAR γ (Kim *et al.* 2008, Puri *et al.* 2008a) and its expression is affected by PPAR γ phosphorylation (Choi *et al.* 2011, Banks *et al.* 2015, Tan *et al.* 2016) mediated through MEK/ERK, we investigated if GH modulates PPAR γ levels and/or activity through these pathways. Although no changes in total PPAR γ protein levels were noted, we did find that the effects on lipolysis are mediated through both MEK/ERK and PPAR γ . A 2-h pre-treatment with 10 μ M U0126,

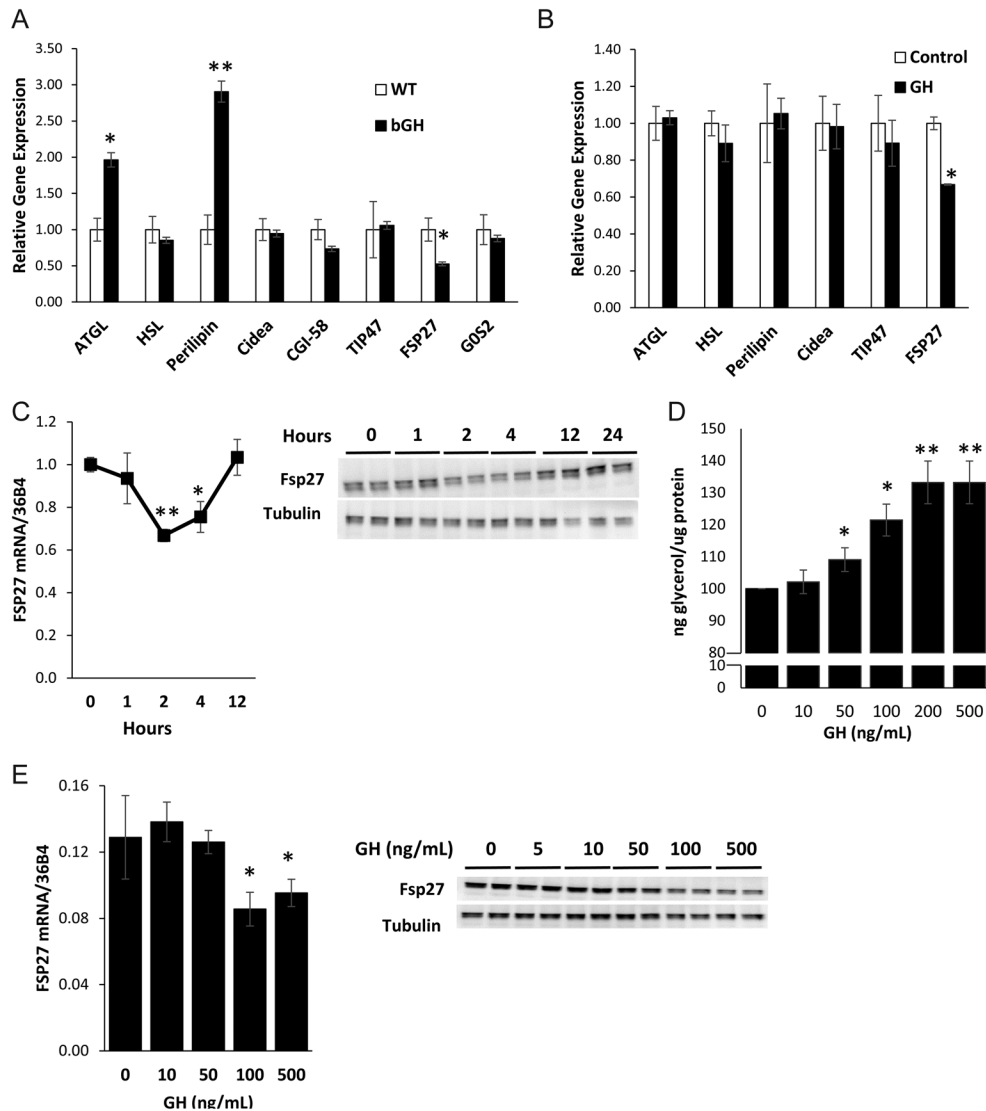


Figure 1

GH acutely induces lipolysis and reduces FSP27 expression in a time and dose-dependent manner. (A) qPCR analysis of mRNA levels of regulators of lipolysis: *ATGL*, *HSL*, *Perilipin*, *Cidea*, Comparative gene identification-58 (*CGI-58*), Tail-interacting protein of 47 kD (*TIP47*), G0/G1 switch gene 2 (*G0S2s*), and *FSP27* mRNA was compared in RNA isolated from perigonadal fat of 4 month old male bGH mice. Data are shown as mean \pm s.e.m. of 8–10 Samples. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$. (B) qPCR analysis of *Atgl*, *Hsl*, *Perilipin*, *Cidea*, *CGI-58*, *TIP47*, and *FSP27* mRNA in RNA isolated from 3T3-L1 adipocytes treated with 500 ng/mL recombinant bovine GH (bGH) for 2 h. Data are shown as mean \pm s.e.m. of three independent experiments. (C) qPCR and Western Blot analysis of FSP27 mRNA and protein isolated from 3T3-L1 adipocytes treated with 500 ng/mL recombinant bovine GH (bGH). Data are shown as mean \pm s.e.m. of three independent experiments. (D) Lipolysis as measured by glycerol release from 3T3-L1 adipocytes treated with bGH for two hours. Data are shown as mean \pm s.e.m. of three independent experiments. (E) qPCR and Western Blot analysis of FSP27 mRNA and protein isolated from 3T3-L1 adipocytes treated with bGH for 2 h. Data are shown as mean \pm s.e.m. of three independent experiments.

to block the phosphorylation of MEK, or 1 μ M rosiglitazone, to fully activate PPAR γ , was sufficient to completely inhibit GH-induced lipolysis (Fig. 2A). Western blot analysis indicates that 20 min of GH treatment induced phosphorylation of ERK on T²⁰²/Y²⁰⁴ and STAT5 on Y⁶⁹⁴ without changes in their total protein levels. Pre-treatment with U0126 abolished ERK phosphorylation without affecting STAT5 phosphorylation, while rosiglitazone

pre-treatment had no effect on the phosphorylation of either ERK1 or STAT5. Phosphorylation of HSL at S⁵⁶³ was induced by GH treatment at the 2 h time point, and this induction was blunted by U0126, but not significantly altered by rosiglitazone pre-treatment (Fig. 2B and Supplementary Fig. 2A).

Interestingly, both U0126 and rosiglitazone pre-treatment not only prevented GH-induced reduction of

FSP27, but led to a GH-dependent three-fold increase in the level of FSP27 mRNA and protein (Fig. 2C and D). Levels of other regulators of lipolysis including *ATGL*, *HSL* and *Cidea* were not significantly altered either by U0126 and rosiglitazone pre-treatment or GH treatment. Furthermore, no changes in total PPAR γ levels were noted (Fig. 3A and Supplementary Fig. 2B, C, D). Taken together, these data indicate that GH suppresses FSP27 expression through MEK/ERK- and PPAR γ -dependent mechanisms.

GH treatment results in PPAR γ translocation to the cytoplasm

MEK has been shown to directly interact with and phosphorylate PPAR γ leading to its nuclear export after stimulation with tetradecanoyl phorbol acetate

or TNF- α stimulation (Burgermeister *et al.* 2007, Burgermeister & Seger 2007, Tan *et al.* 2016). GH also acts through these mechanisms as 3T3-L1 adipocytes treated with 500 ng/mL bGH for 20 min increased phosphorylation of pPPAR γ on S¹¹² without affecting total pPPAR γ levels. Pre-treatment with 10 μ M U0126 abolished this effect, while pre-treatment with rosiglitazone had no effect (Fig. 3A). Nuclear fractionation experiments 1 h after GH treatment, demonstrated that GH leads to a rapid increase in cytoplasmic PPAR γ and a reduction in nuclear PPAR γ . This translocation was inhibited by both U0126 and rosiglitazone (Fig. 3B). These effects were confirmed by immunofluorescence for PPAR γ (green) with nuclei counterstained with DAPI (blue) in 3T3-L1 adipocytes treated with 500 ng/mL bGH for 1 h GH treatment resulted in a rapid translocation of PPAR γ from the nucleus to

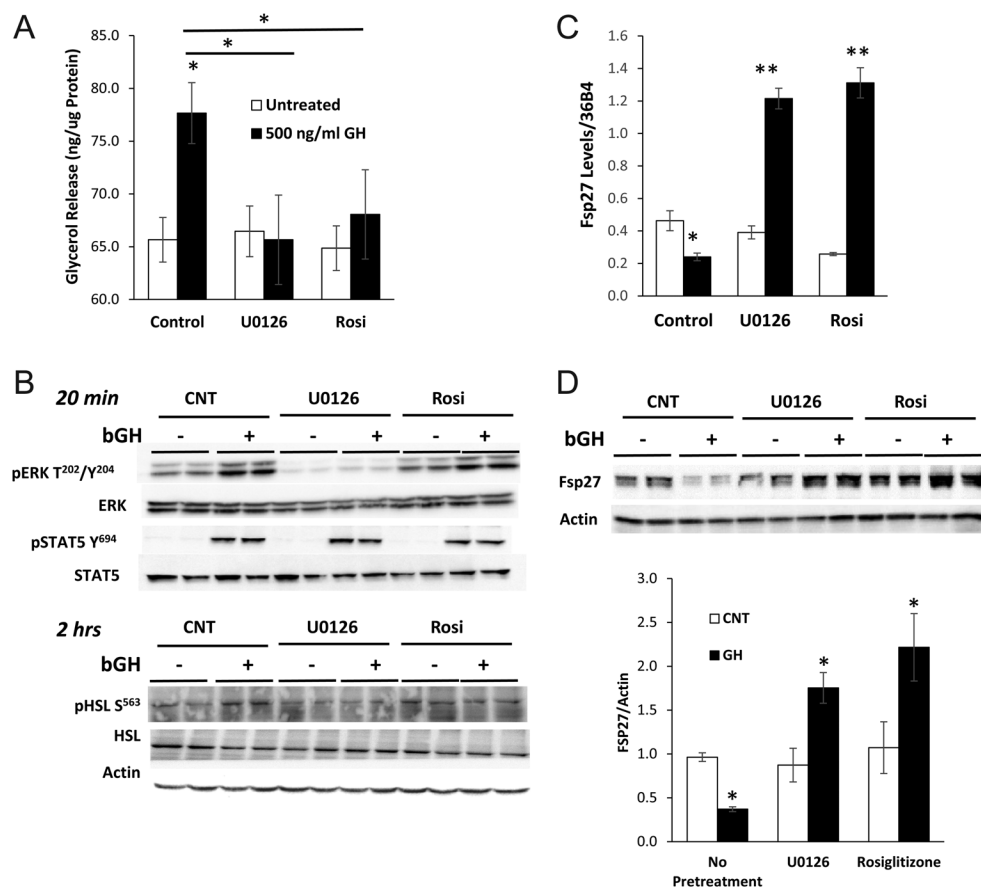


Figure 2

GH regulates lipolysis in a MEK and PPAR γ dependent manner. (A) Lipolysis as measured by glycerol release from 3T3-L1 adipocytes treated with 500 ng/mL bGH for 2 h after 2 hours of pre-treatment with 10 μ M U0126 or 1 μ M Rosiglitazone. Data are shown as mean \pm s.e.m. of three independent experiments. Asterisks indicate a significant differences in all panels * P <0.05; ** P <0.01. (B) Representative Western blot analysis of pERK T²⁰²/Y²⁰⁴, total ERK, pSTAT5 Y⁶⁹⁴, total STAT5, pHSL S⁵⁶³, and total HSL in 3T3-L1 adipocytes treated with 500 ng/mL bGH for either 20 min or 2 h after 2 hours of pre-treatment with 10 μ M U0126 or 1 μ M Rosiglitazone. Actin is used as a loading control. (C and D) qPCR and Western blot analysis of FSP27 mRNA and protein isolated from cells treated with 500 ng/mL bGH for 2 h after 2 hours of pre-treatment with 10 μ M U0126 or 1 μ M Rosiglitazone. Actin is used as a loading control. Quantitation of Western blot data are shown as mean \pm s.e.m. of four independent experiments.

the cytoplasm in 3T3-L1 adipocytes, and pre-treatment with 10µM U0126 or 1µM rosiglitazone blocked this effect (Fig. 3C).

STAT5 increases PPARγ activity on the FSP27 promoter

The 3-fold increase of *FSP27* mRNA in 3T3-L1 adipocytes that we observed upon U0126 or rosiglitazone pre-treatment and subsequent GH treatment (Fig. 2C) lead us to hypothesize that an additional regulatory mechanism is responsible for *FSP27* regulation. Nuclear fractionation indicates that STAT5 translocates to the nucleus in a MEK- and PPARγ-independent manner (Supplementary Fig. 3A). Since STAT5 mediates many GH-dependent effects, we pretreated 3T3-L1 adipocytes with the specific STAT5 inhibitor, CAS 285986-31-4 (Muller *et al.* 2008).

The STAT5 inhibitor reduced GH-induced phosphorylation of STAT5 on Y⁶⁹⁴ by ~70% without changes in total protein levels (Supplementary Fig. 3B and C). Although pre-treatment with the STAT5 inhibitor alone had limited effect on mRNA levels of *FSP27*, pre-treatment with STAT5 inhibitor in combination with GH treatment leads to 60% downregulation of *FSP27* mRNA. In addition, the STAT5 inhibition completely suppressed the upregulation of *FSP27* mRNA that was observed with U0126 and rosiglitazone pre-treatment and subsequent GH treatment and lead to an 80–90% downregulation of *FSP27* mRNA (Fig. 4A). Inhibition of STAT5 increased GH-mediated lipolysis by ~15%; however, these results did not quite reach statistical significance (Fig. 4B). Furthermore, qPCR analysis of RNA isolated from subcutaneous and perigonadal fat of mice, which express hypomorphic forms of both *Stat5a* and *Stat5b*, *STAT5^{AN/AN}*-mutant mice

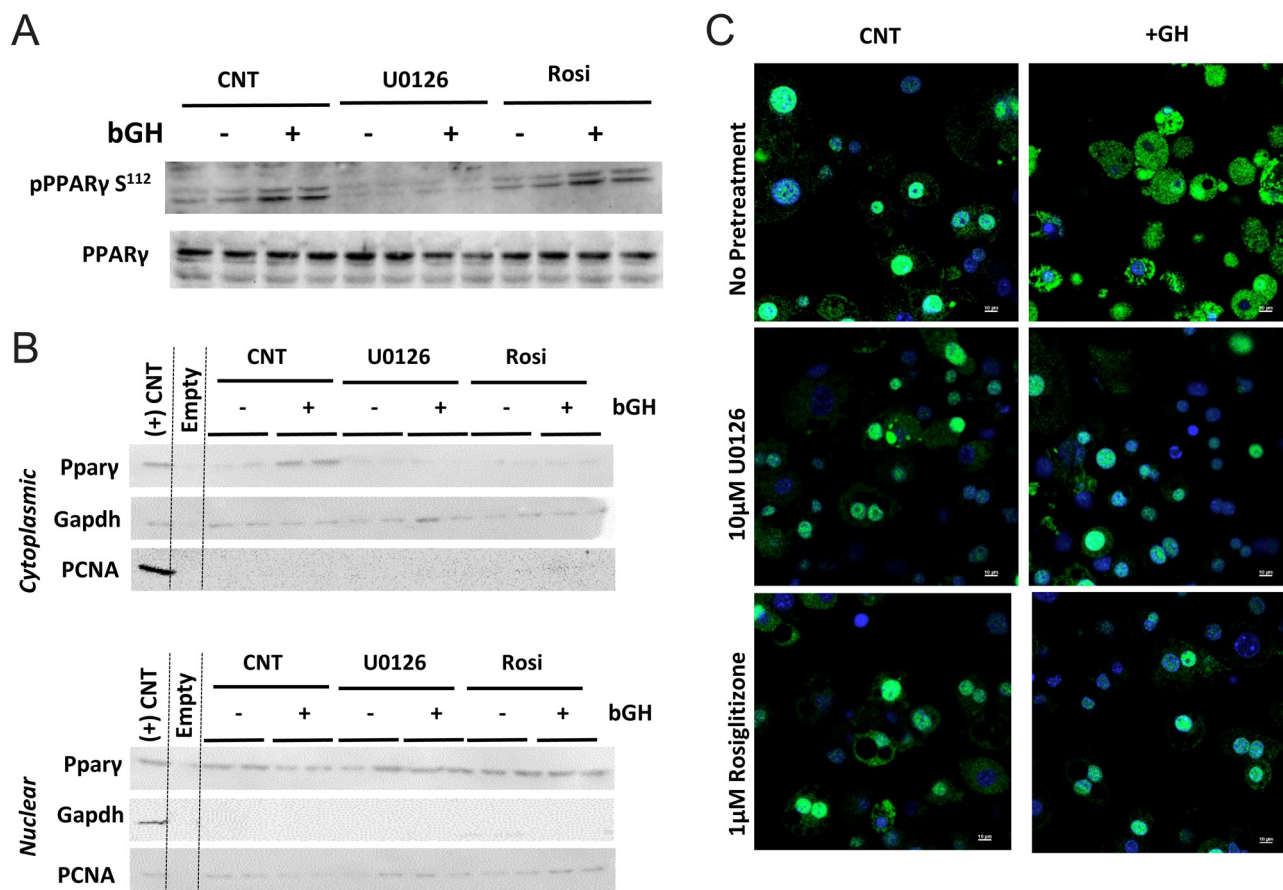


Figure 3 GH treatment results in rapid PPARγ translocation. (A) Representative Western blot analysis of pPPARγ S¹¹² and total pPPARγ in 3T3-L1 adipocytes treated with 500 ng/mL bGH for 20 min after 2 hours of pre-treatment with 10µM U0126 or 1µM Rosiglitazone. (B) Representative Western blot analysis for PPARγ in 3T3-L1 adipocytes treated with 500 ng/mL bGH for 1 h after 2 h of pre-treatment with MEK1 inhibitor, 10µM U0126, or 1µM Rosiglitazone following nuclear fractionation. The positive control is whole cell lysate of 3T3-L1 adipocytes. Gapdh and PCNA are loading controls for the cytoplasmic and nuclear fractions, respectively. (C) Immunofluorescence for PPARγ (green) with nuclei counterstained with DAPI (blue) of in 3T3-L1 adipocytes treated with 500 ng/mL bGH for 1 h after 2 h pre-treatment with 10µM U0126 or 1µM Rosiglitazone.

(Teglund *et al.* 1998, Cui *et al.* 2004), demonstrates that FSP27 is significantly reduced in the perigonadal fat and tends to be reduced in subcutaneous fat (Fig. 4C).

The dependence of FSP27 expression on both STAT5 and PPAR γ prompted us to test whether FSP27 might be directly regulated by the cooperative action of these factors. A reporter construct harboring a 0.9 kb upstream fragment of the human *FSP27* gene linked to a luciferase reporter gene (FSP27-luc) was co-transfected with either a vector control or 25 ng of PPAR γ expression vector and 25 ng of its obligate heterodimer RXR α . The cells were also co-transfected with either a vector control or 25 ng of a *STAT5* expression vector. Luciferase assays demonstrated that basal *FSP27* promoter activity in 293T cells was very low, and no change in activity was seen following *STAT5* expression. The *FSP27*-luc activity was potently activated by co-transfection of PPAR γ and its obligate heterodimer partner RXR α . Although we could not detect direct interaction between PPAR γ and *STAT5* (Supplementary Fig. 3D), co-transfection of *STAT5* with PPAR γ resulted in a synergistic 50% increase in FSP27-luc activation compared to PPAR γ alone (Fig. 4D). Both *STAT5A* and *STAT5B* were equally able to transactivate the *FSP27* promoter construct when co-transfected with PPAR γ , while a constitutively active form of *STAT5B* (Farrar 2010) lead to further transactivation of the promoter construct (Supplementary Fig. 3E).

Examination of the *FSP27* promoter sequence revealed the presence of half of a consensus STAT-binding site (StatRE) adjacent to the known PPAR γ response element (PPRE) (Kim *et al.* 2008). Site-directed mutagenesis of the PPRE (Δ PPRE) in the *FSP27* promoter abolishes its response to both PPAR γ and *STAT5* (Fig. 4E). On the other hand, mutation of the StatRE (Δ SRE) does not significantly affect activation either by PPAR γ or co-transfection of PPAR γ and *STAT5* (Fig. 4F).

FSP27 over-expression prevents GH-induced lipolysis and insulin resistance

Our data indicate that GH-induced lipolysis is associated with a reduction of FSP27 expression. Thus, we hypothesized that FSP27 over-expression might protect adipocytes from GH-induced lipolysis and insulin resistance. To test our hypothesis, we utilized adenovirus to stably over-express human FSP27 in 3T3-L1 adipocytes. Interestingly, FSP27 over-expression in adipocytes almost completely suppressed GH-induced lipolysis (Fig. 5A and B). Furthermore, since lipolysis is associated with lipid droplet fragmentation (Walther

& Farese 2012), we assessed lipid droplet number in control and FSP27 over-expressing cells. Control 3T3-L1 adipocytes each contained ~20 lipid droplets/cell, and 24 h of GH treatment leads to over a five-fold increase in the number of lipid droplets. FSP27 over-expression was sufficient to completely abolish the effect GH on lipid fragmentation (Fig. 5C and Supplementary Fig. 4A). Since the FFAs liberated by lipolysis have long been known to cause insulin resistance (Boden 1997), we assessed the insulin-stimulated phosphorylation of AKT S⁴⁷³ in these adipocytes. Treatment of control adipocytes with 10 nM insulin for 15 min strongly induces phosphorylation of AKT on S⁴⁷³, while pre-treatment with 250 ng/mL GH for 2 h reduced this phosphorylation by ~60%. However, the inhibitory effect of GH on AKT S⁴⁷³ phosphorylation was abolished in adipocytes over-expressing FSP27 (Fig. 5D and Supplementary Fig. 4B). Thus, over-expression of FSP27 is sufficient to prevent GH-induced lipolysis, lipid fragmentation, and insulin resistance.

Discussion

In the present study, we present a molecular mechanism by which GH regulates lipolysis in adipose tissue. These studies are consistent with human studies in adipose tissue, as GH receptor levels have been shown to be directly correlated with FSP27 levels in human subcutaneous adipose tissue (Karastergiou *et al.* 2016) and acute infusion of 30 ng/min of GH in human subjects is sufficient to induce lipolysis and leads to reduction in FSP27 expression at the mRNA and protein levels (Sharma V, *et al.* (submitted manuscript)). Our data demonstrate that GH-induced MEK/ERK activation promotes PPAR γ phosphorylation and translocation from the nucleus. This serves as the dominant pathway controlled by GH and led to reduced transcription of FSP27. As a counter-regulatory mechanism, GH-induced phosphorylation of *STAT5*, which enhances PPAR γ activity on the *FSP27* promoter. Together, these intersecting pathways act together to tightly regulate the lipolytic effects GH (Fig. 5E).

The molecular mechanisms described in this study indicate that GH-induced lipolysis is secondary to its reduction in the expression of FSP27. This mechanism is distinct from previous studies describing pathways by which GH chronically induces lipolysis through HSL activation (Dietz & Schwartz 1991, Bergan *et al.* 2013). In agreement with these previous findings, we also observed an increase in the MEK/ERK-mediated phosphorylation of HSL (Fig. 2B). However, since ATGL

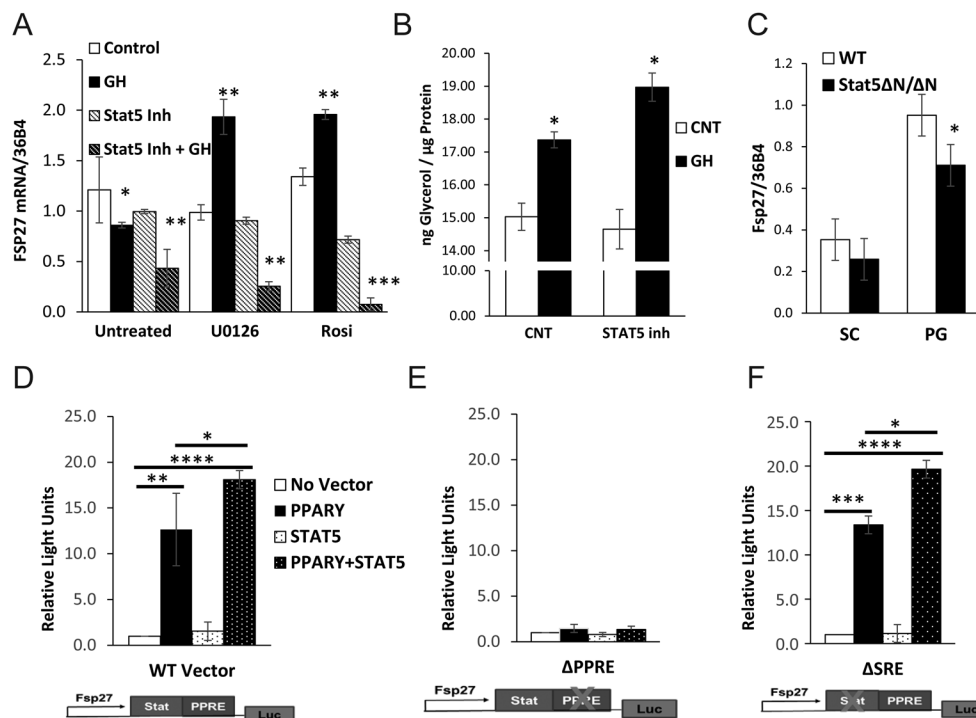


Figure 4

STAT5 and PPAR γ regulate FSP27 expression. (A) qPCR analysis of FSP27 in RNA isolated from 3T3-L1 adipocytes treated with vehicle or 500 ng/mL bGH for 2 h after 2 hours of pre-treatment with 10 μ M U0126, 1 μ M Rosiglitazone, or 200 μ M STAT5 inhibitor. Data are shown as mean \pm s.e.m. of three independent experiments. Asterisks indicate a significant differences in all panels * P <0.05; ** P <0.01; *** P <0.001. (B) Lipolysis as measured by glycerol release from 3T3-L1 adipocytes treated with vehicle or 500 ng/mL bGH for 2 h after 2 hours of pre-treatment with 200 μ M STAT5 inhibitor. Data are shown as mean \pm s.e.m. of two independent experiments, each with three replicates/group. (C) Expression level of FSP27 mRNA was compared using quantitative real-time PCR (qPCR) of RNA isolated from subcutaneous (SC) and perigonadal (PG) fat of 4 month old male *Stat5 Δ N/ Δ N* mice. Data are shown as mean \pm s.e.m. of six samples. (D, E and F) Luciferase activity of 293T cells transfected with a 0.9-kb WT FSP27 luciferase reporter, with the PPAR γ response element mutated, or with a presumptive STAT5 response element mutated. The reporter vector was co-transfected either a vector control or 25 ng of PPAR γ expression vector and 25 ng of its obligate heterodimer RXR α . The cells were also co-transfected with either a vector control or 25 ng of a STAT5 expression vector. Data are shown as mean \pm s.e.m. of three independent experiments, each with three replicates.

hydrolysis of triglycerides is the first and rate-limiting step in lipolysis, it suggests that the changes in the ATGL activity by modulation of FSP27 may be the key regulatory step in control of GH-induced lipolytic rate. Furthermore, except in cases of acromegaly, GH secretion is pulsatile and regulated in diurnal rhythms (Ho *et al.* 1987). This pulsatile secretion leads us to espouse a biological model by which GH acutely and transiently regulates lipolysis over the effects observed in cellular models of chronic GH treatment. Interestingly, FSP27 levels are chronically, rather than transiently, reduced in bGH animals with consistently high GH levels. bGH has similar somatotrophic effects as hGH, but does not bind the prolactin receptor, and thus, does not have the severe lactogenic and reproductive effects observed upon over-expression of hGH effects (Posner 1976, Bartke *et al.* 1992, 1994). In addition to reduced fat mass, bGH mice also have numerous changes within their adipose tissue, including increased immune cell infiltration within adipose tissue,

increased circulating levels of inflammatory cytokines and dramatically altered adipokine expression (Wang *et al.* 2007, Benencia *et al.* 2015, Berryman & List 2017). All these factors have been shown to affect FSP27 expression (Tan *et al.* 2016, Becerril *et al.* 2018) and may contribute to its chronically reduced expression in bGH adipose tissue.

In contrast to the extremely rapid kinetics of catecholamine-induced lipolysis, in which measurable FFA release occurs within minutes, administration of a physiological GH bolus has been shown to stimulate lipolysis after a time lag of 2–3 h (Moller *et al.* 1990, Morimoto *et al.* 2001). Consistent with this, the expression of FSP27 is reduced 2 h after GH treatment (Fig. 1B and C). Since FSP27 protein is very unstable and has a short half-life of ~15 min (Nian *et al.* 2010, Yang *et al.* 2011), our data indicate that the reduced PPAR γ -dependent transcription caused by GH leads to a rapid reduction in its mRNA and protein expression. Importantly, levels of other regulators of lipolysis including *Atgl*, *Hsl*, *Cidea* and *CGI-58* are not

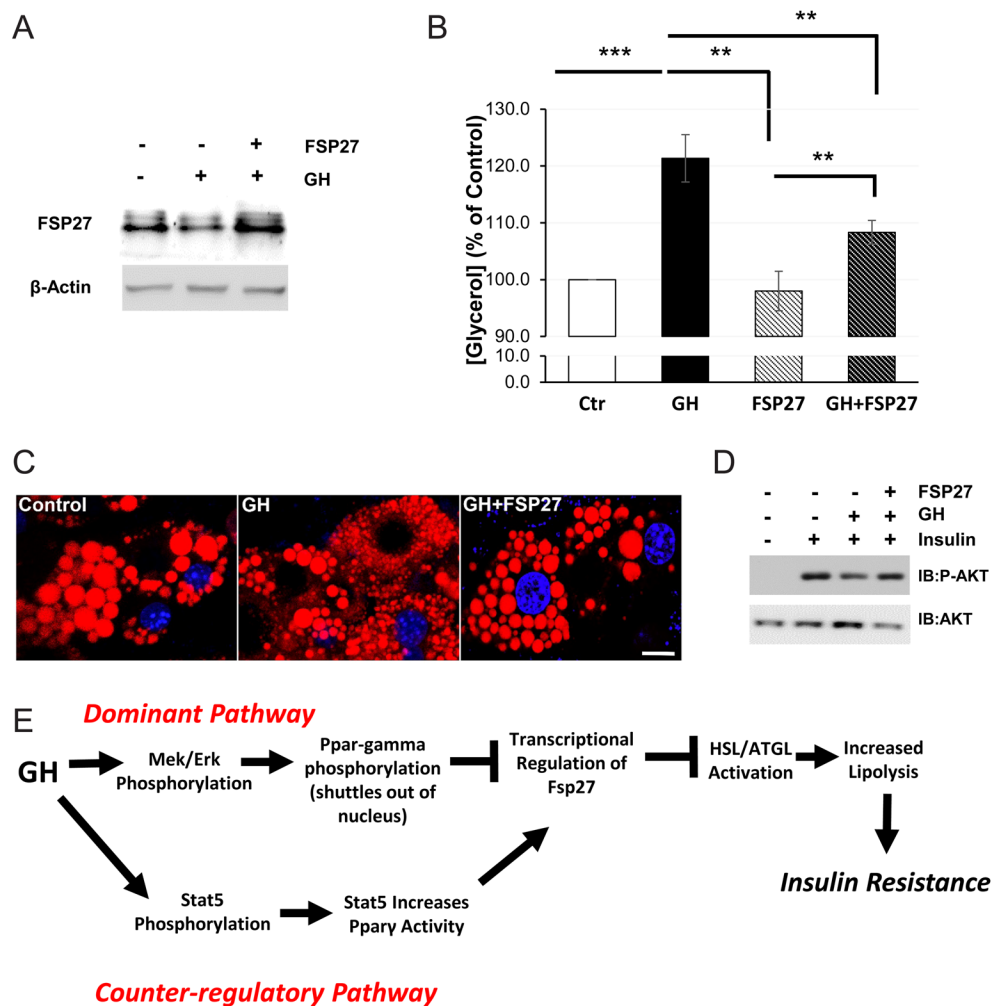


Figure 5

FSP27 over-expression prevents GH-induced lipolysis and insulin resistance. (A) Representative Western blot analysis of FSP27 in WT 3T3-L1 adipocytes and FSP27 over-expressing adipocytes treated with 500 ng/mL bGH for 2 h. (B) Lipolysis as measured by glycerol release from WT 3T3-L1 adipocytes and FSP27 over-expressing adipocytes treated with 250 ng/mL bGH for two hours. Data are shown as mean \pm S.E.M. of three independent experiments. Asterisks indicate a significant differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (C) Immunofluorescence of Nile Red Stained WT and FSP27 over-expressing 3T3-L1 adipocytes treated with vehicle or 500 ng/mL bGH for 24 h. (D) Representative Western blot analysis of pAKT S473 and total AKT in WT 3T3-L1 adipocytes and FSP27 over-expressing adipocytes treated with 250 ng/mL bGH for 2 h and 10 nM insulin for 15 min. (E) Proposed mechanism of GH-induced lipolysis.

acutely changed by GH treatment. Furthermore, although chronic GH treatment upregulates transcription of PPAR γ mRNA in a STAT5-dependent manner (Kawai *et al.* 2007), the acute lipolytic effects of GH occur in the absence of increased PPAR γ expression (Fig. 4A). In addition, these effects are also independent of *Igf1*, as levels of IGF-1 remain unchanged after 2 h of GH treatment in cultured adipocytes (Supplementary Fig. 2A). Although we could not detect direct protein-protein interaction of STAT5 and PPAR γ (Supplementary Fig. 3D), our data clearly indicate that STAT5, in a GH-dependent manner, is critical for maintaining FSP27 expression and may play a role in moderating the magnitude of GH-induced lipolysis

(Fig. 4A and B). Treatment with the STAT5 inhibitor tended to increase lipolysis; this difference did not quite reach statistical significance. This may be due to the secondary role of STAT5 pathway in this process, as the MEK/ERK is the predominant pathway. In addition, the STAT5 inhibitor did not fully suppress STAT5 phosphorylation, thus limiting its effect on lipolysis. Elucidating the exact molecular mechanism by which STAT5 controls PPAR γ activity will be of importance in future studies.

Our data demonstrate that reduction of lipolysis by over-expression of FSP27 blocks the GH-mediated suppression of insulin signaling in adipocytes. These results are in agreement with previous reports that

indicate circulating FFAs are the dominant inducer of insulin resistance and non-suppressible hepatic glucose production in patients with T2DM (Titchenell *et al.* 2016). Furthermore, both current and early studies have consistently demonstrated that acute administration of GH has a strong diabetogenic effect that is primarily due to its lipolytic action (Houssay 1936, Segerlantz *et al.* 2003, Moller *et al.* 2009, Salgin *et al.* 2009, Cornford *et al.* 2012). These diabetogenic effects of GH are also manifested in acromegalic patients with high levels of GH that display increased rates of insulin resistance, hyperinsulinemia and type 2 diabetes (Rizza *et al.* 1982, Hansen *et al.* 1986, Melmed *et al.* 2009). This induction of insulin resistance is, in all likelihood, a critical part of the response of adipocytes to GH, as it serves to inhibit the anti-lipolytic action of insulin (Okada *et al.* 1994) and allows lipolysis to proceed. Although previous experiments in mice have shown that the diabetogenic effect of GH can, at least in part, be explained by alterations in the ability of insulin to activate PI-3 kinase in insulin target cells (Dominici *et al.* 2005, del Rincon *et al.* 2007), studies in humans have questioned the role of PI-3 kinase in GH-induced insulin resistance (Jessen *et al.* 2005, Nielsen *et al.* 2008, Krusenstjerna-Hafstrom *et al.* 2011a,b). Importantly, the 'dawn phenomenon', which describes an early morning increase in insulin resistance in patients with diabetes, can be almost entirely corrected by reducing GH levels or GH-induced lipolysis (Campbell *et al.* 1985, Davidson *et al.* 1988, Salgin *et al.* 2009). Although the dawn phenomenon was first described in type 1 diabetes mellitus (T1DM), recent studies utilizing the advent of continuous glucose monitoring systems has demonstrated the dawn phenomenon occurs in ~50% of patients with both T1DM and T2DM, significantly increases HbA1c levels (~0.4% in T2DM) and leads to frequent hyperglycemic episodes (Monnier *et al.* 2012, 2013, Bouchonville *et al.* 2014). Taken together, these studies demonstrate that GH-mediated lipolysis is a critical regulator of insulin resistance in both healthy subjects and patients with diabetes.

Clinical studies have consistently demonstrated that GH treatment reduces visceral fat mass and improves metabolic parameters in GH-deficient patients, as well as those with visceral obesity (Beauregard *et al.* 2008, Bredella *et al.* 2013). This reduction in visceral fat mass is presumably, at least in part, due to lipolytic action of GH. On the other hand, in diabetic patients, GH-induced lipolysis leads to frequent hyperglycemic episodes in the dawn phenomenon (Monnier *et al.* 2012, 2013, Bouchonville *et al.* 2014). Thus, understanding the molecular mechanisms which underlie GH-induced

lipolysis is crucial for the treatment of a wide variety of patients. Our studies identify several molecular targets of intervention to manipulate GH-induced lipolysis. Many of these targets already have pharmacological agents in clinical use, including MEK inhibitors (Trametinib), PPAR γ agonists (Thiazolidinediones) and anti-lipolytic agents (Acipimox). Therefore, we strongly believe that these data have clear bench to bedside ramifications and can have significant and immediate clinical impact.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-18-0282>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

R S, Q L, V M S, M H, B H, A C, and D E B designed and performed experiments; J J K, J O L J, N J and V P designed experiments and analyzed data; K Y L designed and performed experiments, analyzed data and wrote the paper.

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