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# FGF21 attenuates lipolysis in human adipocytes – A possible link to improved insulin sensitivity

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**Abstract Fibroblast growth factor 21 (FGF21) is active in murine adipocytes and has beneficial metabolic effects in animal models of type 2 diabetes mellitus. We assessed whether FGF21 influences lipolysis in human adipocytes and 3T3-L1 cells. FGF21 had no short-time effect (h) while a 3-day incubation with FGF21 attenuated hormone-stimulated lipolysis. FGF21 did not influence the mRNA expression of genes involved in regulating lipolysis, but significantly reduced the expression of the lipid droplet-associated phosphoprotein perilipin without affecting differentiation. Via reduced release of fatty acids into the circulation, the anti-lipolytic effect could be a mechanism through which FGF21 promotes insulin sensitivity in man.**


*Keywords:* Adipocyte; Lipolysis; Differentiation; Adipogenesis; Perilipin; β-Klotho

## 1. Introduction

Fibroblast growth factor 21 (FGF21) is predominantly expressed in murine liver and thymus [1]. Little was known regarding the bioactivity of this molecule until it was demonstrated that FGF21 stimulates non-insulin dependent glucose uptake in adipocytes [2]. This effect is mediated via increased expression of glucose transporter-1 (GLUT1). However, no effect on GLUT 1 expression or glucose uptake can be observed in non-differentiated adipose precursor cells or muscle and liver cell lines. In vivo administration of FGF21 in rodent models of diabetes lowers both plasma glucose and triglyceride levels and improves insulin sensitivity and glucose clearance. Transgenic mice over expressing FGF21 from the liver display resistance to diet-induced obesity and have improved glycemic control. FGF21 administration in diabetic monkeys results in a similar beneficial phenotype including improvements in lipoprotein profiles [3]. In diabetic rodents, FGF21 directly affects the endocrine function of the pancreas by enhancing insulin production and beta cell survival and reduces the maladaptive glucagon release [4]. Recent data have also shown that FGF21 can stimulate lipolysis in murine adipocyte [5]. FGF21 requires the presence of the transmembrane protein β-klotho for its effects [6]. So far, there have been no reports regarding the expression of this gene in human adipocytes.

Insulin sensitivity is influenced by different metabolic processes. In obesity and other insulin resistant conditions, a dysregulation of adipocyte lipolysis has been shown to be an important pathogenic process (reviewed in [7]). Via increased lipolysis in white adipose tissue, circulating non-esterified fatty acids (NEFA) are transported to the liver in elevated concentrations, which increases the production of very low density lipoprotein particles followed by hyperlipidemia. In addition, NEFAs are preferentially oxidized by muscle thereby attenuating glucose uptake in this tissue. Finally, NEFAs may have direct negative effects on beta cell function and insulin release (i.e. lipotoxicity).

Lipolysis is regulated by several proteins including perilipin (PLIN), a phosphoprotein that coats the intracellular lipid droplet and is phosphorylated by protein kinase A (PKA) upon lipolytic stimulation. It is believed that this results in the recruitment of lipases (mainly hormone sensitive lipase, HSL) to the surface of the lipid droplet for subsequent triglyceride hydrolysis [7]. PLIN phosphorylation increases the molecular weight of the protein which corresponds to a small transition of PLIN protein on SDS–PAGE (by approx. 2 kDa) [8].

Given the rather selective effects of FGF21 on adipocytes we assessed whether FGF21 could influence lipolysis in human fat cells.

## 2. Materials and methods

### 2.1. FGF21, tissue material, cell isolation and lipolysis

Human and murine FGF21 was obtained as described earlier [2]. Subcutaneous white adipose tissue was obtained from individuals undergoing surgery for non-malignant disorders. They were otherwise healthy and free of medication. The studies were approved by the ethical committee at Karolinska Institutet and explained in detail to each subject and informed consent was obtained. Isolation of mature fat cells, in vitro differentiation of primary preadipocytes and subsequent lipolysis experiments were performed exactly as described [9]. Human mesenchymal stem cells (hMSCs) were seeded at 10 000 cells/well into 96-well plates and cultured for 2 days under supplier (Biowhitaker) growth recommendations followed by 10 days of culture under differentiation conditions ±FGF21. Medium for adipogenic differentiation included combinations of 1 μM dexamethasone, 0.2 mM indomethacin, 1.7 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, St.

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*Abbreviations:* qRT-PCR, quantitative reverse-transcribed polymerase chain reaction; PPARγ, peroxisome proliferators-activated receptor gamma; HSL, hormone sensitive lipase; PLIN, perilipin; PKA, protein kinase A; PDE3B, phosphodiesterase 3B; ANP, atrial natriuretic peptide; NA, noradrenaline

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Louis, MO, USA), and 10 μM rosiglitazone (Eli Lilly, Indianapolis, IN). The cells were then fixed and stained with 2 mM Nile red and 2 mM Syto 16 green (Molecular Probes), and fluorescence was measured and analyzed with Acumen instrument and software. 3T3-L1 cells were maintained at subconfluence and differentiated into adipocytes as previously described [2]. Cells were differentiated for 14–21 days, incubated for 16 h in DMEM/0.1% bovine serum albumin (BSA) and treated with FGF21 for 8 h at the indicated concentrations in DMEM/0.1% BSA prior to undergoing the assay. Glycerol content of the culture media was quantitated using an Adipolysis Assay Kit (Millipore, Billerica, MA) according to the manufacturer's instructions and absorbance was measured at 540 nm using a SpectraMax 190 spectrophotometric plate reader (Molecular Devices, Sunnyvale, CA).

### 2.2. RNA analysis and protein expression

Total RNA extraction, primer sequences for quantitative reverse-transcribed polymerase chain reaction (qRT-PCR) and protein isolation from adipocyte cultures are described previously [9]. The PLIN antibody was from Progen Biotechnik (Heidelberg, Germany) and β-actin from Sigma and Western blots were performed as described previously [10]. Antigen–antibody complexes were detected by chemiluminescence (Supersignal®, Pierce, Rockford, USA) and specific bands were measured using a Chemidoc XRS system (BioRad, Germany). Images were analyzed using the Quantity One Software supplied by the manufacturer (BioRad).

### 2.3. MAPK assay

Total ERK phosphorylation was assessed using an AlphaScreen *SureFire* Phospho-ERK1/2 Assay Kit (Perkin–Elmer, Waltham, MA, USA) according to the manufacturer's instructions. An EnVision Multilabel Microplate Reader Model 2103 with the AlphaScreen HTS Turbo option was used for signal detection (Perkin–Elmer).

### 2.4. Statistical analysis

Values are means ± standard deviation (S.D.) and compared with Student's paired *t*-test unless otherwise stated. $P < 0.05$ was considered as statistically significant.

## 3. Results

### 3.1. FGF21 attenuates stimulated lipolysis in human adipocytes in culture

Differentiated primary human adipocytes were incubated with different concentrations of FGF21 ranging up to 150 nmol/l for different time periods. Glycerol release (lipolysis index) was measured. Incubations for 2–12 h had no effect on basal or catecholamine-stimulated lipolysis (values not shown) but a 72 h-incubation with FGF21 significantly attenuated noradrenaline (NA) action (Fig. 1A). This was a post-receptor effect since lipolysis induced by the adenylate cyclase activator forskolin was decreased by FGF21. FGF21 also inhibited atrial natriuretic peptide (ANP)-stimulated lipolysis in separate experiments (Fig. 1B) but did not influence basal glycerol release (Fig. 1A and B). The maximum FGF21 effect was observed in cells pre-treated with 150 nmol/l (data not shown). Therefore, this concentration of FGF21 was used in all further experiments. Acute (2 h) experiments were also performed with suspensions of freshly isolated fat cells. NA caused a three-fold induction of lipolysis (Fig. 1C). This effect was significantly attenuated by co-incubation with insulin but there was no effect on lipolysis with FGF21.

### 3.2. Human adipocytes express β-klotho, a required protein for FGF21 signalling

To assess whether human adipocytes express the necessary components for FGF21 response we performed qRT-PCR in cell fractions of white adipose tissue. This showed that β-klotho is expressed predominantly in the adipocyte fraction and less in the stroma-vascular portion (containing undifferentiated preadipocytes, endothelial cells and immune cells) (Fig. 2A).

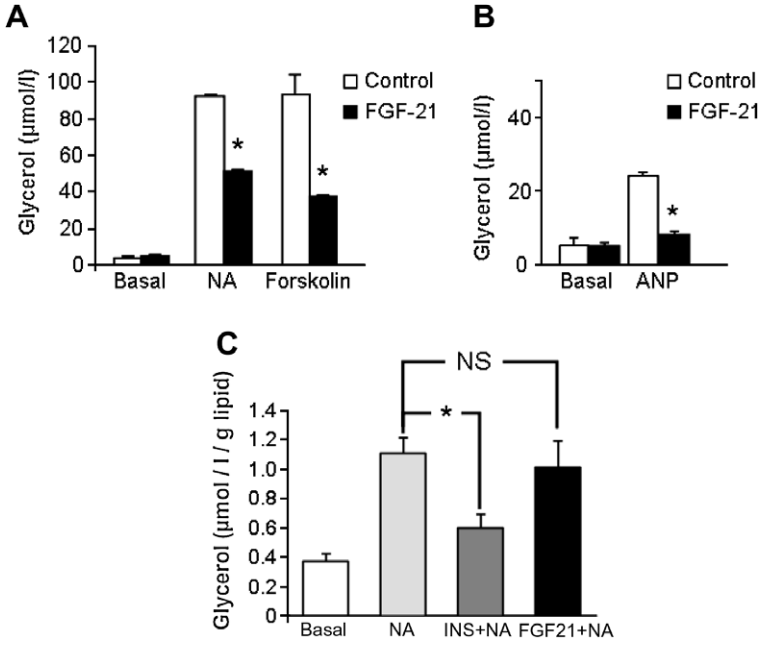


Fig. 1. Lipolysis experiments. (A) Preadipocytes were differentiated for 12 days. At this time-point, 150 nmol/l FGF21 was added to the cultures for 72 h, parallel incubations without FGF21 served as controls as indicated. After incubation, cells were washed and incubated without (Basal) or with $10^{-6}$ mol/l noradrenaline (NA) or $10^{-5}$ mol/l Forskolin for 2 h after which the media was removed and analyzed for glycerol concentration by bioluminescence. (B) Cells treated as in (A) but lipolysis was stimulated with $10^{-6}$ mol/l ANP. (C) Freshly isolated fat cells were isolated by collagenase extraction and incubated in suspension in the presence of $10^{-6}$ nmol/l NA without or with a 2 h preincubation with $10^{-6}$ mol/l insulin (INS + NA) or FGF21 (FGF21 + NA). Glycerol release was analyzed. All experiments were repeated at least five times in triplicates with cells from different donors. $^{*}P < 0.05$ by Student's paired *t*-test for control versus FGF21 treated cells.