Original article

MOLECULAR METABOLISM

Fibroblast growth factor 21 is elevated in metabolically unhealthy obesity and affects lipid deposition, adipogenesis, and adipokine secretion of human abdominal subcutaneous adipocytes

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ABSTRACT

Objective: Serum concentrations of the hepatokine fibroblast growth factor (FGF) 21 are elevated in obesity, type-2 diabetes, and the metabolic syndrome. We asked whether FGF21 levels differ between subjects with metabolically healthy vs. unhealthy obesity (MHO vs. MUHO), opening the possibility that FGF21 is a cross-talker between liver and adipose tissue in MUHO. Furthermore, we studied the effects of chronic FGF21 treatment on adipocyte differentiation, lipid storage, and adipokine secretion.

Methods: In 20 morbidly obese donors of abdominal subcutaneous fat biopsies discordant for their whole-body insulin sensitivity (hereby classified as MHO or MUHO subjects), serum FGF21 was quantified. The impact of chronic FGF21 treatment on differentiation, lipid accumulation, and adipokine release was assessed in isolated preadipocytes differentiated *in vitro*.

Results: Serum FGF21 concentrations were more than two-fold higher in MUHO as compared to MHO subjects (457 ± 378 vs. 211 ± 123 pg/mL; $p < 0.05$). FGF21 treatment of human preadipocytes for the entire differentiation period was modestly lipogenic ($+15\%$; $p < 0.05$), reduced the expression of key adipogenic transcription factors (*PPARG* and *CEBPA*, -15% and -40%, respectively; $p < 0.01$ both), reduced adiponectin expression (-20%; $p < 0.05$), markedly reduced adiponectin release (-60%; $p < 0.01$), and substantially increased leptin ($+60\%$; $p < 0.01$) and interleukin-6 ($+50\%$; $p < 0.001$) release.

Conclusions: The hepatokine FGF21 exerts weak lipogenic and anti-adipogenic actions and marked adiponectin-suppressive and leptin and interleukin-6 release-promoting effects in human differentiating preadipocytes. Together with the higher serum concentrations in MUHO subjects, our findings reveal FGF21 as a circulating factor promoting the development of metabolically unhealthy adipocytes.

Keywords FGF21; Hepatokine; Adiponectin; Adipokine; Secretome; Type-2 diabetes

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Abbreviations: AMPK, AMP-activated protein kinase; BMI, body mass index; C/EBP-α, CCAAT/enhancer-binding protein-α; CIDEA, cell death-inducing DNA fragmentation factor-like effector a; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; hasc, human abdominal subcutaneous; IL-6, interleukin-6; MHO, metabolically healthy obesity; MUHO, metabolically unhealthy obesity; qPCR, quantitative polymerase chain reaction; PGC-1α, PPAR-γ coactivator-1α; PPAR-γ, peroxisome proliferator-activated receptor-γ; rh, recombinant human; UCP-1, uncoupling protein-1

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1. INTRODUCTION

Fibroblast growth factor (FGF) 21, FGF19 (murine homologue: FGF15), and FGF23 constitute a subfamily of FGFs with hormone-like rather than growth factor functions [1].

In mice, FGF21 is produced by liver, pancreas, skeletal muscle, brown and white adipose tissue, and major stimuli for FGF21 release are fasting and ketogenic diets, cold exposure, and exercise [2]. In murine models of obesity and diabetes, and less evident or even absent in normal mice, pharmacological administration or transgenic overexpression of FGF21 exerts beneficial metabolic effects, i.e., reduction of body weight and liver fat, improvement of insulin sensitivity, hyperlipidaemia, and hyperglycaemia, and this is most probably due to the induction of thermogenesis and stimulation of energy expenditure [3–5].

In humans, FGF21's role in physiology and metabolic disease is more complicated and far from being understood: circulating FGF21 is mainly produced by the liver and is independent of nutritional status [1]; furthermore, its plasma concentrations are elevated in abdominal obesity, fatty liver disease, hyperlipidaemia, insulin resistance, type-2 diabetes, metabolic syndrome, and coronary artery disease [6–10]. Since this is in sharp contrast to data obtained in mice and the existence of a postulated FGF21-resistant state in humans is still insufficiently proven, there is a clear need for further studies to better understand FGF21's role in humans.

We previously succeeded in describing metabolically healthy and unhealthy obesity (MHO and MUHO) with hepatic fat content and inflammation representing major determinants of these states [11,12]. In a very recent metabolomics study, we have shown that human abdominal subcutaneous (hasc) preadipocytes differentiated *in vitro* to adipocytes reveal intra- and extracellular metabolite signatures that also allow discriminating MUHO from MHO [13]. Thus, these cells reveal stable, mitotically inherited properties that may reflect and/or contribute to these states.

In this study, we asked whether serum FGF21 concentrations differ between MHO and MUHO subjects. This would open the possibility that FGF21 acts as a cross-talker between the liver and adipose tissue affecting (pre)adipocyte properties in a way that could explain metabolic features of MUHO versus MHO. Therefore, we studied the effects of chronic FGF21 treatment on typical adipocyte functions, such as adipose conversion, lipid storage, and adipokine secretion, in hasc preadipocytes differentiated *in vitro*.

2. MATERIAL AND METHODS

2.1. Study participants

The 20 hasc adipose tissue donors reported earlier [13] were morbidly obese (body mass index (BMI) > 40 kg/m^2 all), well matched for gender, age, and body fat content (as measured by bioelectrical impedance), but discordant for their whole-body insulin sensitivity (as estimated by a five-point oral glucose tolerance test-derived insulin sensitivity index calculated as 10,000/[fasting glucose {mmol/L} x fasting insulin {pmol/L} x mean glucose {mmol/L} x mean insulin {pmol/L}]$^{1/2}$). According to their insulin sensitivity, they were stratified into ten MHO (insulin-sensitive; insulin sensitivity index $\geq 5.0 \times 10^{19}$ L^2 x mol^{-2}) and ten MUHO (insulin-resistant; insulin sensitivity index $\leq 3.5 \times 10^{19}$ L^2 x mol^{-2}) subjects, and the clinical characteristics of the two groups were reported recently [13]. The study adhered to The Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed written consent to the study, and the study protocol was approved by the local ethics board.

2.2. Preadipocyte culture, differentiation, and treatment

Preadipocytes were isolated from the 20 hasc adipose tissue explants as described earlier [14] and expanded in α-MEM/Ham's nutrient mixture F12 (1:1) containing 20% fetal calf serum, 1% chicken embryo extract (Sera Laboratories, Haywards Heath, UK), 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 0.5 μg/mL fungizone, and 2 mmol/L glutamine. Second-pass cells were used for experiments. At confluence, adipose conversion was induced by shifting the cells into DMEM/Ham's nutrient mixture F12 (1:1), 5% fetal calf serum, 17 μmol/L pantothenate, 1 μmol/L biotin, 2 μg/mL apo-transferrin, 1 μmol/L human insulin, 1 μmol/L dexamethasone, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 0.5 μg/mL fungizone, and 2 mmol/L glutamine (differentiation medium) supplemented with 0.5 mmol/L 3-isobutyl-1-methyl-xanthine, 2 nmol/L triiodo-thyronine, and 50 μmol/L indomethacin for seven days. Thereafter, the cells were allowed to terminally differentiate for another 11 days in differentiation medium alone. Differentiating preadipocytes were left untreated or were chronically treated for the whole 18-day period with 50 ng/mL recombinant human (rh)FGF21 (PeproTech, Rocky Hill, NJ, USA). Culture media and supplements were obtained from Lonza (Basel, Switzerland) and Biochrom (Berlin, Germany).

2.3. Staining of intracellular neutral lipids

Differentiation of preadipocytes to adipocytes was qualitatively monitored by microscopy after Oil Red O staining according to Greenberger et al. [15]. For quantification of intracellular neutral lipids, Oil Red O was extracted with isopropanol and photometrically measured at 500 nm as described earlier [16].

2.4. RNA isolation and real-time quantitative polymerase chain reaction (qPCR)

(Pre)adipocytes were harvested with Qiazol lysis reagent (Qiagen, Hilden, Germany). Total RNA was isolated with miRNeasy columns (Qiagen), treated with RNase-free DNase I, and reverse transcribed into cDNA using Qiagen's QuantiTect reverse transcription kit. Real-time qPCR was performed in technical duplicates with QuantiTect primer assays on a LightCycler™ 480 II (Roche Diagnostics, Mannheim, Germany). All mRNA data were normalized to the housekeeping gene *RPS13* using the ΔCt method.

2.5. Enzyme-linked immunosorbent assays

FGF21 in serum and adiponectin, leptin, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in the conditioned cell culture media were measured with immunoassays from R&D Systems (Wiesbaden-Nordenstadt, Germany) according to the manufacturers' instructions.

2.6. Protein extraction and immunoblotting

Cellular protein was extracted using RIPA buffer (50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mmol/L phenyl-methyl-sulfonyl-fluoride, 1 mmol/L dithiothreitol) containing a protease and phosphatase inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). Cell lysates were sonicated and cleared by centrifugation. Protein concentration was determined with the RC DC kit from Bio-Rad (Hercules, CA, USA). Equal amounts of protein (30 μg/lane) were loaded onto 10-% sodium dodecyl sulfate polyacrylamide gels. After electrophoresis, proteins were transferred to polyvinylidene fluoride membranes and incubated with antibodies against phospho-threonine 172 of the catalytic α-subunit of AMP-activated protein kinase (AMPK), AMPKα protein, phosphorylated extracellular signal-regulated kinases (ERK) 1 and 2, ERK1 and 2 protein, or glyceraldehyde 3-phosphate dehydrogenase

