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Serum concentrations of fibroblast growth factor 21 are elevated in patients with congenital or acquired lipodystrophy

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ABSTRACT

Objective: Patients with lipodystrophy (LD) suffer from loss of subcutaneous adipose tissue accompanied by dysregulation of several adipocyte-secreted factors. However, regulation of adipocyte-expressed fibroblast growth factor (FGF) 21 which acts in an insulin-mimetic, lipid-lowering, and anti-atherogenic manner has not been investigated in non-human immunodeficiency virus (HIV) LD.
Material and methods: Circulating serum FGF21 levels were quantified in 37 patients with non-HIV LD and 37 controls matched for age, gender, and body mass index. Moreover, FGF21 plasma levels and mRNA expression were measured in LD mice and control animals. Additionally, serum FGF21 levels were assessed in 10 LD patients before and during metreleptin therapy.
Results: Median FGF21 serum concentrations were significantly higher in LD patients (381.2 ng/l) as compared to the control group (231.2 ng/l; $p = 0.023$). There was an independent and positive association between circulating FGF21 and serum triglycerides (TG), as well as fibrate treatment, in multiple linear regression analysis. LD mice showed significantly upregulated FGF21 plasma levels (4.5-fold), as well as mRNA expression in various adipose tissue depots and liver as compared to controls ($p < 0.05$). Metreleptin treatment did not significantly alter circulating FGF21 levels in human subjects.
Conclusions: Serum concentrations of FGF21 are elevated in patients with non-HIV LD with adipose tissue and liver being potential sources of increased production. TG and fibrate treatment are independent positive predictors of circulating FGF21.

1. Introduction

Adipose tissue plays a crucial role in regulating overall energy homeostasis besides energy storage in the form of lipids [1]. Both too much and too little adipose tissue contributes to severe metabolic abnormalities. Thus, obesity, which is characterized by hyperplasia and hypertrophy of adipocytes, is linked to insulin resistance, diabetes mellitus, hyperlipidemia, hepatic steatosis, and premature coronary heart disease [2]. Interestingly, the same complications can be found in lipodystrophy (LD) which is a heterogeneous disease group of congenital or acquired origin and characterized by the selective reduction of subcutaneous adipose tissue (SAT) [3]. Similar to obesity, LD patients suffer from consecutive risks of metabolic derangement which include pancreatitis, liver cirrhosis, and myocardial infarction [3].

Dysregulation of several adipocyte-derived proteins - so called adipokines - has been described as a pathogenetic factor of both obesity and LD. Thus, both disease states are characterized by downregulation of the insulin-sensitizing adipokine adiponectin [4,5]. Furthermore, resistance to leptin, an appetite-suppressive adipokine, develops in obesity whereas leptin is decreased due to adipose tissue loss in LD patients [4,5]. Both mechanisms contribute to hyperphagia which is present in both obesity and LD [2,6]. Interestingly, leptin administration in LD resulted in significant improvements in glucose homeostasis and lipid metabolism in affected humans [7] whereas adiponectin supplementation has only been tested in rodents so far [8].

Abbreviations: BAT, brown adipose tissue; BMI, body mass index; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; FFA, free fatty acids; FG, fasting glucose; FGF, fibroblast growth factor; FI, fasting insulin; HbA1c, glycosylated hemoglobin A1c; HDL, high density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment of insulin resistance; LD, lipodystrophy; LDL, low density lipoprotein; SAT, subcutaneous adipose tissue; TG, triglycerides; VAT, visceral adipose tissue; WHR, waist-to-hip ratio.

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Fibroblast growth factor (FGF) 21 is another adipokine besides leptin and adiponectin that has recently been implied in the pathogenesis of obesity complications. FGF21 was cloned in 2000 and belongs to the FGF family which currently consists of 22 members [9]. Besides adipose tissue, FGF21 is produced by skeletal muscle, pancreatic β cells, and liver [9]. It acts through cell-surface receptors, i.e. FGF receptor isotypes 1–4, and activation of FGF receptors depends on the co-receptor β-Klotho [10]. FGF21 has potent insulin-mimetic and lipid-lowering effects in both rodents and monkeys [11,12]. Furthermore, anti-atherogenic properties of the adipokine have been described recently [13]. Despite these beneficial metabolic and vascular effects, our group and others have consistently shown upregulation of circulating FGF21 in various vascular and metabolic disease states including obesity, non-alcoholic fatty liver disease, insulin resistance, type 2 diabetes, dyslipidemia, hypertension, polycystic ovarian syndrome, renal impairment, and human immunodeficiency virus (HIV)-associated LD [14–22].

In contrast to obesity-associated disease states and HIV-associated LD, the adipokine FGF21 has not been investigated in congenital or acquired non-HIV LD until now. Therefore, we measured FGF21 serum concentrations in 37 patients with congenital or acquired non-HIV LD as compared to 37 controls matched for age, gender, and body mass index (BMI). Moreover, FGF21 serum concentrations were correlated to parameters of inflammation, as well as glucose homeostasis and lipid metabolism. To define the sources of FGF21, we analyzed FGF21 mRNA expression in liver, muscle and adipose tissue of LD mice as compared to control animals. Furthermore, we measured FGF21 serum concentration in 10 LD patients before and after 6 months of metreleptin therapy. We hypothesized that FGF21 is elevated in patients with non-HIV LD and that treatment with metreleptin reverses this upregulation.

2. Material and methods

2.1. Patients and control group

We included 37 patients with non-HIV LD in our study as recently described [23]. In brief, medical history of LD was obtained by using a standardized questionnaire. Moreover, LD patients underwent comprehensive physical examination. The control group consisted of 37 subjects without LD and was matched for age, gender, and BMI. Patients and controls were recruited from the outpatient clinic of the Endocrine Department, University of Leipzig.

2.2. Metreleptin treatment study

Ten of the 37 patients with LD met inclusion criteria for metreleptin therapy. Inclusion and exclusion criteria, as well as metreleptin dosing, has been described in detail recently [23]. Patients underwent clinical examination and laboratory investigation before and at 1 week, 1 month, 3 months, 6 months, and 1 year of metreleptin treatment. Since metreleptin's effect on glucose homeostasis and lipid metabolism was most pronounced after 6 months treatment, we chose this time point for investigating the influence of metreleptin treatment on FGF21 serum concentration in LD.

2.3. Patient characterization and laboratory assessment

BMI was calculated as weight in kilogram divided by squared height in meters. After assessment of waist and hip circumferences, waist-to-hip ratio (WHR) was evaluated. Age of the whole study population ranged from 16 to 75 years. BMI was between 17.4 and 46.1 kg/m^2. Calculation of homeostasis model assessment of insulin resistance (HOMA-IR) was performed as previously described [24]. The CKD-EPI equation [25] was used for calculation of the renal function marker estimated glomerular filtration rate (eGFR) after assessment of serum creatinine. Approval by the local Ethics Committee and written informed consent of all subjects before taking part in the study was obtained.

Blood was taken in the fasted state after at least 8 h fasting. Commercially available enzyme-linked immunosorbent assays were used for determining serum concentrations of adiponectin (Mediagnost, Reutlingen, Germany), FGF21 (Biovendor, Modrice, Czech Republic), and leptin (Mediagnost, Reutlingen, Germany) according to the manufacturerś instructions. Parameters of glucose homeostasis, i.e. fasting insulin (FI), fasting glucose (FG), as well as glycosylated hemoglobin A1c (HbA1c), of renal function, i.e. creatinine, of lipid metabolism, i.e. free fatty acids (FFA), triglycerides (TG), total, high density lipoprotein (HDL), as well as low density lipoprotein (LDL) cholesterol, and C reactive protein (CRP) were determined in a certified laboratory by standard laboratory methods.

2.4. Animal experiments and characterization

All animal experiments were conducted in the Medical Experimental Center, University of Leipzig. Tg(aP2-SREBF1c)9884Reh/0 mice (Jackson Laboratory, Bar Harbor, ME) were used as an animal model of lipodystrophy and C57BL/6NTac mice (F15; Jackson Laboratory, Bar Harbor, ME) served as controls. Both mouse models were on a low-density lipoprotein receptor knockout background, housed on a 12:12 h (6 AM/6 PM) light/dark cycle with 21 ± 1 °C room temperature, and they were maintained on a cholesterol-enriched semisynthetic Clinton/Cybulsky diet (V1534, Sniff, Soest, Germany). After euthanasia at 20 weeks of age, insulin-sensitive tissues including liver, muscle, as well as brown (BAT), visceral (VAT), and subcutaneous (SAT) adipose tissue samples, were removed and immediately frozen in liquid nitrogen. The local ethics committee approved all animal experiments (approval no. TVV37/12).

Commercially available enzyme-linked immunosorbent assays were used for quantification of insulin (Mercodia, Uppsala, Sweden), adiponectin (Mediagnost, Reutlingen, Germany), leptin (Crystalchem, Zaandam, Netherlands), and FGF21 (Biovendor, Brno, Czech Republic) according to the instructions of the manufacturer. Lipids were extracted from mouse hepatic tissue according to Ref. [26]. Liver cholesterol and TG content were assessed by a colorimetric assay kit (Wako, Neuss, Germany).

Quantitative real-time RT-PCR for determination of FGF21 mRNA synthesis relative to *36B4* was performed with a protocol described in [27]. Mouse primer sets were as follows: FGF21: 5′-AGATCAGGGAGGATGGAACA-3′, and 5′-TCAAAGTGAGGCGATC CATA-3′; *36B4*: 5′-AAGCGCGTCCTGGCATTGTCT-3′, and 5′-CCGCAG GGGCAGCAGTGGT-3′ (forward and reverse, respectively).

2.5. Statistical analysis

All statistical analyses were performed with SPSS Statistics Version 20.0 (IBM, Armonk, NY). Differences between LD patients and controls were determined by Mann–Whitney-*U* test and differences between LD mice and controls were determined by unpaired Student's *t*-test. For correlations, the Spearmańs rank correlation method was used. Shapiro–Wilk W test was performed to test for normal Gaussian distribution of parameters. Multivariate linear regression analysis was performed for identification of independent relationships after logarithmic transformation of non-normally distributed parameters. Wilcoxon signed rank test was used to assess differences in the course of metreleptin therapy. $p < 0.05$ was considered as statistically significant.