Brief Communication

MOLECULAR METABOLISM

FGF21 resistance is not mediated by downregulation of beta-klotho expression in white adipose tissue

Kathleen R. Markan [1,2], Meghan C. Naber [1,2], Sarah M. Small [1,2], Lila Peltekian [1,2], Rachel L. Kessler [1,2], Matthew J. Potthoff [1,2,*]

ABSTRACT

Objective: Fibroblast growth factor 21 (FGF21) is an endocrine hormone that regulates metabolic homeostasis. Previous work has suggested that impairment of FGF21 signaling in adipose tissue may occur through downregulation of the obligate FGF21 co-receptor, β-klotho, which leads to "FGF21 resistance" during the onset of diet-induced obesity. Here, we sought to determine whether maintenance of β-klotho expression in adipose tissue prevents FGF21 resistance and whether other mechanisms also contribute to FGF21 resistance in vivo.

Methods: We generated adipose-specific β-klotho transgenic mice to determine whether maintenance of β-klotho expression in adipose tissue prevents FGF21 resistance in vivo.

Results: β-klotho protein levels are markedly decreased in white adipose tissue, but not liver or brown adipose tissue, during diet-induced obesity. Maintenance of β-klotho protein expression in adipose tissue does not alleviate impaired FGF21 signaling in white adipose or increase FGF21 sensitivity in vivo.

Conclusions: In white adipose tissue, downregulation of β-klotho expression is not the major mechanism contributing to impaired FGF21 signaling in white adipose tissue.

Keywords FGF21; Resistance; Betaklotho; Obesity; Adipose

1. INTRODUCTION

Fibroblast growth factor 21 (FGF21) is a member of the FGF19 subfamily of fibroblast growth factors (FGFs) and is an important regulator of nutrient and energy homeostasis. Pharmacological administration of FGF21 to obese and diabetic animal models has significant therapeutic effects including improving insulin sensitivity and decreasing body weight (reviewed in [1]). In humans, FGF21 analogs also reduce body weight and improve metabolic profiles in obese and diabetic patients [2,3]. In contrast to its pharmacological actions, circulating levels of FGF21 are regulated physiologically by various nutritional cues and cellular stress. For example, plasma FGF21 levels are elevated in both rodents and humans in response to high carbohydrate levels [4–6], protein restriction [7,8], fasting [9–14], and obesity and insulin resistance [14–22].

FGF21 signals to target cells through a receptor complex consisting of the FGF receptor (FGFR), FGFR1c, and a co-receptor termed β-klotho [23,24]. Although FGFR1c is ubiquitously expressed, β-klotho expression is restricted to specific metabolic tissues including adipose tissues, liver, pancreas, and specific regions of the brain [25,26] and confers specificity for FGF21 signaling. Activation of the FGF21 receptor complex initially activates phosphorylation of FGF receptor substrate 2α (FRS2α) and the MAPK signaling cascade resulting in ERK1/2 phosphorylation [23,24,27]. Multiple studies have implicated adipose tissues in mediating the physiological and pharmacological effects of FGF21 on metabolism. A role for FGF21 in regulating metabolism was originally identified in a screen for factors that induce glucose uptake in white adipocytes [28]. Subsequent studies found that FGF21 markedly improves insulin sensitivity in rodents both acutely and following chronic administration [29–31], and adipose tissues were implicated in mediating these metabolic effects. For example, elimination of either FGFR1 [32] or β-klotho [33] from adipose tissues impairs the acute insulin sensitizing effects of FGF21. In addition, the metabolic effects of FGF21 [34] and FGFR1-agonists (activating antibodies) [35] in lowering triglyceride and glucose levels are markedly impaired in lipodystrophic mice. Transplantation of white adipose tissue from wild-type mice into lipodystrophic mice subsequently restored FGF21 responsiveness [34]. Finally, increases in adipose tissue 'browning' [30,36] and brown adipose tissue (BAT) glucose and triglyceride uptake [33,37,38] have been observed in response to FGF21 administration.

Pharmacological administration of FGF21 has pronounced metabolic effects even though circulating endogenous levels of FGF21 are

[1]Department of Pharmacology, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA [2]Fraternal Order of Eagles Diabetes Research Center, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA

*Corresponding author. University of Iowa Carver College of Medicine, 169 Newton Road 3322 PBDB, Iowa City, IA 52242, USA. Fax: +1 319 335 8930. E-mail: matthew-potthoff@uiowa.edu (M.J. Potthoff).

Received February 8, 2017 • Revision received March 17, 2017 • Accepted March 22, 2017 • Available online 27 March 2017

http://dx.doi.org/10.1016/j.molmet.2017.03.009

markedly elevated in obese rodents [15–19], monkeys [39], and humans [20–22]. The elevated FGF21 levels during obesity have led to the postulation that obesity is a "FGF21-resistant state" [17]. However, the concept of FGF21 resistance is controversial as a subsequent study demonstrated that FGF21 sensitivity is not impaired in DIO WT and *ob/ob* mice compared to lean controls [18]. Both studies identified a reduction in β-klotho expression in adipose tissue of obese rodents [17,18], an effect also observed in white adipose tissue of obese humans [40]. Thus, downregulation of β-klotho expression in adipose tissue could impair endogenous FGF21 action and contribute to FGF21 resistance. Here we show that while β-klotho expression in white adipose tissue is markedly reduced during diet-induced obesity, maintenance of β-klotho expression in adipose tissue, through the generation of adipose-specific β-klotho transgenic mice, does not increase FGF21 sensitivity or significantly improve metabolic parameters during obesity. We propose that selective downregulation of β-klotho expression in white adipose tissue may function as a beneficial adaptation, rather than a pathological impairment, in FGF21 action to regulate energy homeostasis.

2. MATERIALS AND METHODS

2.1. Animals

Adiponectin-cre transgenic mice have been reported previously [41]. To generate inducible KLB transgenic (TG) mice, a full-length mouse β-klotho cDNA clone was purchased (Thermo Scientific, Inc.) and cloned into the MSP universal transgenic construct [42] (kindly provided by Dr. Curt Sigmund, Univ. of Iowa). The β-klotho-MSP-Universal transgenic construct was then linearized via SphI and XhoI restriction sites and sent to the University of Iowa Genome Editing Core for injection and founder generation. To achieve adipose-specific β-klotho expression, different lines of inducible KLB TG mice were crossed with Adiponectin-Cre mice (JAX Labs) to generate KLB AdipoTG mice. Three lines of KLB AdipoTG mice were generated and screened for transgene expression levels. Line 2 was selected and then backcrossed four generations to C57Bl/6 mice before crossing to Adiponectin-cre mice for the reported studies. All mice used in these studies were male mice that were maintained on either standard chow (2920X; Envigo) or 60% high fat diet (HFD; Research Diets, D12492i) for the indicated time. To induce obesity, mice were placed on HFD starting at 4–6 weeks of age. All experiments were approved by the University of Iowa IACUC.

2.2. Glucose and insulin tolerance tests

Body composition was measured using a rodent-sized NMR machine (Bruker Minispec LF50) prior to glucose tolerance tests (GTTs) for determination of lean mass. Following an overnight (16 h) fast, time 0 blood was collected via tail bleed followed by an intraperitoneal (i.p.) injection of glucose (2 g glucose/kg lean body weight). Tail blood was then collected into 300K2E microvette EDTA tubes (Sarstedt) over the course of 120 min and then centrifuged at 3000 rpm for 30 min at 4 °C for the separation of plasma. Plasma glucose was then measured using the Autokit Glucose Reagent (WAKO) per manufacturer's instructions. For insulin tolerance tests (ITTs), mice were fasted 5–6 h. Time 0 blood was obtained via tail bleed followed by an intraperitoneal (i.p.) injection of insulin (at the indicated dose). Tail blood was then collected and plasma glucose analyzed as described above.

2.3. In vivo FGF21 signaling

Food was removed 2 h prior to the start of these experiments. Each mouse received an intraperitoneal injection of vehicle or recombinant human FGF21 (0.1 mg/kg total body weight). Fifteen minutes post-injection, mice were euthanized by decapitation for tissue collection. All tissues were snap frozen in liquid nitrogen and then stored at −80 °C until analysis.

2.4. Gene expression and protein analysis

Gene expression analysis was performed as described [4]. QPCR primer sequences are as follows: *Klb*: 5′-GATGAAGAATTTCCTA AACCAGGTT-3′, 5-AACCAAACACGCGGATTTC-3′; *Fgfr1c*, 5′-GCCAGA-CAACTTGCCGTATG-3′, 5′-ATTTCCTTGTCGGTGGTATTAACT-3′; *Tdtomato*, 5′-CGAGGAGGTCATCAAAGAGTTC-3′, 5′-GGGAAGGACAGCTTCT TGTAAT-3′; *U36b4*, 5′-CGTCCTCGTTGGAGTGACA-3′, 5′-CGGTGCGTC AGGGATTG-3′; *Fasn*: 5′-GCTGCGGAAACTTCAGGAAAT-3′, 5′-AGA-GACGTGTCACTCCTGGACTT-3′; *Fgf21*: 5′-CCTCTAGGTTTCTTTGCCAA-CAG-3′, 5′-AAGCTGCAGGCCTCAGGAT-3′; *Pck1*: 5′-CACCATCACCTCC TGGAAGA-3′, 5′-GGGTGCAGAATCTCGAGTTG-3′; *Hmgcr*: 5′-CTTGTGG AATGCCTTGTGATTG-3′, 5′-AGCCGAAGCAGCACATGAT-3′; *Srebf1*: 5′-GGAGCCATGGATTGCACATT-3′, 5′-GGCCCGGGAAGTCACTGT-3′; *Scd1*: 5′-TGCCCCTGCGGATCTT-3′, 5′-GCCCATTCGTACACGTCATT-3′.

For protein analysis, tissues were homogenized on ice in lysis buffer containing 10 mM Tris–HCl, pH 7.4, 5 mM EDTA, 5 mM EGTA, 150 mM NaCl, 10% glycerol, 1% NP-40, 0.5% Triton X-100, and protease inhibitors. Samples were centrifuged for 5 min at 0.5×*g* at 4 °C and infranatant collected. An appropriate volume of 6X Laemmli buffer was added and all samples incubated at 100 °C for 10 min and then briefly placed on ice. Sample protein concentration was determined by Bradford assay and then equal quantity of sample resolved by SDS-PAGE. Proteins were transferred to a PVDF membrane before being probed with the specified antibodies. Antibody information: β-klotho (R&D Systems, #AF2619), β-actin (Sigma, #A5316), IR-β (Santa Cruz, #sc-711), phospho-FRS2α (Cell Signaling, #3864), phospho-ERK1/2 (Cell Signaling, #9101), total ERK1/2 (Cell Signaling, #9102), phospho-MEK (Cell Signaling, #9121), and total MEK (Cell Signaling, #9122).

For adipose tissue fractionation data, white adipose tissue was harvested from the indicated mice and processed using the Minute Adipose Tissue Fractionation Kit (Invent Biotechnologies, #AF-023) per the manufacturer's instructions.

2.5. Plasma and tissue analysis

Mouse FGF21 (Biovendor) and mouse insulin (Crystal Chem) were measured using commercially available ELISAs. Blood was collected into 300K2E microvettes (Sarstedt) and spun at 3,000 rpm for 30 min 4 °C to separate plasma. Plasma glucose levels were measured using the glucose autokit (Wako Chemicals). Plasma triglycerides and cholesterol were measured using colorimetric assays (Infinity™, Thermo Scientific). All measurements were performed according to the manufacturer's instructions.

Hepatic triglycerides were quantified via Folch extraction. Mouse livers were collected, snap-frozen, pulverized, and stored at −80 °C prior to analysis. Pulverized liver tissue was thoroughly homogenized for 30 s per sample in 4 ml of a 2:1 v/v chloroform/methanol mix then allowed to equilibrate at room temperature for 30 min. After adding 1 ml of 50 mM NaCl to each sample, the samples were vortexed for 15 s and centrifuged for 10 min at 1,000×*g* at room temperature. The organic phase was isolated, and 1 ml of 0.36 M $CaCl_2$/Methanol/H_2O mix (1:1 v/v Methanol/H_2O) was added to the samples, vortexed, and centrifuged as before. The organic layer was isolated and placed into 5 ml glass volumetric flasks. The flasks were then volumed up to the 5 ml mark with fresh chloroform, capped, and left overnight at room temperature. The following day, any traces of water were carefully aspirated from the samples. In new test tubes, 10 μl of a 1:1 v/v Triton-X

