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Fibroblast growth factor 21, fibroblast growth factor receptor 1, and β-Klotho expression in bovine growth hormone transgenic and growth hormone receptor knockout mice

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ABSTRACT

Objective: Although growth hormone (GH) and fibroblast growth factor 21 (FGF21) have a reported relationship, FGF21 and its receptor, fibroblast growth factor receptor 1 (FGFR1) and cofactor β-Klotho (KLB), have not been analyzed in chronic states of altered GH action. The objective of this study was to quantify circulating FGF21 and tissue specific expression of *Fgf21*, *Fgfr1*, and *Klb* in mice with modified GH action. Based on previous studies, we hypothesized that bovine GH transgenic (bGH) mice will be FGF21 resistant and GH receptor knockout (GHR−/−) mice will have normal FGF21 action.

Design: Seven-month-old male bGH mice ($n = 9$) and wild type (WT) controls ($n = 10$), and GHR−/− mice ($n = 8$) and WT controls ($n = 8$) were used for all measurements. Body composition was determined before dissection, and tissue weights were measured at the time of dissection. Serum FGF21 levels were evaluated by ELISA. Expression of *Fgf21*, *Fgfr1*, and *Klb* mRNA in white adipose tissue (AT), brown AT, and liver were evaluated by reverse transcription quantitative PCR.

Results: As expected, bGH mice had increased body weight ($p = 3.70E^{-8}$) but decreased percent fat mass ($p = 4.87E^{-4}$). Likewise, GHR−/− mice had decreased body weight ($p = 1.78E^{-10}$) but increased percent fat mass ($p = 1.52E^{-9}$), due to increased size of the subcutaneous AT depot when normalized to body weight ($p = 1.60E^{-10}$). Serum FGF21 levels were significantly elevated in bGH mice ($p = 0.041$) and unchanged in GHR−/− mice ($p = 0.88$). Expression of *Fgf21*, *Fgfr1*, and *Klb* mRNA in white AT and liver were downregulated or unchanged in both bGH and GHR−/− mice. The only exception was *Fgf21* expression in brown AT of GHR−/−, which trended toward increased expression ($p = 0.075$).

Conclusions: In accordance with our hypothesis, we provide evidence that circulating FGF21 is increased in bGH animals, but remains unchanged in GHR−/− mice. Downregulation or no change in *Fgf21*, *Fgfr1*, and *Klb* expression are seen in white AT, brown AT, and liver of bGH and GHR−/− mice when compared to their respective controls, except for an increase in brown AT *Fgf21* expression in GHR−/− mice, which could suggest a possible link to increased thermogenic potential in these mice. Overall, these results suggest possible modulation of FGF21 by GH resulting in FGF21 resistance or changes in FGF21 levels due to GH induced changes in liver size or kidney function.

1. Introduction

Fibroblast growth factor 21 (FGF21) has been implicated as a possible therapeutic agent for the treatment of obesity and type 2 diabetes mellitus (T2DM) [2,10,28,35,59]. As a regulator of glucose and lipid metabolism, FGF21 greatly improves metabolic profiles and can even result in reversal of obesity [2,10,28,35,59]. Under normal physiologic conditions, FGF21 has a role in increasing energy expenditure without affecting food intake, decreasing free fatty acid oxidation, and decreasing inflammation [27]. Interestingly, a study using FGF21 transgenic mice reports that along with an improved metabolic profile, the mice have an increased lifespan compared to their littermate controls [59]. Negative effects of FGF21 include decreased fertility and bone loss, although

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these effects have only been reported in FGF21 transgenic mice or mice treated with very high levels of FGF21 [59]. FGF21 is produced mainly in the liver but also in white adipose tissue (AT), brown AT, pancreas, skeletal muscle, and thymus [39]. These sites of production are also the main tissues where FGF21 induced signaling is reported to occur [27]. FGF21 requires a transmembrane cofactor, β-Klotho (KLB), to bind to a transmembrane FGF receptor (FGFR) (FGFR1 in AT and liver) [34,36,53] resulting in induction of intracellular signaling [1,3,34]. The importance of white AT in FGF21 action has been recently shown in several studies, as mice with lipodystrophy [54], deletion of FGFR1 in AT [3], and deletion of KLB in AT [17] are all refractory to the benefits of FGF21 treatment. Collectively, these studies suggest that FGF21 action on AT is necessary to see the whole-body benefits of FGF21 treatment. Levels of the receptor components are also important to consider because there is some evidence that animals and humans with obesity and T2DM are FGF21 resistant [15,25,29,45,51,58], and that FGFR1 and KLB are downregulated and thus the limiting factors for FGF21 function [4,15,25,30,51]. These results all indicate the importance of learning more about AT and liver expression of FGF21, FGFR1, and KLB in disease states in order to evaluate the possible therapeutic potential of FGF21 in obesity and T2DM.

Growth hormone (GH) has profound effects on AT and decreases adiposity by promoting lipolysis and reducing lipogenesis [8]. GH is also diabetogenic with excess GH causing insulin resistance [8]. As such, mice with excess GH action through expression of a bovine GH transgene (bGH) have increased body weight but decreased fat mass [6,47]. They are hyperinsulinemic when young [16,33] and have a decreased lifespan [5]. On the other hand, mice with no GH action due to disruption of the GH receptor gene (GHR −/− mice) have a decreased body weight and increased percent fat mass [6,7,12], but are insulin sensitive and have been shown to have increased lifespan [12]. Together, these two mouse lines with extremes of GH action reveal a link between GH and longevity [5,12].

There is evidence that GH and FGF21 levels may be inter-related. That is, injection of GH has been shown to increase FGF21 levels in circulation in mice [11], cattle [57], and GH deficient patients [41] and increase both mRNA [11,57] and protein [11] levels in liver. This increase has been shown to be lipolysis dependent, with inhibitors of lipolysis blocking the GH-induced increase in FGF21 [11,43]. However, GH deficient patients have been characterized as having normal FGF21 levels, indicating that while GH may induce FGF21 expression, it is not critical for maintaining basal FGF21 levels [41]. FGF21 has also been shown to result in inhibited GH activity. This has been shown through decreased insulin-like growth factor 1 (IGF-1) expression in three circumstances: (1) in FGF21 overexpressing transgenic mice [32,59], (2) in C57Bl/6 mice following FGF21 injection [32,46], and (3) in clinical cases, such as anorexia nervosa, associated with increased circulating FGF21 [24]. Decreased phosphorylated signal transducer and activator of transcription 5 (P-STAT5) and increased suppressor of cytokine signaling 2 (SOCS2) have also been reported in FGF21 overexpressing transgenic mice and after FGF21 injection, further supporting downregulation of GH action by FGF21 [32]. In FGF21 transgenic mice, this regulation has been linked to decreased body size despite increased circulating GH levels, supporting a case for GH resistance in these mice [32,59]. The decreased GH action and IGF-1 levels in FGF21 overexpressing transgenic mice could be responsible for the improved metabolic profiles and increased lifespan observed in these mice [32,59]. The strong evidence that GH and FGF21 are inter-related makes the GH status of the organism important to consider when evaluating FGF21 as a therapeutic target.

Despite the evidence for an important relationship between GH and FGF21, no reports to our knowledge have quantified FGF21, or possibly more importantly FGFR1 and KLB, levels in mice with altered GH action. Thus, the purpose of this study was to establish levels of FGF21, FGFR1, and KLB in two mouse strains with extremes in GH action, bGH and GHR −/− mice. Due to evidence that hyperinsulinemia leads to FGF21 resistance [15,25,29,45,51,58] and the role of GH in increasing FGF21 expression [11,41,57], we hypothesized that bGH mice will be FGF21 resistant, with increased circulating FGF21 and downregulation of tissue *Fgfr1* and *Klb* expression. As GH deficient individuals have been reported to have normal serum FGF21 [41], we expected GHR −/− mice to have normal FGF21 action, indicated by normal circulating FGF21 and tissue expression of *Fgf21*, *Fgfr1*, and *Klb*.

2. Materials and methods

2.1. Animals

Two genetically modified mouse lines were used in this study: bGH and GHR −/− mice. Both mouse lines have been previously described [6,12]. The mouse strains were produced either on a pure C57BL/6 J background or backcrossed more than ten generations into a C57BL/6 J background. Animals used include 9 bGH and 10 littermate controls, and 8 GHR −/− mice and 8 littermate controls. One bGH mouse was excluded from the study due to abnormal body weight and composition measures. All mice used were 7 month old males. These animals were bred and housed up to four animals per cage at the Ohio University animal facility with a 10-h light/14-h dark cycle. The vivarium was temperature-controlled at 23 °C. After weaning, mice had *ad libitum* access to water and standard rodent chow (ProLab RMH 3000, PMI Nutrition International, Inc., St. Louis, MO) throughout the study. Animal protocols for these mice have been approved by the Institutional Animal Care and Use Committee at Ohio University and were in accordance with all standards set forth by federal, state, and local authorities, including the NIH guide for the care and use of laboratory animals.

2.2. Blood and tissue collection

Body weight and body composition measurements were taken the week of tissue collection using a bench top quantitative nuclear magnetic resonance apparatus (Minispec; Bruker Optics). Animals were fasted for 12 h overnight prior to blood and tissue collection. Blood was clotted at room temperature for 30 min and then centrifuged at 7000 rpm for 10 min at 4 °C to separate and isolate serum, which was stored at −80 °C until time of analysis. Tissue samples were dissected after sacrifice by cervical dislocation, flash frozen in liquid nitrogen, and stored at −80 °C until further processing. Tissues collected included subcutaneous AT, epididymal AT, mesenteric AT, retroperitoneal AT, brown AT, and liver.

2.3. Serum FGF21

Serum samples were thawed on ice. The R&D Systems Mouse/Rat FGF-21 Quantikine ELISA Kit (cat. no. MF2100) with an intra-assay coefficient of variation (CV) of 4.3% and inter-assay CV of 6.7% was used to assess levels of FGF21 in serum samples following the manufacturer's protocol.

2.4. Reverse transcription quantitative PCR

Levels of mRNA expression of *Fgf21*, *Klb*, and *Fgfr1* were evaluated in subcutaneous white AT, mesenteric white AT, retroperitoneal white AT, epididymal white AT, brown AT, and liver of all mice described previously. Total RNA was isolated from frozen tissues using TRIzol reagent following the manufacturer's protocol (Life Technologies, Grand Island, NY; Catalog number 15,596–026). The quality and quantity of total RNA was analyzed by visual inspection of the 18S and 28S RNA on an agarose gel and by measuring absorbance at 260 and 280 nm with a ratio of ≥1.8 using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific). RNA was converted to cDNA using Thermo Scientific Maxima First Strand cDNA Synthesis Kit (cat. no. K1671). Two step reverse