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Plasma FGF21 Concentrations, Adipose Fibroblast Growth Factor Receptor-1 and β-Klotho Expression Decrease with Fasting in Northern Elephant Seals

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Abstract

Fibroblast growth factor (FGF)-21 is secreted from the liver, pancreas, and adipose in response to prolonged fasting/starvation to facilitate lipid and glucose metabolism. Northern elephant seals naturally fast for several months, maintaining a relatively elevated metabolic rate to satisfy their energetic requirements. Thus, to better understand the impact of prolonged food deprivation on FGF21-associated changes, we analyzed the expression of FGF21, FGF receptor-1 (FGFR1), β-klotho (KLB; a co-activator of FGFR) in adipose, and plasma FGF21, glucose and 3-hydroxybutyrate in fasted elephant seal pups. Expression of FGFR1 and KLB mRNA decreased 98% and 43%, respectively, with fasting duration. While the 80% decrease in mean adipose FGF21 mRNA expression with fasting did not reach statistical significance, it paralleled the 39% decrease in plasma FGF21 concentrations suggesting that FGF21 is suppressed with fasting in elephant seals. Data demonstrate an atypical response of FGF21 to prolonged fasting in a mammal suggesting that FGF21-mediated mechanisms have evolved differentially in elephant seals. Furthermore, the typical fasting-induced, FGF21-mediated actions such as the inhibition of lipolysis in adipose may not be required in elephant seals as part of a naturally adapted mechanism to support their unique metabolic demands during prolonged fasting.

Keywords

fibroblast growth factor 21; fasting; food deprivation; elephant seal

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1. Introduction

Northern elephant seal pups endure a post-weaning fast for 2–3 months, after which they set out to sea to forage, before returning to the rookery and fasting again for 1–2 months for molting (Riedman, 1990). Throughout fasting, the maintenance of their metabolic rate and energetic requirements are primarily satisfied by fat oxidation in the subcutaneous blubber that is the main site of fat storage (Iverson, 2002; Champagne et al., 2005; Kelso et al. 2012; Crocker et al., 2014).

During prolonged food deprivation in terrestrial mammals, fibroblast growth factor (FGF)-21 is secreted mainly from liver and adipose (Kharitonenkov and Shanafelt, 2009). FGF21 belongs to the group of vertebrate endocrine FGFs, which include FGF15/19, 21, and 23 (Itoh and Ornitz, 2011). FGF21 exerts its metabolic actions in adipose via FGF receptor-1 (FGFR1) in the presence of the co-activator, β-klotho (KLB), which is required for the FGF21-induced activation of target cells (Adams et al., 2013).

Food deprivation shifts fuel utilization from carbohydrates (glucose and glycogen) to lipids. White adipose tissue (WAT) is the main energy storage site in the body, and after hepatic glycogen is depleted, the triacylglycerides (TG) stored in WAT are oxidized to fatty acids and used for energy production. Derived free fatty acids (FFA) stimulate FGF21 secretion mainly from the liver and the WAT (Muise et al., 2008; Chen et al., 2011). FGF21 may initiate adaptive physiological responses during food-deprived condition such as hepatic ketogenesis (Badman et al., 2007), somatic growth suppression, and hibernation-like behavior (Inagaki et al., 2008; Potthoff et al., 2012). Additionally, FGF21 attenuates lipolysis in WAT via a negative feedback loop (Hotta et al., 2009). Collectively, this suite of cellular events contributes to survival during starvation or protracted periods of food deprivation; however, data on the effects of prolonged fasting on FGF21 in a naturally adapted mammal are lacking.

If FGF21 were to contribute to metabolism during prolonged fasting in elephant seals, FGF21 and its receptor would be enhanced with fasting duration; however, the conditions in fasting seals may contradict those required for typical FGF21-mediated cellular events. FGF21 inhibits lipolysis in WAT during fasting (Hotta et al., 2009) and FGF21 pharmacologically stimulates glucose uptake and ameliorates glucose tolerance and insulin sensitivity (Xu et al., 2009; Lin et al., 2013). However, fasting elephant seals rely on lipid oxidation in the subcutaneous blubber and hepatic gluconeogenesis in tandem with the Cori cycle and an insulin resistance (IR)-like condition via reduction of glucose uptake (Champagne et al., 2005; Viscarra et al., 2011; Houser et al., 2012). In addition, FGF21 promotes torpor to conserve energy (Inagaki et al., 2008); however, fasting elephant seal pups remain metabolically and physically active, normo-thermic, and maintain high levels of energy expenditure without increasing ketoacids despite high rates of beta-oxidation of fatty acids (Champagne et al., 2005; Crocker et al. 2014). FGF21 also regulates energy homeostasis by activating mitochondrial oxidative function that enhances the oxidative capacity of adipose via an AMPK-SIRT1-PGC-1α-dependent pathway (Chau et al., 2010). Conversely, fasting duration is associated with increased AMPK activation in adipose in the elephant seals (Viscarra et al. 2011) suggesting that FGF21 may be up-regulated, at least

locally, with fasting in seals. Nonetheless, these contradictory conditions that may be potentially imparted by FGF21 provide an intriguing situation, which may extend our understanding of the cellular events mediated by FGF21. Therefore, we hypothesized that the expression of FGF21, FGFR1, and KLB decrease with fasting in northern elephant seal pups.

2. Material and Methods

All procedures were reviewed and approved by the Institutional Animal Care and Use Committees of both the University of California Merced and Sonoma State University, USA. All work was performed under National Marine Fisheries Service marine mammal permit no. 87-1743.

2.1 Sample Collection

Adipose (subcutaneous blubber) biopsies, and plasma samples were collected from northern elephant seal pups at Año Nuevo State Park, CA during the autumn haul-out that follows the first trip to sea in 2011 and 2012. Different cohort of animals were sampled within the first week (early; N = 8) and after 3–4 weeks of fasting (late; N = 9). Sampling procedures were performed as described previously (Vázquez-Medina et al., 2011). Briefly, animals were sedated with tiletamine hydrochloride and zolazepam hydrochloride, which were administered intramuscularly. Immobilization was maintained by injecting ketamine as required. After immobilization, a spinal needle was inserted into the extradural spinal vein, and blood samples were collected, placed on ice, and centrifuged on site to separate the plasma. Tissue samples were rinsed with ice-cold sterile saline solution and placed in cryogenic vials. Plasma and tissue samples were frozen by immersion in liquid nitrogen and stored at −80°C until analyzed.

2.2 Quantitative RT-PCR

RNA extraction, cDNA synthesis and qPCR were performed according to a previously reported procedure (Suzuki et al., 2013). Briefly, RNA was extracted from adipose with TRIzol (Life Technologies, Grand Island, NY). cDNA was synthesized with 1 μg of RNA and oligo (dT) primer using a QuantiTect® Reverse Transcription Kit (Qiagen, Valencia, CA). Quantitative PCR reactions were performed for KLB (158 bp, accession no.: AB986562), FGFR1 (110 bp, AB986561), and FGF21 (184 bp, AB986560) using gene-specific primers, which were designed to amplify a region that covered several exons (Table 1). GAPDH expression was also quantified as an internal standard. Each of the PCR fragments was sub-cloned and sequenced, as described previously (Suzuki et al., 2010). Real time PCR was performed using 1 μL of the cDNA for each gene with the plasmid as a standard.

2.3 Measurement of plasma FGF21, glucose, and 3-hydroxybutyrate

Plasma FGF21 concentration was measured using a commercially available ELISA kit (Abnova, Taipei, Taiwan) according to the manufacturer's instructions. Plasma concentrations of glucose and 3-hydroxybutyrate were measured with an Analox GM7 analyser (Analox Instruments, London, UK). Due to the limited plasma volume that

remained after the FGF21 analyses, the glucose and 3-hydroxybutyrate (3-HBA) measurements were performed on 5 samples per fasting period.

2.4 Statistical analysis

The differences in mean values (± SEM) between the early and late fasting periods were compared using the Student's *t*-test. Differences were considered significant at $p < 0.05$. Two aberrant values were rejected from the plasma FGF21 concentration data as detected by Smirnov–Grubbs test.

3. Results

3.1 Expression of Adipose FGFR1, KLB, and FGF21

To evaluate changes in FGF21-mediated signaling in adipose, the mRNA expressions of FGFR1, KLB, and FGF21 were measured by qRT-PCR. The expressions of FGFR1 and KLB mRNA decreased 98% and 43%, respectively, with fasting (Figure 1A and 1B). While not statistically significant ($p = 0.17$), mean adipose FGF21 mRNA expression decreased 80% with fasting (Figure 1C).

3.2 Changes in the plasma FGF21 concentration

The plasma FGF21 concentrations were measured to better assess the relevance of the cellular events. The average and individual data of plasma FGF21 levels are shown in Figure 2. Similar to the adipose FGF21 expression levels, plasma FGF21 concentrations decreased 39% (early fasting: 0.33 ± 0.05 ng/mL vs. late fasting: 0.20 ± 0.02 ng/mL) with fasting duration, corroborating the decreasing tendency in adipose mRNA levels.

3.3 Changes in plasma glucose and ketone concentrations

Measurements of plasma glucose and 3-hydroxybutyrate concentrations were performed to further assess the potential shifts in substrate metabolism. Neither plasma glucose (early: 148 ± 6 mg/mL vs. late: 142 ± 6 ng/mL) nor 3-HBA (early: 223 ± 24 μM vs. late: 226 ± 7 μM) were significantly altered with fasting duration.

4. Discussion

While prolonged food deprivation/starvation typically stimulates FGF21-mediated signaling to suppress glucose and lipid metabolism to manage substrate utilization during a period of energetic burden, this study demonstrates a unique and atypical response in a mammal naturally adapted to protracted fasting. Unlike other mammals, FGF21 is suppressed with fasting duration in northern elephant seals. These data suggest that FGF21 may not contribute to the regulation of lipid and glucose metabolism during prolonged food deprivation in seals and/or that FGF21 may only contribute to cellular metabolism during the very early stage of their long-term fasting.

The co-activator, KLB, is essential for FGF21 activation, thus cells without KLB cannot respond to FGF21 (Suzuki et al., 2008). Therefore, the down-regulation of adipose KLB in the presence of decreasing plasma FGF21 levels during late fasting strongly suggests that

FGF21-mediated signaling may not contribute to lipid and/or glucose metabolism in adipose during prolonged food deprivation in seals. In rat and human adipocytes, TNFα can suppress KLB expression and impair FGF21 signaling as a consequence of insulin resistance (Díaz-Delfín et al. 2012). Previously, we reported an increase in muscle TNFα (Suzuki et al., 2013) and IR-like condition in adipose (Viscarra et al., 2011) during late fasting in elephant seal pups suggesting that a local increase in TNFα and/or induction of an IR-like condition may contribute to the down-regulation of KLB. Consistent with the decrease in KLB, adipose FGFR1 expression was also suppressed with fasting duration. FGFR1 expression is stimulated by FGF21 in the mouse (Adams et al., 2013). The parallel decreases in adipose FGFR1 and plasma FGF21 levels during late fasting suggest that the FGF21 receptor content is mediated by circulating levels. The suppression of adipose FGFR1 and KLB in the presence of decreased plasma FGF21 is indicative of down-regulation of cellular FGF21-mediated signaling. Furthermore, while physical activity may increase FGF21 (Cuevas-Ramos et al., 2011), during this time of fasting northern elephant seals maintain a relatively high metabolic rate (hypermetabolism) and are physically active (Kelso et al. 2013) suggesting that the suppression of FGF21 and its associated cellular cascade is more a function of fasting dynamics in seals than a change in physical activity and/or metabolic rate.

Circulating FGF21 is primarily secreted from the liver and adipose (Kharitonenkov and Shanafelt, 2009). Because an examination of hepatic FGF21 expression in elephant seals is not be possible, we cannot assess the contribution of each organ to plasma FGF21; however, given that adipose constitutes approximately 35–38% of a juvenile elephant seal's body mass and is metabolically active (Kelso et al. 2012), the parallel decreases in adipose FGF21 expression and plasma FGF21 suggest that adipose is a significant source of FGF21 in elephant seals. The levels measured here are similar to those in humans, although levels in humans vary greatly (Gälman et al., 2008).

In other mammals, PPARγ activation induces FGF21 expression pharmacologically (Muise et al., 2008; Wang et al., 2008). In elephant seals, adipose PPARγ expression decreases with prolonged fasting (Viscarra et al., 2011) suggesting that the decreased adipose FGF21 expression may have resulted from reduced PPARγ expression. Paradoxically, fasting in seals is associated with suppressed FGF21, and this suppression is not likely to contribute to substrate metabolism as evident by the lack of changes in plasma glucose and 3-HBA. These data are coincident with previous studies demonstrating that the maintenance of blood glucose and inconsequential accumulation of ketoacids via the Cori cycle and an IR-like condition in the seals (Champagne et al., 2005; Viscarra and Ortiz, 2013; Crocker et al., 2014) may facilitate the conservation of circulating glucose by relying on lipid oxidation for energy.

Collectively, these data suggest that, unlike other fasted/starved mammals, prolonged fasting attenuates FGF21-mediated signaling in elephant seals, a mammal naturally adapted to such a behavior. FGF21 is a potent activator of glucose uptake via GLUT1 through PPARγ activation in adipocytes (Moyers et al., 2007; Muise et al., 2008). In fasting elephant seal pups, an IR-like condition develops in adipose with prolonged food deprivation with PPARγ downregulation (Viscarra et al., 2011). The decreases in plasma FGF21 and the expressions

of adipose FGFR1 and KLB with fasting duration may contribute to IR via down-regulation of PPARγ expression. This atypical FGF21 response to food deprivation may be an important adapted mechanism that contributes to the maintenance of elevated circulating glucose to support the fasting metabolism of elephant seals. Thus, the typical fasting responses mediated by FGF21 activation, including the inhibition of lipolysis in adipose and the induction of torpor, are not evident in elephant seals.

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Highlights

- We tested effects of fast on FGF21-associated changes in elephant seal.
- Expression of FGF receptor-1 and its co-activator in adipose was decreased by fast.
- Plasma FGF21 levels were also decreased with a trend of decrease in adipose FGF21.
- Their atypical metabolism responses during fast do not require FGF21 functions.

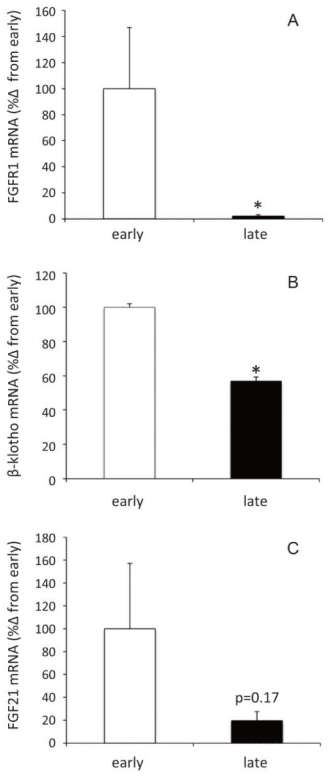

Figure 1.

Changes in mRNA expressions of fibroblast growth factor receptor-1 (A), β-Klotho (B), and fibroblast growth factor 21 (C) in adipose of early- and late-fasted northern elephant seal pups. * denotes significant difference at $p<0.05$.

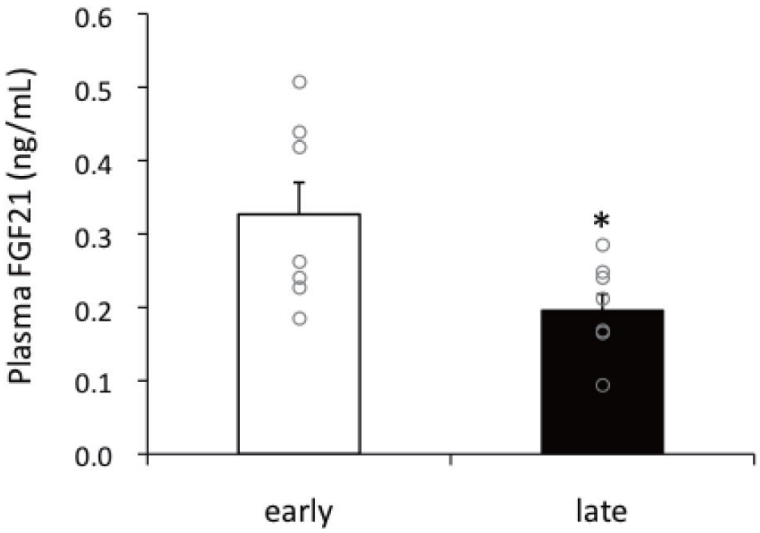

Figure 2.

Change in mean (± SEM) plasma fibroblast growth factor 21 concentration between early- and late-fasted northern elephant seal pups. Circles are shown individual data. * denotes significant difference at $p<0.05$.

Table 1

Primer sequences used for qPCR.

Primers	
FGF21 FW	5’ -cgacagcggtacctctacac-3’?
FGF21 RV	5’ -ggccctggcacaggaacc-S’
β-klotho FW	5’ -gagaatggctggttcacagacagtc-3’
β-klotho RV	5’ -gccattcaaagccatccaggagaga-3’
FGFR1 FW	5’ -ccttctagtgggactcctaacc-3’
FGFR1 RV	?’ -atgctccaggtggcgtaacg-3’