HHS Public Access

Author manuscript

Front Neuroendocrinol. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Front Neuroendocrinol. 2018 October ; 51: 125–131. doi:10.1016/j.yfrne.2018.06.002.

Homeostatic sensing of dietary protein restriction: A case for FGF21

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Abstract

Restriction of dietary protein intake increases food intake and energy expenditure, reduces growth, and alters amino acid, lipid, and glucose metabolism. While these responses suggest that animals 'sense' insufficient consumption of amino acids, the basic physiological mechanism mediating the adaptive response to protein restriction has been largely undescribed. In this review we make the case that the liver-derived metabolic hormone FGF21 is the key signal which communicates and coordinates the homeostatic response to dietary protein restriction. Support for this model centers on the evidence that FGF21 is induced by settings of insufficient dietary protein or amino acid intake and is required for adaptive changes in metabolism and behavior. FGF21 occupies a unique endocrine niche, being induced when energy intake is adequate but protein and carbohydrate are imbalanced. Collectively, the evidence thus suggests that FGF21 is the first known endocrine signal of dietary protein restriction.

Keywords

macronutrient; FGF21; dietary protein; nutrition

1. Introduction

The ability to sense and respond to nutrient restriction is one of the most essential physiological functions in biology. Survival is dependent on the organism procuring sufficient nutrients to meet metabolic needs and doing so in a fashion that responds to changes in the external environment or internal physiology. However, free-feeding animals often face a complex nutritional landscape where individual food sources vary in macronutrient content, energy density, palatability, and availability. Effectively navigating this nutritional landscape requires choices that maximize nutrient intake while minimizing procurement cost. Although considerable progress has been made in understanding the neural regulation of feeding behavior, much of this work has focused on food intake in terms of total food (grams) or energy (calories). Yet it seems intuitive that we eat for more than

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just calories/energy. Roughly 6 years ago we summarized the evidence for the homeostatic regulation of dietary protein intake while also noting that the neuroendocrine mechanisms governing this behavior were largely undefined (Morrison et al., 2012). Here we provide an update by making the case that the metabolic hormone FGF21 is a key component in the homeostatic mechanism mediating responses to protein restriction.

2. Impact of Dietary Protein Restriction on Behavior and Metabolism

Consumption of essential amino acids is required for life, and regulatory systems exist to detect insufficient protein intake and coordinate adaptive changes in feeding behavior and metabolism. For instance, a large amount of evidence indicates that reduced dietary protein content increases total food intake across a range of species, while also increasing energy expenditure, reducing growth, and altering the expression of a variety of genes associated with amino acid, glucose and lipid metabolism within the liver and elsewhere (Berthoud et al., 2012; Morrison et al., 2012; Anthony et al., 2013; Davidenko et al., 2013; Ghosh et al., 2014; Gosby et al., 2014; Martens & Westerterp-Plantenga, 2014; Morrison & Laeger, 2015). Additional evidence suggests that variations in amino acid composition can also influence feeding behavior, such that animals increase their consumption of diets that are moderately restricted in one or a few amino acids, avoid diets that are severely imbalanced in AA composition, and selectively find and consume the missing amino acid when faced with multiple food options (Hrupka et al., 1997; Torii & Niijima, 2001; Anthony & Gietzen, 2013; Cummings et al., 2017; Wanders et al., 2017). Finally, the specific response is often dependent on the magnitude of the dietary restriction, with methionine restriction producing adaptive changes in food intake and metabolism within a specific ‘window’ of dietary restriction (Forney et al., 2017a; Forney et al., 2017b). Methionine intakes higher than this threshold producing no overt phenotype and methionine intakes below producing negative physiological outcomes that are more accurately described as deprivation.

In addition to changes in feeding behavior, early work by Rothwell and Stock demonstrated that increases in food intake during protein restriction were not accompanied by the predicted increase in body weight (Rothwell et al., 1982, 1983). More recent work provides clear evidence that protein restriction increases whole-body energy expenditure, both at room temperature and thermoneutrality, and that these effects are associated with activation of brown adipose tissue, increases in UCP1, and the browning of white fat (Rothwell & Stock, 1987; Laeger et al., 2016; Hill et al., 2017). Although the physiological reason for this increase in energy expenditure is unclear, it could be argued that the increase in energy expenditure allows the animal to burn off excess calories in an effort to procure the missing amino acids (Felicetti et al., 2003; Sorensen et al., 2008). However, we recently demonstrated that preventing hyperphagia in protein-restricted mice increased weight loss and did not block the increase in energy expenditure, while deletion of UCP1 blocked both the increase in both food intake and the increase in energy expenditure during protein restriction (Hill et al., 2017). Taken together, these data not only suggest that increased energy expenditure is independent of hyperphagia, but that increased energy expenditure may in fact drive food intake.

As expected, dietary protein restriction also influences metabolism within multiple tissues, including muscle, fat and liver. The liver in particular responds to reduced amino acid intake by inhibiting protein synthesis and amino acid catabolism and increasing amino acid biosynthesis (Anthony et al., 2004; Kilberg et al., 2005), effects which buffer against dramatic or persistent falls in circulating amino acids during protein restriction (Anderson et al., 1990; Kalhan et al., 2011; Laeger et al., 2014b). Protein restriction promotes other changes within the liver, altering the expression of genes associated with amino acid and lipid metabolism, enhancing insulin sensitivity, and increasing autophagy (Mortimore & Schworer, 1977; Guo & Cavener, 2007; Hasek et al., 2013; Stone et al., 2014; Fontana et al., 2016; Henagan et al., 2016; Maida et al., 2016; Cummings et al., 2017). The liver is also uniquely positioned to sense and respond to alterations in dietary protein content due to its direct sensing of absorbed amino acids via the portal circulation. The observations led our lab to focus on the possibility that the liver produced a signal that communicated protein status, and this effort eventually led to the link between FGF21 and dietary protein restriction (Laeger et al., 2014a).

3. Nutritional Regulation of Liver FGF21 Expression

FGF21 is a member of a large group of fibroblast growth factors that influence an array of physiological and cellular functions (Nishimura et al., 2000). While most FGFs act in a paracrine fashion, FGF21, FGF15/19 and FGF23 form a subgroup of ‘endocrine FGFs’ which circulate in appreciable amounts within the bloodstream and thereby act as true endocrine hormones (Angelin et al., 2012; Itoh et al., 2015). Initial interest in FGF21 stemmed from the discovery that FGF21 promoted glucose uptake in adipocytes (Kharitonenkov et al., 2005). Soon it was demonstrated that FGF21 treatment reduced body weight, glucose and lipid concentrations in models of obesity (Kharitonenkov et al., 2005; Kharitonenkov et al., 2007; Coskun et al., 2008), leading to substantial interest within both the basic research and pharmaceutical communities regarding FGF21’s potential as treatment for obesity/diabetes. Multiple prior reviews thoroughly cover the discovery of FGF21 and the early description of its metabolic effects (Potthoff et al., 2012; Kharitonenkov & Adams, 2014; Fisher & Maratos-Flier, 2016; Kharitonenkov & DiMarchi, 2017; Potthoff, 2017).

In addition to defining the pharmacological potential of FGF21, significant effort also focused on defining the physiological significance of FGF21. Early work in rodent models shaped the FGF21 narrative by demonstrating that circulating FGF21 is induced by fasting and ketogenic diets and principally produced by the liver, that PPARα was the key molecular regulator of FGF21 expression, and that FGF21 contributed to the adaptive responses to fasting (Badman et al., 2007; Inagaki et al., 2007; Badman et al., 2009; Potthoff et al., 2009; Markan et al., 2014). However, the effect of fasting and ketogenic diets to increase FGF21 is not nearly as robust in humans as initially observed in mice (Galman et al., 2008; Christodoulides et al., 2009; Dushay et al., 2010). FGF21 is also increased in settings of obesity (Zhang et al., 2008; Chavez et al., 2009; Dushay et al., 2010), which appears contradictory for a fasting hormone which lowers body weight and improves glucose homeostasis. The elevated FGF21 levels observed during obesity has led some to suggest that obesity is an FGF21 resistant state, and it has been demonstrated that the FGF21 co-

receptor beta-Klotho (Klb) is significantly downregulated and FGF21 signaling attenuated in liver and fat of obese models (Fisher et al., 2010; Markan et al., 2017). However, subsequent studies indicate that FGF21's effects on metabolic endpoints are not impaired in models of obesity (Hale et al., 2012; Laeger et al., 2017), and it has been speculated that loss of FGF21 signaling in white adipose tissue could represent an adaptive effort to shunt lipids to brown adipose during obesity (Schlein et al., 2016; Markan et al., 2017). Finally, several recent studies suggest that alcohol intake also increases FGF21 and that FGF21 inhibits alcohol intake (Schumann et al., 2016; Talukdar et al., 2016; Desai et al., 2017; Soberg et al., 2018; Song et al., 2018). The connection between liver FGF21 secretion and obesity, steatosis, alcohol consumption and metabolic stress has led to a broader view of FGF21 as a signal of metabolic or cellular stress (Maratos-Flier, 2017). Indeed, various manipulations which trigger cellular stress increase FGF21 production, even in tissues such as muscle which normally do not produce significant quantities of FGF21 (Kim et al., 2012; Brahma et al., 2014; Keipert et al., 2014; Guridi et al., 2015; Harris et al., 2015; Touvier et al., 2015; Vandanmagsar et al., 2016; Pereira et al., 2017b). These data collectively suggest that diverse signals are capable of regulating FGF21 production in liver and other tissues, but considerable debate remains regarding the relevance of these various inputs in a physiological context. In the next section we suggest that one key physiological effect of FGF21 is to signal macronutrient imbalance, particularly insufficient protein in the context of high energy/carbohydrate.

3.1 Increased FG21 in response to high carbohydrate.

The first connection between carbohydrate intake and FGF21 derived from a study demonstrating that refeeding with high carbohydrate diets induced a robust increase in liver and circulating FGF21 (Sanchez et al., 2009). This effect appeared to be specific for liver, as treatment of primary rat hepatocytes with glucose also increased FGF21 mRNA expression (Iizuka et al., 2009). However, the role of carbohydrate as a regulator of FGF21 gained significant momentum when it was demonstrated that fructose ingestion acutely increased circulating FGF21 in humans (Dushay et al., 2015). Multiple studies have since demonstrated that both acute ingestion of carbohydrate (generally sucrose, fructose or glucose), as well as longer-term exposure to high carbohydrate diets, leads to increases in both hepatic FGF21 mRNA expression and circulating FGF21 protein (Solon-Biet et al., 2016; von Holstein-Rathlou et al., 2016; Fisher et al., 2017; Iroz et al., 2017; Lundsgaard et al., 2017; Maekawa et al., 2017; Pereira et al., 2017a). The mechanism of this carbohydrate-induced increase in FGF21 in the liver appears to be primarily driven by carbohydrate response element binding protein (ChREBP). The FGF21 promoter contains ChREBP elements, carbohydrate intake induces ChREBP binding to the FGF21 promoter, and deletion of ChREBP blocks the carbohydrate-induced increase in FGF21 (Iizuka et al., 2009; von Holstein-Rathlou et al., 2016; Fisher et al., 2017; Iroz et al., 2017). Interestingly, the transcription factor PPARα is also required for glucose-induced increases in FGF21 expression, and available evidence highlights an important interaction between PPARα and ChREPB signaling in the context of FGF21 regulation (Iroz et al., 2017). Taken together, these data provide compelling evidence that dietary carbohydrates engage a specific transcriptional pathway to control FGF21 production.

3.2 Regulation of FGF21 by dietary protein restriction.

The identification of FGF21 as a signal of dietary protein restriction was initiated by De Sousa-Coelho and colleagues (De Sousa-Coelho et al., 2012). Their work demonstrated that the FGF21 promoter contained amino acid response elements (AARE) and that depletion of the amino acid leucine promoted binding of the transcription factor ATF4 to these AAREs, leading to robust increases in FGF21 expression in vitro and in vivo. Subsequent work demonstrated that FGF21 was increased by ER stress via the same ATF4-dependent pathway (Schaap et al., 2013; Jiang et al., 2014), thereby supporting ATF4 as an alternative mechanism for FGF21 regulation in the liver. ATF4 is a key molecular mediator of the classic integrated stress response (ISR), which coordinates the cellular response to various stressors, including amino acid depletion (Wek et al., 2006; Kilberg et al., 2009). This connection between FGF21, amino acid restriction and the ISR led our lab to hypothesize that perhaps the restriction of dietary protein contributed to the increases in FGF21 observed during fasting. Our work demonstrated that liver FGF21 expression and circulating FGF21 protein levels are increased by protein restriction in mice, rats and humans, and that this effect is independent of any change in energy intake (Laeger et al., 2014a). In fact, restricting energy intake without restricting protein decreased FGF21 expression. Subsequent work by multiple groups has confirmed that FGF21 is robustly increased by dietary protein restriction (Fournier et al., 2014; Ozaki et al., 2015; Chalvon-Demersay et al., 2016; Gosby et al., 2016; Maida et al., 2016; Pezeshki et al., 2016; Solon-Biet et al., 2016; Larson et al., 2017; Pereira et al., 2017a). This effect to increase FGF21 also extends to other situations of amino acid restriction, including the restriction of leucine, methionine/cysteine, the depletion asparagine, and the restriction of multiple non-essential amino acids (De Sousa-Coelho et al., 2012; Wanders et al., 2015; Wilson et al., 2015; Maida et al., 2016; Wanders et al., 2017). Contrastingly, available evidence suggests that the restriction of branched-chain amino acids may not increase FGF21, at least not persistently (Fontana et al., 2016; Cummings et al., 2017). Importantly, multiple groups have independently extended this observation to humans, suggesting that increases in FGF21 during dietary protein restriction is conserved across mammalian species (Laeger et al., 2014a; Fontana et al., 2016; Gosby et al., 2016; Maida et al., 2016).

Currently the mechanisms driving increases in liver FGF21 in response to protein restriction are not fully clear. Initial work implicated the classic integrated stress response pathway, particularly activation of the amino acid sensor GCN2, increased elF2a phosphorylation, the binding of ATF4 to the FGF21 promoter, and increases in FGF21 expression (De Sousa-Coelho et al., 2012). Indeed, deletion of GCN2 markedly attenuates the increase in FGF21 during protein restriction as well as in a model of asparagine depletion (Wilson et al., 2015; Laeger et al., 2016). However, GCN2-deficient mice appear to compensate over time, such that FGF21 expression eventually increases even in the absence of GCN2 (Laeger et al., 2016). Consistent with changes in FGF21 levels, the metabolic response to protein restriction is also impaired in GCN2-deficient mice for only the first few weeks of dietary protein restriction. The identity of this compensatory, GCN2-independent mechanism is currently unclear, but it seems possible that other components of the integrated stress response could contribute. For instance, PERK is known to promote FGF21 expression in response to classic ER stress signals and could potentially compensate for loss of GCN2

(Schaap et al., 2013), while other integrated stress response components such as Nupr1 and IRE1α-XBP1 have been implicated on FGF21 regulation (Jiang et al., 2014; Maida et al., 2016). GCN2 seems to be wholly unnecessary for increases in FGF21 in the context of methionine restriction, and while initial work suggested that PERK was activated, PERK is also dispensable for changes in FGF21 expression and metabolism during methionine restriction (Wanders et al., 2016; Pettit et al., 2017). Contrastingly, PPARα-deficient mice fail to increase FGF21 during protein restriction (Laeger et al., 2014a), and therefore PPARα is required for the effects of protein restriction, fasting and carbohydrate excess to increase FGF21 (Badman et al., 2007; Inagaki et al., 2007; Iroz et al., 2017). Although PPARα is required, there is no evidence that PPARα signaling is activated by protein or amino acid restriction (Ghosh et al., 2014; Laeger et al., 2014a), an outcome which suggests that PPARα likely plays a structural or constitutive role in FGF21 transcriptional activity during protein restriction, just as it appears to do in response to carbohydrate excess (Iroz et al., 2017). In summary, multiple lines of evidence suggest that protein restriction potently and persistently increases liver FGF21 expression and circulating FGF21 concentrations.

3.3 FGF21 as a signal of protein:carbohydrate imbalance

The above discussion suggests that FGF21 is not simply a fasting hormone, but is regulated by multiple nutritional inputs. One complication of many nutritional studies, including our own, is that protein and carbohydrate are often concomitantly altered to maintain a diet that is isocaloric with the control. As such, low protein diets are also marginally higher in carbohydrate, and it could be argued that this carbohydrate contributes to the increase in FGF21 induced by low protein diets. However, FGF21 is also increased by the restriction of individual amino acids acids (De Sousa-Coelho et al., 2012; Wanders et al., 2015; Wilson et al., 2015; Maida et al., 2016; Wanders et al., 2017; Cummings et al., 2018), and in this situation there is no change in carbohydrate content. High carbohydrate cannot explain the effect of ketogenic diets to increase FGF21, as ketogenic diets are virtually devoid of carbohydrate. Interestingly, several studies demonstrate that ketogenic diets increase FGF21 only when protein is reduced (Laeger et al., 2014a; Stemmer et al., 2015), and therefore dietary protein or amino acid restriction is sufficient to increase FGF21 regardless of the carbohydrate content of the diet. A similar argument could be made of pure sucrose/glucose/fructose/alcohol solutions, as these treatments are devoid of protein and therefore dilute protein intake. However, a recent study demonstrated that high carbohydrate diets increase FGF21 even when protein intake is controlled (Lundsgaard et al., 2017), and the specific, critical role played ChREBP in mediating the effects of glucose provides a specific mechanism linking carbohydrate intake to FGF21 expression.

Collectively these data suggest that FGF21 is independently regulated by multiple macronutrient inputs, and this conclusion is supported by a study using the geometric framework to define the macronutrient regulation of FGF21 in 858 mice eating 25 diets which varied in protein, carbohydrate, fat, and energy density (Solon-Biet et al., 2016). This study provides strong evidence that low protein and high carbohydrate independently increase FGF21, but energy intake itself does not influence FGF21. Taken together these observations suggest that FGF21 is primarily regulated by an imbalance between protein and carbohydrate intake, being particularly increased when protein intake is restricted and

carbohydrate intake is excessive. FGF21 therefore responds to a nutritional state that is different from leptin and other energy balance signals. Two alternative nutritional scenarios can be envisioned which highlight these unique roles. The first scenario is one in which food is generally scarce, resulting in the restriction of energy intake. In this scenario, classic energy balance signals (leptin, insulin, ghrelin, etc.) govern metabolic and behavioral adaptions to dietary (energy) restriction. The second scenario is one in which low protein, high carbohydrate foods are readily available, but protein rich foods are scare. In this case energy balance signals are not engaged because energy intake is sufficient, but FGF21 is specifically increased because protein intake is low but carbohydrate intake is high. It is thus attractive to hypothesize that the induction of FGF21 in this state provides an endocrine mechanism to coordinate adaptive responses to imbalanced, protein-poor diets (Felicetti et al., 2003; Sorensen et al., 2008; Morrison et al., 2012). However, for this scenario to be relevant FGF21 must be physiologically required for adaptive responses to protein restriction.

4. FGF21 is essential for metabolic responses to protein restriction

It is generally accepted that physiological systems sense ‘nutrient restriction’ and engage adaptive mechanisms which both mitigate the consequences of restriction and promote a restoration of physiological function once food becomes available. Powerful examples exist for responses to the restriction of energy, sodium and water. While available evidence suggests that animals sense and respond to protein restriction, the primary mechanisms mediating the adaptive response to protein restriction is largely unknown (Morrison et al., 2012; Morrison & Laeger, 2015). However, work from our lab and others increasingly suggest that FGF21 is robustly increased by dietary protein restriction and required for adaptive responses to low protein diets.

The initial observations demonstrating FGF21’s required role stems from straightforward studies assessing the impact of low protein diets in FGF21-deficient mice. Protein restriction in mice produces an array of metabolic responses, including reduced body weight and adiposity and increased energy expenditure and food intake. These effects are lost in FGF21-deficient mice, whose growth rate is not reduced by the low protein diet (Laeger et al., 2014a). These data therefore indicate that FGF21 is the missing endocrine signal which coordinates adaptive behavioral and metabolic responses to dietary protein restriction. Subsequent work from multiple groups has replicated these core observations. Metabolic responses to protein restriction persist beyond 6 months of exposure, and FGF21 is fully required for this persistent response (Laeger et al., 2016). FGF21 also contributes to the effects of protein restriction on other metabolic endpoints, most notably improving insulin sensitivity in both diet and genetic models of obesity (Maida et al., 2016). The contribution of FGF21 to dietary protein restriction has also been extended to methionine restricting diets, where it was shown that FGF21 was required of the changes in energy expenditure and glucose homeostasis during MR, but not effects on lipid metabolism (Forney et al., 2017a; Wanders et al., 2017).

We have also indirectly validated the contribution of FGF21 via a separate set of studies focusing on the mechanism for FGF21 induction during protein restriction (Laeger et al.,

2016). As described above, the amino acid sensor GCN2 is required for acute increases in FGF21 during protein restriction, such that GCN2-KO mice phenocopy FGF21-KO in their failure to respond to protein restriction, at least initially. However, GCN2-KO mice exhibit a delayed response to protein restriction, and this delayed metabolic response is explained by a delayed increase in FGF21 (Laeger et al., 2016). Because the response to protein restriction in GCN2-KO mice is tied to the increase in FGF21, this observation provides independent evidence that FGF21 is required for metabolic responses to protein restriction. Therefore, an increase in circulating FGF21 levels during protein restriction is required for the animal to 'sense' protein restriction and adapt metabolically and behaviorally, and this novel mechanism may provide a window into the biology underlying this robust but poorly understood response.

5. Biology of FGF21 action in the context of a low protein diet.

FGF21 is required for metabolic responses to protein restriction, but the mechanisms through which FGF21 produces these metabolic responses are less clear. More generally, understanding where and how FGF21 acts to regulate metabolic endpoints, both pharmacologically and physiologically, remains an important scientific question. While a full review of the FGF21 signal transduction cascade and the gamut of effects and sites of action implicated in the response to pharmacological FGF21 treatment is beyond the scope of this review, the growing consensus from this work suggests FGF21 largely acts through a heterodimer of FGFR1 and the co-receptor beta-Klotho (Klb) (Ding et al., 2012; Lee et al., 2018). Klb appears to be essential for the biological effects of FGF21, with adipose tissue and brain being key targets (Sarruf et al., 2010; Yang et al., 2012; Owen et al., 2013; Owen et al., 2014; BonDurant et al., 2017). Indeed, the brain has proven to be an essential mediator of FGF21 action in the context of pharmacological treatment (Sarruf et al., 2010; Bookout et al., 2013; Owen et al., 2014; von Holstein-Rathlou et al., 2016), although effects on adipose tissue or an interacting effect between multiple tissues remains a possibility (BonDurant et al., 2017). To date none of these questions have been rigorously tested within the context of protein restriction. Currently the mechanisms through which the brain senses and regulates macronutrient specific intake is poorly understood (Berthoud et al., 2012), but recent work has implicated FGF21 in this context by demonstrating that FGF21 acts to specifically reduce sweet taste (Talukdar et al., 2016; von Holstein-Rathlou et al., 2016) and alcohol consumption (Schumann et al., 2016; Talukdar et al., 2016). Similarly, genetic studies have linked FGF21 to macronutrient intake (Chu et al., 2013; Tanaka et al., 2013; Heianza et al., 2016; Schumann et al., 2016; Soberg et al., 2017). Finally, we contend that dietary protein restriction represents a valuable physiological context in which to test the mechanism of FGF21 action, both because it provides an alternative to studies using pharmacological doses/administration and because signaling of protein restriction represents an important physiological role for FGF21.

6. Conclusions and Future Directions

The above discussion highlights three main conclusions: First, dietary protein restriction triggers an array of adaptive responses that include changes in feeding behavior, energy expenditure, growth rate, and metabolism. Second, protein restriction produces a large

increase in circulating levels of FGF21 within a range of mammalian species. Third, the metabolic effects of protein restriction appear, in large part, to require increased circulating FGF21. Collectively, these data provide strong evidence that FGF21 is the first known hormone that coordinates adaptive behavioral and metabolic responses to protein restriction. However, many unanswered questions remain. First, the specific mechanisms connecting dietary protein intake to FGF21 production remain unclear. For instance, are certain amino acids preferentially sensed or are all amino acids (essential and non-essential) treated equally, and what are the intracellular signaling events that drive increased FGF21 expression in the liver during protein restriction. It will also be important to define how these mechanisms transcriptionally interact with other nutritional signals that also engage FGF21. As noted earlier in this review, FGF21 is independently increased by high carbohydrate intake and low protein intake, and could therefore be more broadly labeled as a hormone signaling macronutrient imbalance. Second, if FGF21 indeed functions to coordinate adaptive responses to protein restriction/macronutrient imbalance, then how does FGF21 signaling interact with classic signals of energy balance such as leptin? To what extent do these ‘protein’ and ‘energy’ signals interact to control feeding behavior or metabolism? A third set of questions surround the mechanism through which FGF21 produces its metabolic effects in the context of dietary protein restriction. In which tissues (brain, fat, liver) does FGF21 primarily act; how does FGF21 signaling translate into changes in energy expenditure, feeding or glucose homeostasis; which of these effects are primary and which are secondary. A fourth set of questions surround the extent to which signals beyond FGF21 contribute to the overall adaptive response to protein restriction, as it seems unlikely that a single hormone mediates the broad and extensive effects that protein restriction induces. Finally, although evidence demonstrates that protein restriction also increases FGF21 in humans, the extent to which the specific metabolic effects produced by protein restriction in rodents translate to humans is an important question. Within the fields of obesity and metabolism it is almost axiomatic that adequate or even high protein diets are ‘healthy’, as they tend to reduce food intake and promote fat loss while also supporting the maintenance of lean mass. However, recent studies in flies and rodents suggest that high protein diets may have negative effects on insulin sensitivity and longevity while low protein diets exert metabolic benefits and extend lifespan (Grandison et al., 2009; Piper et al., 2011; Levine et al., 2014; Solon-Biet et al., 2014; Solon-Biet et al., 2015; Fontana et al., 2016; Cummings et al., 2017; Piper et al., 2017). These latter studies support the longstanding consensus that dietary restriction promotes healthspan and lifespan and suggest that the restriction of protein may be a contributing mechanism. Future studies are therefore needed to specifically define whether FGF21 contributes to these effects, and how these novel pathways can be leveraged to improve health in humans.

Acknowledgments

Funding

This work was supported by the National Institutes of Health R01DK105032 to CDM and F32DK115137 to CMH.

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Highlights

1. Dietary protein restriction triggers adaptive changes in metabolism and behavior
2. FGF21 is increased by excess carbohydrate and/or insufficient protein intake
3. Adaptive responses to protein restriction require intact FGF21