Minireview

MOLECULAR METABOLISM

FGF21, energy expenditure and weight loss — How much brown fat do you need?

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ABSTRACT

Background: Fibroblast growth factor 21 (FGF21) belongs to the large family of fibroblast growth factors (FGFs). Even though FGF signaling has been mainly implicated in developmental processes, recent studies have demonstrated that FGF21 is an important regulator of whole body energy expenditure and metabolism, in obesity.
Scope of review: Given the fact that obesity has developed epidemic proportions, not just in industrialized countries, FGF21 has emerged as a novel therapeutic avenue to treat obesity as well as associated metabolic disorders. While the metabolic effects of FGF21 are undisputed, the mechanisms by which FGF21 regulate weight loss have not yet been fully resolved. Until recently it was believed that FGF21 induces brown fat activity, thereby enhancing energy expenditure, which concomitantly leads to weight loss. Novel studies have challenged this concept as they could demonstrate that a part of the FGF21 mediated effects are retained in a mouse model of impaired brown adipose tissue function.
Major conclusions: The review illustrates the recent advances in FGF21 research and discusses the role of FGF21 in the regulation of energy expenditure linked to brown fat activity.

Keywords FGF21; UCP1; Brown fat; Energy expenditure

1. INTRODUCTION

Higher energy intake than expenditure causes excessive accumulation of fat and leads to the development of obesity and associated metabolic disorders, which has reached epidemic proportions not only in developed countries but worldwide [1]. Furthermore, obesity can lead to the development of various co-morbidities, like type 2 diabetes, cardiovascular diseases, neurodegenerative disorders and some types of cancers, that severely impact life quality and expectancy [2,3]. While treatments for the obesity related co-morbidities are available, albeit with varying success, no therapeutic avenues, with the exception of highly invasive bariatric surgery, exist to treat obesity directly. Several approaches that target the central nervous system to reduce food intake have failed in this context due to massive side effects [4].
As it can both store and burn calories, adipose tissue has gained renewed attention recently as a possible target for the treatment of obesity due to its Janus-faced character. In this context, adipose tissue can be functionally divided into two main depots, namely white and brown adipose tissue. White adipose tissue is characterized by the presence of large unilocular adipocytes whose main function is the storage of energy and the release of fatty acids during fasting and starvation. Brown adipose tissue is characterized by the presence of smaller cells with multilocular lipid droplets. In contrast to that of white adipose tissue, the primary function of brown adipose tissue is to produce heat, a feature which is achieved through the function of uncoupling protein 1 (UCP1). UCP1 effects a proton leak from the mitochondrial intermembrane space to the mitochondrial matrix, effectively short circuiting the electron transport chain [5]. Thus, as its name suggests, UCP1 uncouples oxidation from phosphorylation, driving a futile cycle that produces heat. In addition, to classical brown adipocytes, which are mainly localized to the interscapular depot, other brown like adipocytes, termed either beige or brite adipocytes, can be found in predominantly white adipose tissue depots [6,7]. These cells have received substantial attention in recent years due to the fact that their appearance is dependent on induction. The origin of these cells, however, remains a matter of debate [8—10].
During cold exposure the sympathetic system activates brown fat via secretion of catecholamines that stimulate β3-adrenergic receptors on the adipocyte surface. In rats, brown adipose tissue has been shown to be responsible for over 60 percent of the excess heat production during cold stimulation [11]; also in newborn humans, the role of brown fat in protecting against hypothermia has been appreciated for a long time [12,13]. In addition, several independent groups recently demonstrated that brown fat is also activated by cold and contributes to heat production through non-shivering thermogenesis in adult humans [14—18]. Yoneshiro et al. showed in 2013 that repeated cold exposure leads to recruitment of brown fat in humans and increases cold-induced gains of energy expenditure [19]. Further corroborating a functional role for brown fat in the adult human was gained from a study by Cypess et al., which demonstrated that β3-adrenergic receptor agonists can not only increase glucose uptake of brown fat but also increase resting metabolic rate by approximately 13% [20].

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Brown fat activation is achieved by a variety of factors, including but not limited to natriuretic peptide A, β3-adrenergic receptor agonists and other circulating factors such as fibroblast growth factor 21 (FGF21). Until recently, the energy expenditure enhancing and weight loss effects of FGF21 were thought to be mediated mainly by brown fat thermogenesis, because FGF21 has been demonstrated to directly stimulate thermogenic gene expression and to induce browning of white fat. Thus, FGF21 seemed to be a suitable candidate for both the recruitment of brown cells in the white fat, as well as for the activation of endogenous brown fat.

2. IDENTIFICATION OF FGF21

FGF21 is a member of the prominent fibroblast growth factor (FGF) superfamily, which currently encompasses 22 members [21]. Based on various genetic mutations, FGFs were first implicated in embryonic development, regulating proliferation and differentiation as well as organ morphogenesis. The spectrum has been expanded recently, and it is now accepted that FGF signaling plays an important role in the pathogenesis of several diseases. This is especially true for FGF21, which has emerged as a new therapeutic target for the regulation of whole body metabolism.

FGF21 was first cloned in 2000 as a novel hepatokine with unknown function [22]. Only five years later it was shown by the Lilly Research Laboratories that FGF21 is an important factor regulating glucose uptake [23] as FGF21 knockout mice exhibit a mild weight gain, increased adipocyte size and slightly impaired glucose homeostasis [24]. Furthermore, when challenged with a ketogenic diet, FGF21KO mice develop hepatosteatosis and gain weight concomitant with an impairment in ketogenesis and glucose homeostasis, as well as a downregulation of PGC1α and PGC1β both in liver and white fat. Conversely, systemic injection or transgenic overexpression of FGF21 leads to protection from diet-induced obesity, concomitant with a significant reduction in blood glucose and triglyceride levels [23,25]. Interestingly, FGF21 overexpression also causes a marked increase in food intake under chow and high fat conditions, suggesting that either energy expenditure and/or nutrient uptake in these animals is induced. Taken together, these facts identify FGF21 as a major regulator of metabolic utilization of energy containing metabolites; therefore FGF21 is considered a novel therapeutic target for the treatment of obesity and its associated metabolic disorders [6,26–29].

3. REGULATION OF FGF21 EXPRESSION

FGF21 is expressed in liver, pancreas, fat tissue and muscle [22]. In adult humans, fructose ingestion acutely stimulates circulating FGF21 levels [30] and in liver, FGF21 expression is induced after birth in response to intake of milk during suckling [31] through the action of PPARα. Due to its high expression in brown adipose tissue several studies have addressed the regulation of FGF21 expression and secretion in response to activation of this organ. For example, Hondares et al. showed that higher expression leads to increased secretion by analyzing arteriovenous differences in FGF21 concentration across interscapular brown fat [32]. In contrast, work from Chartoumpekis et al. could not demonstrate an increase in FGF21 serum levels after an acute cold induction at the end of a 12 h starvation period [33]. Keipert et al. compared FGF21 mRNA levels in muscle, heart, liver, subcutaneous white and brown fat from animals at different housing temperatures. Interestingly, at thermoneutrality, only liver seems to express FGF21 at a level comparable to that in activated brown adipose tissue. In Ucp1 genetically ablated mice (UCP1KO), FGF21 expression is induced in brown adipose tissue as well as in circulation [34,35]. This increase only occurs during cold adaptation, suggesting that brown fat communicates with other organs, maybe to compensate for the loss in thermogenic capacity. In contrast, transgenic overexpression of UCP1 switches on FGF21 production in muscle suggesting that this phenotype is specific for brown fat and might be diametrically opposite in other organs [36].

4. FGF21 SIGNALING

FGF21 signaling utilizes the classical intracellular FGFR signaling pathway, but, in contrast to other FGFs, it does not bind directly to the FGFR. Instead, FGF21 mediates its function via the transmembrane receptor β-Klotho, which is a FGFR co-receptor with a high expression in liver, fat and the central nervous system. Whole body deletion of β-Klotho leads to a complex phenotype including developmental defects such as growth retardation and a counterintuitive increase in glucose tolerance and insulin sensitivity. In brown adipose tissue of these mice, however, UCP1 levels and body temperature are reduced, suggesting that β-Klotho deficient mice have less energy expenditure than wild-type mice. Since it was shown that other FGFs such as FGF19 can also signal via β-Klotho, this finding could be explained by a broader ligand spectrum for this surface receptor [37]. The cell autonomous effect of FGF21 on adipocytes was first reported in 2005 by Kharitonenkov, et al. [23], who demonstrated an FGF21 mediated induction of glucose uptake in mature adipocytes. In 2012, Ding et al. showed that aP2-cre mediated ablation of β-Klotho leads to blockade of the acute insulin-sensitizing effect [38]. However, given the fact that aP2-cre mediated deletion targets multiple cells within the adipose tissue including adipocyte precursors, macrophages and endothelial cells [39–41], it is unclear whether this effect is due to FGF21 signaling in adipocytes.

Since brown fat activity is tightly regulated by the sympathetic nervous system it remains unclear whether the systemic effects of FGF21 are due to a cell autonomous regulation of brown fat activity or if this effect is mediated via the CNS. A central effect of FGF21 has been demonstrated by intracerebro-ventricular (i.c.v.) injection of FGF21 [42], which, similar to systemic FGF21 injection, led to increased energy expenditure, food uptake and insulin sensitivity. However, central FGF21 administration failed to reduce body weight and size of adipose tissue, suggesting that FGF21 effects are mediated both peripherally and centrally. Further evidence for the importance of FGF21 in regulating central pathways comes from animal models with hypothalamic- and hindbrain-specific genetic deletion of β-Klotho in conjunction with an FGF21 overexpression model, which demonstrated that centrally or peripherally administered FGF21 increases sympathetic activation in brown adipose tissue and that this effect can be blunted by i.c.v. injection of an FGFR1 inhibitor (PD173074) [43]. In addition to its endocrine function, FGF21 may have paracrine or autocrine functions, as fat-specific knockout of FGF21 inhibits browning of white adipose tissue in adaptive thermogenesis [44].

5. FGF21 AND BROWN FAT UNCOUPLING – NEW INSIGHTS

Recent reports of Samms et al. and Veniant et al. [35,45] investigate the causal link between FGF21 increased browning and the loss of body weight. In accordance with previous results [44], the study by Veniant et al. demonstrates that FGF21 injection leads to an increase in brown adipose tissue mass concomitant with an increase in UCP1 expression in brown adipose tissue, while the effect in inguinal white adipose tissue is lost at thermoneutrality. Interestingly, the effect on

weight loss is retained even when FGF21 is injected into UCP1KO mice, suggesting that the effects of FGF21 on weight loss are independent of brown fat activation. A closer look at the data, however, reveals that this is a simplified assumption. Scrutiny of the energy expenditure data suggests that in both studies the effect of FGF21 is reduced in UCP1KO mice, a finding that is especially evident in the study by Samms et al. in which FGF21 induced energy expenditure seems to be almost completely abolished in UCP1KO mice. Similar evidence can be gained from circulating metabolic parameters. While the effect of FGF21 on FFA and cholesterol are retained in UCP1KO mice, the effect on circulating glucose seem to be reduced or completely blunted. One important point to note is the effect of FGF21 on food intake in the context of Ucp1 ablation. The study by Veniant et al. observes a reduced induction of food intake in UCP1KO while the study by Samms et al. doesn't observe an induction of food intake by FGF21 in wild type mice and actually reports a reduction of food intake in UCP1KO mice when treated with FGF21; the discrepancy could be due to differences in the dosing paradigm and housing temperature. Taking into account that UCP1KO mice have a reduced food intake upon FGF21 treatment, one would expect them show a reduction in body weight, a finding that is not observed. Therefore, it is possible that this difference is due to the effect of FGF21 on weight loss is mediated through the activation of brown adipose tissue.

In UCP1KO mice both brown fat FGF21 mRNA levels and circulating levels of FGF21 are increased significantly [34]. Since some effects of FGF21 are abolished in UCP1KO animals the question remains how FGF21 reduces body weight independent of UCP1. A more detailed look at the regulation of energy balance is required. First of all, most tissues contribute to the metabolic rate and oxygen consumption of an organism and in the adult state the majority of calories ingested, are finally lost as heat. The contribution of different tissues to mammalian metabolic rate (independent of exercise) mainly depends on the genetic background and the environmental temperature. Based on the reported functions for FGF21 and given the literature of FGF21 and brown fat activation, the main question that arises from the recent studies is whether FGF21 acts as a physiological feedback molecule from thermogenically active tissues to integrate information about the available capacity and, if so, whether this alteration sensitizes other tissues to the action of FGF21.

To answer these questions it is necessary to review the literature on UCP1 mediated and brown fat mediated uncoupling in detail. It is well established that the genetic ablation of UCP1 induces obesity in mice living at thermoneutrality [46]. Furthermore, overexpression of UCP1 under the aP2 and the human skeletal actin promoter protects against development of genetic and diet-induced obesity, respectively [47,48]. These data, which have been reviewed comprehensively [49], suggest that UCP1 function is the main driver of brown adipose tissue thermogenesis and that loss of UCP1 leads to a decrease in energy expenditure and concomitant obesity. However, several reports do not fit this paradigm. One example is a study by Liu et al. that demonstrates that loss of UCP1 can protect from diet induced obesity under certain conditions [50]. Based on this controversy, several studies have addressed the UCP1-independent adrenergic heat production with somewhat controversial results and opinions range from 20 to 50% of brown fat capacity [51,52] and it has been suggested that these effects could be due to UCP1-independent increases in thermogenesis in white adipose tissue [53,54]. How these thermogenic processes are regulated is unclear at the moment; however, futile cycling as well as fatty acid mediated uncoupling of the mitochondrial membrane have been suggested as possible mechanisms. For example, in mammalian muscle, sarcolipin was identified as a regulator of non-shivering thermogenesis [55]. Also, She et al. reported increased energy expenditure associated with the activation of a futile protein turnover cycle in peripheral branched-chain amino acid metabolism [56]. Furthermore, simultaneous activation of lipogenesis and lipolysis as well as prolonged beta3-adrenergic receptor activation can induce futile cycling both in white and brown adipose tissue [57] [58].

6. CONCLUSIONS

FGF21 induces weight loss and leads to reduction of some key metabolic parameters in UCP1KO mice. While some of these effects seem to be conserved and thus independent of UCP1, others seem to be blunted. In particular, the FGF21 mediated changes in food intake seem to be dependent on UCP1, suggesting a compensatory central regulation maybe through the induction of circulating FGF21. In line with this notion recent work by Schultz et al. might be important, as it suggests a fixed thermogenic capacity, which is tightly regulated by compensatory effects [59]. Thus, it is possible that under physiological conditions the effects of FGF21 are mainly mediated by brown adipose tissue through UCP1, while under abnormal conditions such as UCP1 ablation, these effects might be mediated via other tissues. In light of these findings, and although UCP1-dependent thermogenesis is the most potent and possibly the most relevant physiological regulator of non-shivering thermogenesis, other mechanisms that have been proven to play a role in thermogenesis should be considered in the context of FGF21 signaling.

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