CLINICAL STUDY

Impact of short-term high-fat feeding and insulin-stimulated FGF21 levels in subjects with low birth weight and controls

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Abstract

Objective: Fibroblast growth factor 21 (FGF21) is a metabolic factor involved in glucose and lipid metabolism. However, little is known about the physiological role of FGF21 during a dietary challenge in humans.

Research design and methods: Twenty healthy low birth weight (LBW) with known risk of type 2 diabetes and 26 control (normal birth weight (NBW)) young men were subjected to 5 days of high-fat (HF) overfeeding (+50%). Basal and clamp insulin-stimulated serum FGF21 levels were examined before and after the diet, and *FGF21* mRNA expression was measured in muscle and fat biopsies respectively. **Results:** Five days of HF overfeeding diet significantly ($P < 0.001$) increased fasting serum FGF21 levels in both the groups ($P < 0.001$). Furthermore, insulin infusion additionally increased serum FGF21 levels to a similar extent in both the groups. Basal mRNA expression of *FGF21* in muscle was near the detection limit and not present in fat in both the groups before and after the dietary challenge. However, insulin significantly ($P < 0.001$) increased *FGF21* mRNA in both muscle and fat in both the groups during both diets.

Conclusion: Short-term HF overfeeding markedly increased serum FGF21 levels in healthy young men with and without LBW but failed to increase muscle or fat *FGF21* mRNA levels. This suggests that the liver may be responsible for the rise of serum FGF21 levels during overfeeding. In contrast, the increase in serum FGF21 levels during insulin infusion may arise from increased transcription in muscle and fat. We speculate that increased serum FGF21 levels during HF overfeeding may be a compensatory response to increase fatty acid oxidation and energy expenditure.

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Introduction

The incidence of chronic metabolic diseases such as type 2 diabetes, atherosclerosis, and obesity is rapidly becoming a global health concern. A sedentary lifestyle and consuming a high-calorie diet are believed to be the primary causes of these illnesses (1).

Recently, a part of the fibroblast growth factor (FGF) family proteins have been described to be involved in the regulation of metabolic processes and function in an endocrine manner (2, 3, 4, 5, 6). These proteins include FGF19, 21, and 23 and are distinguished from the rest of the FGF proteins by lacking the heparin-binding domain, allowing them to diffuse away from their tissue of origin (7). Pharmacologically, FGF21 has been shown to be a regulator of glucose and lipid metabolism in both rodents and primates (2, 3, 4, 5, 6). Transgenic (tg) mice overexpressing *Fgf21* are resistant to diet-induced

obesity, have a lower fasting blood glucose level, as well as improved glucose tolerance compared with their wild-type littermates (2). In the fed state, where ketogenesis is normally suppressed by insulin, the *Fgf21* tg mice have increased ketogenesis compared with control mice (6). The *Fgf21* knockout mice display mild obesity, hepatic steatosis, and glucose intolerance as well as impaired ketogenesis in response to fasting (3, 8).

Pharmacologically, FGF21 induces GLUT1 (SLC2A1)-mediated glucose uptake in adipocytes *in vitro* and treatment of diabetic animal models with recombinant FGF21 lowers the blood glucose level, corrects dyslipidemia, and increases energy expenditure, making FGF21 an attractive pharmaceutical target for the management of type 2 diabetes (9, 10, 11). However, the physiological role of FGF21 is still not fully understood. Expression of *FGF21* has predominantly

been detected in liver and pancreatic tissues, but low levels are also present in muscle and fat (4, 12, 13, 14). In human muscle tissue, insulin has been shown to upregulate *FGF21* mRNA expression during a hyperinsulinemic euglycemic clamp (13). In mice, liver *FGF21* mRNA expression as well as *FGF21* secretion from the liver is strongly upregulated by fasting in a PPAR α (PPARA)-dependent manner (5). In human subjects, fasting for 7 days showed increase in the plasma level of *FGF21*, whereas no effect was found for 3 days of fasting (15, 16). It has recently been shown that *FGF21* increases hepatic expression of peroxisome proliferator-activated receptor gamma coactivator protein 1 α (PGC1 α (PPARGC1A)), a key transcriptional regulator of energy homeostasis, and causes a corresponding increase in fatty acid oxidation (8). This suggests an important role for *FGF21* in the regulation of fatty acid metabolism during fasting. However, plasma levels of *FGF21* have also been reported to be upregulated by high-fat (HF) feeding in mice (17), in animal models of obesity and type 2 diabetes (14), as well as in patients with type 2 diabetes (18, 19). Overall, the plasma level of *FGF21* is highly associated with different insulin-resistant states including body mass index (BMI) (20), dyslipidemia, and nonalcoholic fatty liver disease (16, 21).

It has previously been shown that individuals born with low birth weight (LBW) have an increased risk of developing type 2 diabetes and the metabolic syndrome. Indeed, we and others have found several metabolic defects including impaired insulin-stimulated glucose uptake in muscle (22), decreased whole-body insulin-stimulated glycolytic flux (23), hepatic insulin resistance (23), as well as changes in muscle and fat insulin signaling proteins (24, 25) in young and otherwise healthy men born with LBW. Furthermore, LBW subjects exhibit an elevated rate of fat oxidation after a dietary challenge (26). As *FGF21* has been shown to be upregulated in subjects with type 2 diabetes and may play an important role in fatty acid oxidation, we aimed to examine the short-term effects of a westernized diet, i.e. HF overfeeding of *FGF21* serum levels and expression in young LBW men and normal birth weight (NBW) controls in a previously published study addressing the effects of HF overfeeding on glucose and fat metabolism (26, 27, 28). *FGF21* is supposed to play an important role in the control of homeostatic metabolic changes including fasting, and to the best of our knowledge, the effect of HF overfeeding on *FGF21* has not been examined in humans before and may provide valuable information about the role of *FGF21* and its regulation in response to dietary changes in subjects with and without risk of developing type 2 diabetes. In addition, we speculated whether the increased risk of developing insulin resistance and type 2 diabetes in LBW subjects might be related to changes in *FGF21* levels.

Subjects and methods

Twenty healthy, young, lean, male volunteers with LBW (BW <10 percentile) and 26 control subjects with NBW (BW between the 50 and 90 percentile) were recruited from the Danish Medical Birth Registry as described previously (23, 26, 27). All subjects were matched according to age and BMI. The subjects had no family history of diabetes in two generations, and subjects with a BMI higher than 30 kg/m² or a high physical activity level (>10 h/week) were excluded. *In vivo* data on these two groups including muscle oxidative phosphorylation (OXPHOS) gene expression and metabolic responses to HF overfeeding in both NBW and LBW subjects were published previously (23, 26, 27). The protocol conformed to the declaration of Helsinki and was approved by the ethics committee for Copenhagen County, and all subjects signed an informed consent form.

Experimental protocol

The study was a randomized crossover study with a washout period of 6–8 weeks, as described previously (26, 27). In brief, subjects were examined twice after intake of a 5-day HF overfeeding diet (mean 17.6 $\pm$ 1.4 MJ) containing 50% extra calories and 60% fat as well as after a control diet including 30% fat (mean 11.9 $\pm$ 1.1 MJ). The study activities were carried out over 3 days, and 5 days before each examination period, all subjects were standardized with regard to alcohol consumption, physical activity, and food intake. Furthermore, subjects were asked to remain weight stable between the two examination periods. Besides the standardization period, the control experiment was optimized even further, by providing all foods to the subjects 3 days before the first examination day (26, 27).

Energy expenditure

Energy expenditure was assessed during a 24-h period by indirect whole-body calorimetry using a 14.7 m³ respiratory chamber as described previously (29) on the first examination day. Measurements were initiated at 0900 h in the morning on study day 1 and ended at 0900 h the following morning (day 2) according to a standardized protocol; only sedentary activities were allowed with the exception of scheduled physical activity (two sessions of 15 min cycling on ergometer bicycles (Monark; 14E, Monark AB, Varberg, Sweden) and two sessions of walking back and forth 25 times within the chamber). Meals were served at 0900, 1300, and 1900 h and the basal metabolic rate was assessed on day 2 from 0800 to 0900 h after an overnight fast. Spontaneous physical activity (SPA) was assessed by microwave radar detectors (Scissor Mini-Radar; Static Input System SA, Lausanne, Switzerland) and defined

as the amount of time (percentage) of which the subject was active to a detectable degree. Heart rate was registered by a portable ECG device (Dialogue 2000, type 2070-14 XTNJ; Dania Electronics, Roedovre, Denmark) and gas exchange in the chamber was measured by the concentrations of oxygen and carbon dioxide at the outlet of the chamber. Urine was collected throughout the 24-h period and used to adjust the respiratory measurements for nitrogen excretion.

Hyperinsulinemic euglycemic clamp

The clamp was performed on the third examination day and analyzed as described previously (27). A primed continuous infusion of tritiated 3-³H glucose was initiated at 0 h, and an insulin infusion of 80 mU/m² per min was used throughout the 180 min clamp to maintain euglycemia. Basal and insulin-stimulated glucose and fat oxidation rates were measured using indirect calorimetry (30). Muscle and fat biopsies were obtained from the musculus vastus lateralis and abdominal subcutaneous adipose tissue before and after the 3-h insulin infusion under local anesthesia (lidocaine) using a Bergström needle in 35 of the 46 subjects. The tissues were immediately frozen in liquid nitrogen and stored at −80 °C.

FGF21 serum detection

FGF21 was measured using an ELISA kit from Biovendor (Czech Republic). The lowest detection level within the linear range in this ELISA is 7 pg/ml, and therefore, values of FGF21 detected below 7 pg/ml are set to 7 pg/ml. Both serum and plasma levels were measured without any detectable difference.

Quantitative real-time PCR

Total RNA was extracted from muscle biopsies using TRI reagent (Sigma–Aldrich), whereas fat biopsies were purified using RNeasy Mini Kit (Qiagen). cDNA was synthesized using iScript RT kit (Bio-Rad). mRNA levels

were detected with the ABI 7900 sequence detection system using gene-specific probe/primer pairs for FGF21 (HS00173927) and GAPDH (HS99999905) (Applied Biosystems, CA, USA). All samples were run in triplicates and expression was calculated as a function of 2^{−C_t} and normalized to GAPDH expression.

Statistical analysis

Data calculation and statistical analysis was performed using GraphPad Prism version 5.0 (La Jolla, CA, USA). Data are presented as mean ± S.E.M. Two-tailed Student's *t*-test was used to identify significant differences between NBW and LBW subjects (unpaired) and between diets (paired). Differences between groups were evaluated by two-way ANOVA for repeated measures. Significance was accepted at the *P* < 0.05 level. FGF21 levels did not follow a Gaussian distribution, and therefore, non-parametric tests are used for the statistical analysis.

Results

The effects of HF overfeeding on glucose, lipid, and insulin resistance have previously been published by Brøns *et al.* (26, 27, 28) and are summarized in Table 1. In short, on the control diet, LBW subjects had elevated fasting glucose and insulin levels compared with NBW subjects. Furthermore, no difference in glucose or fat oxidation rates at basal or after insulin infusion was observed. When challenged with HF overfeeding, both groups had significantly increased blood glucose levels and decreased free fatty acids (FFA) levels, whereas there was no change in serum insulin. The LBW subjects were already hepatic insulin resistant on the control diet (23), whereas the NBW group developed hepatic insulin resistance during the dietary challenge (27). The LBW subjects further developed peripheral insulin resistance and had an increased rate of fat oxidation under the insulin infusion as well as a reduced PPARGC1A and OXPHOS gene expression compared with the NBW subjects (26). Furthermore, energy expenditure was

Table 1 Subject characteristics of the NBW and LBW groups. Data are mean ± S.D. and are shown for NBW (*n* = 26) vs LBW (*n* = 20) subjects. M value, peripheral insulin resistance. Summarized from references (26, 28).

	NBW		LBW	
	Control diet	HF overfeeding	Control diet	HF overfeeding
Birth weight (g)	3893 ± 207	—	2688 ± 269 [‡]	—
Basal blood glucose (mM)	4.59 ± 0.46	5.05 ± 0.40 [‡]	4.96 ± 0.46*	5.18 ± 0.33 [‡]
Basal serum insulin (pM)	30.9 ± 14.1	43.3 ± 29.2	40.7 ± 14.4*	42.9 ± 18.8
Serum insulin clamp (pM)	870 ± 232	867 ± 181	816 ± 114	842 ± 125
Basal plasma FFA (mM)	334 ± 136	205 ± 82 [‡]	401 ± 118	188 ± 91 [‡]
M value (mg/kg FFM per min)	13.73 ± 2.32	13.29 ± 3.32	13.43 ± 2.98	11.89 ± 3.57 [‡]
Hepatic insulin resistance (mg/kg FFM per min)	68.7 ± 34.1	113.7 ± 61.5 [‡]	99.3 ± 49.1*	108.7 ± 55.5

*Significant differences between NBW and LBW subjects at *P* ≤ 0.05. Paired comparisons between control and overfeeding include *n* = 25 (NBW) and *n* = 18 (LBW) subjects. Significant differences between the control and overfeeding diet are indicated as follows: [‡]*P* ≤ 0.05; [‡]*P* ≤ 0.001.

significantly increased ($P < 0.001$) in both the groups after the dietary challenge independent of birth weight (NBW, 7.14 ± 0.12 – 7.36 ± 0.11 KJ/min and LBW, 7.12 ± 0.16 – 7.38 ± 0.16 KJ/min).

The HF overfeeding diet resulted in an approximately threefold increase in basal fasting FGF21 serum levels for both LBW (138.9 ± 33.1 vs 306.8 ± 39.9 pg/ml) and NBW subjects (78.5 ± 13.9 vs 254.8 ± 22.6 pg/ml), and with no significant differences between the two groups (Fig. 1A). Furthermore, no difference was found between plasma and serum FGF21 measurements (data not shown). As shown in Fig. 1A, there was a large individual variation in serum FGF21 levels both on the control diet and especially after the dietary challenge. In general, the variation in serum FGF21 level was more pronounced in the LBW group, but almost all (44 but two) subjects responded to HF overfeeding by an increase in their serum FGF21 levels.

All subjects underwent hyperinsulinemic euglycemic clamps before and after the dietary challenge, and as

shown in Fig. 1B, a significant increase in serum FGF21 levels in both NBW and LBW subjects was found in response to insulin independent of diet. As shown in Fig. 1B, no difference in response to insulin-stimulated serum FGF21 was seen between NBW and LBW subjects.

As previously reported (23, 26, 27), the group of LBW subjects exhibited increased hepatic glucose production as well as hepatic insulin resistance already on the control diet compared with NBW subjects. This was followed by an increase in fasting plasma insulin levels compared with NBW subjects. As FGF21 has been reported to be increased in several conditions of insulin resistance and elevated insulin levels, we examined whether there was a correlation between fasting insulin levels and FGF21 levels. Interestingly, on control diet, basal serum FGF21 levels were significantly correlated with fasting serum insulin levels in the NBW but not in the LBW subjects (Fig. 2A). This correlation disappeared in both the groups on the HF overfeeding diet (Fig. 2B).

To further explore whether the observed increase in serum FGF21 levels after HF overfeeding or after insulin infusion arose from muscle or fat tissue, the mRNA expression of *FGF21* was examined in muscle (musculus vastus lateralis) and adipose tissue (subcutaneous from abdomen) biopsies before and after HF overfeeding as well as before and after insulin stimulation (clamp). At baseline, the *FGF21* mRNA expression was very low in muscle and no FGF21 was detected in fat independent of diet and birth weight (Fig. 3A and B). These observations did not change when the mRNA expression was corrected for lean or fat mass (data not shown). Therefore, the observed increase in plasma FGF21 after the HF challenge does not arise from muscle or fat.

Insulin infusion significantly increased the *FGF21* mRNA expression in both muscle and fat, but HF overfeeding did not change insulin-stimulated *FGF21* mRNA expression in either muscle or fat (Fig. 3A and B), which was also the case for the serum levels of FGF21 (Fig. 1B). To unmask any potential differences after the dietary challenge between NBW and LBW subjects, the insulin-stimulated *FGF21* mRNA expression in muscle was normalized to the basal expression (Fig. 3C). As shown, there is a trend toward a lower fold induction of insulin-stimulated *FGF21* mRNA expression with HF overfeeding independent of birth weight, although not significant. To examine whether the observed increase in serum FGF21 upon insulin stimulation was related to the observed increase in muscle and fat mRNA expression, a correlation analysis between mRNA expression and serum levels in each subject was performed. However, no correlation was found independent of tissue and birth weight. As energy expenditure increased with the dietary challenge and it has been shown that mice treated with recombinant FGF21 exhibit increased energy expenditure (10), we also examined whether there was a

Figure 1 FGF21 serum levels before and after 5 days of HF overfeeding. (A) Individual variations in FGF21 serum levels on control diet (open symbols) and after 5 days of HF overfeeding (closed symbols) in NBW and LBW subjects. *** $P < 0.001$ paired Student's *t*-test, no significant differences between NBW and LBW. (B) FGF21 serum levels in NBW and LBW before (open bars) and after hyperinsulinemic euglycemic clamp (closed bars) on control or HF overfeeding diet. Insulin significantly increases FGF21 serum levels in both NBW and LBW subjects independent of dietary challenge. Data are mean $\pm$ S.E.M. * $P < 0.05$ two-way ANOVA; *** $P < 0.001$, paired Student's *t*-test.

Figure 2 Correlation of basal FGF21 and insulin serum levels at control (A) and HF overfeeding diets (B). FGF21 serum levels in NBW (filled circle, $n=26$) and LBW (open circle, $n=18$) subjects correlated with their fasting insulin levels. Correlation analysis was performed using Spearman's correlation test, not assuming normal distribution. There is only a significant correlation between insulin and FGF21 levels in the NBW group on control diet ($r^2=0.47$, $P<0.05$).

correlation between serum FGF21 levels and energy expenditure. As shown in Fig. 4, we did not find a correlation between the increase in FGF21 serum levels and the increase in energy expenditure independent of birth weight. This result did not change when energy expenditure was adjusted for lean body mass, fat mass, SPA, or 24-h energy intake (data not shown).

Discussion

In this study, we found that the plasma FGF21 level markedly increased in response to 5 days of HF feeding in young healthy men with NBW and LBW. Increased *FGF21* transcription in muscle or fat seemed not to contribute to the increased serum levels during HF feeding suggesting a role for the liver, whereas the consistent insulin-stimulated increase in the plasma FGF21 level in both the groups and during both the diets was associated with increased *FGF21* mRNA expression in skeletal muscle and adipose tissue.

The physiological role of FGF21 in humans is not well understood, but mounting recent (including the

current) data suggest that FGF21 exhibit important endocrine functions central to energy metabolism and possible type 2 diabetes. Our study demonstrated that 5-day intake of a HF diet with 50% extra calories resulted in a threefold increase in the serum FGF21 level, suggesting that FGF21 may play an important role in order for humans to adapt to a new feeding situation. NBW and LBW subjects showed the same upregulation

Figure 3 *FGF21* mRNA expression before and after 5 days of HF overfeeding at baseline and during hyperinsulinemic euglycemic clamp. (A) *FGF21* mRNA expression in muscle biopsies (musculus vastus lateralis) in NBW ($n=21$ on control diet and during overfeeding) and LBW ($n=17$ on control diet and $n=13$ during overfeeding) subjects at baseline (open bars) and during hyperinsulinemic euglycemic clamp (closed bars). (B) *FGF21* mRNA expression in fat (subcutaneous) in NBW ($n=21$ on control diet and during overfeeding) and LBW ($n=17$ on control diet and $n=13$ during overfeeding) subjects at baseline (open bars) and during hyperinsulinemic euglycemic clamp (closed bars). (C) Fold change in *FGF21* mRNA expression in muscle during hyperinsulinemic euglycemic clamp vs basal on control diet (open bars) and during overfeeding (closed bars). Data are mean $\pm$ s.e.m. *** $P<0.001$ two-way ANOVA.

Figure 4 FGF21 levels after 5 days of HF overfeeding do not correlate with energy expenditure. (A) FGF21 serum levels in NBW (filled circle, $n=26$) and LBW (open circle, $n=18$) subjects correlated with their energy expenditure (EE) after 5 days of high-fat overfeeding. (B) The delta increase in FGF21 levels after 5 days of HF overfeeding correlated with the delta increase in energy expenditure after the dietary challenge in NBW (filled circle) and LBW (open circle) subjects. No significant correlations were found using Spearman's correlation analysis not assuming normal distribution.

of FGF21 in serum in response to the dietary challenge and insulin infusion, and no differences in muscle or fat FGF21 mRNA levels were found between LBW and NBW subjects. Accordingly, these data do not suggest any major role for FGF21 in conferring increased risk of type 2 diabetes in subjects born with LBW.

The previously observed decrease in fasting triglyceride (TG) and FFA levels after the short-term HF overfeeding diet in this study cohort (26) has also been reported in other short-term overfeeding studies (31, 32). Although slightly increased fasting plasma insulin levels in the NBW subjects may contribute to suppress lipolysis, fasting insulin levels did not increase in the LBW subjects, and increased insulin secretion is thus unlikely to be the full explanation for the marked reduction of fasting plasma FFA levels during HF overfeeding (27). Interestingly, FGF21 administered in pharmacological doses has been shown to acutely inhibit lipolysis in the adipose tissue (6, 33, 34) as well as to increase fatty acid oxidation in the liver (5). Accordingly, we speculate that the increased serum

FGF21 levels may explain (or at least contribute to) the somewhat counter-intuitive marked reduction of plasma FFA levels during short-term overfeeding by inhibition of lipolysis as well as by increasing liver and whole-body fat oxidation capacity and oxidative FFA extraction. The lower fasting plasma TG levels induced by overfeeding may subsequently be explained by lower FFA availability for TG synthesis in the liver. In absolute terms, unaltered whole-body fat oxidation rate during HF overfeeding in both the groups should similarly be considered in the context of the markedly reduced plasma FFA levels consistent with the idea of an increased fat oxidation capacity.

It was recently shown that FGF21 follows a circadian rhythm (35, 36) and that the diurnal changes in FFA is related to the diurnal changes of FGF21 in humans, where the nocturnal increase in FFA is followed by a peak of FGF21. As soon as plasma FGF21 peaks, FFA starts to drop (35). This further support the idea that the increase in plasma FGF21 levels, and the parallel decrease in TG and FFA levels, may be closely connected. The lack of any direct correlation between FFA and FGF21 levels in this study is not surprising taking the complexity of the metabolic changes induced by HF feeding – including an increased hepatic fat extraction rate – into account. Another explanation may be the diurnal changes in FFA and FGF21 levels. In addition, obesity in mice is a state of FGF21 resistance, with low expression of FGF21 receptors in white adipose tissue and liver (17). This could *per se* lead to a decreased turnover rate of FGF21 and hence increased plasma levels, even though the major clearance of FGF21 has been indicated to be renal (37).

Plasma FGF21 levels are strongly correlated with hepatic steatosis (16, 21), and the presence of hepatic insulin resistance in both the groups after HF overfeeding (26) suggests that at least some of the excess fat has been disposed of as liver TGs and that the observed increase in serum FGF21 levels may be a compensatory mechanism in order to inhibit lipolysis and to increase FFA oxidation. Additional support for the hypothesis that the observed increase in FGF21 in this study is a compensatory response to the HF overfeeding is that the transgenic mice overexpressing *Fgf21* are resistant to diet-induced obesity (2).

Two weeks of treatment with recombinant FGF21 in diet-induced obese mice has been shown to increase their energy expenditure as well as their uncoupling protein 1 (UCP1) (10). In our study, both the groups independent of birth weight exhibited increased energy expenditure with the dietary challenge. However, we did not find any correlation between serum FGF21 levels and energy expenditure (Fig. 4), indicating that the metabolic regulatory mechanisms are much more complex, including probably the concept of FGF21 resistance even at a very early stage of HF overfeeding.

Our study further showed that the increase in serum FGF21 levels in response to the dietary challenge is most

likely not to be due to an increased mRNA expression in muscle and fat. Thus, almost no mRNA expression was detected in muscle in the basal state and no mRNA expression of *FGF21* at all was found in fat independent of diet and birth weight. Interestingly, insulin stimulation during clamp significantly upregulated the serum levels of FGF21 as well as the mRNA expression of *FGF21* in both muscle and fat (Figs 1 and 3). Hojman *et al.* (13) have previously reported increased plasma FGF21 levels and increased *FGF21* mRNA expression in muscle biopsies contrasting the supposed role of FGF21 as a starvation hormone. This study documents that the insulin effect on *FGF21* mRNA expression is present in both muscle and fat under supraphysiological levels during a hyperinsulinemic euglycemic clamp (Table 1 and Fig. 3). Whether acute insulin at physiological levels stimulates the same upregulation of *FGF21* mRNA expression in muscle and fat still needs to be examined. Like Hojman *et al.*, we did not find a correlation between insulin-stimulated *FGF21* mRNA expression and insulin-stimulated FGF21 serum levels, suggesting that the FGF21, produced by muscle and fat, may play a different role than FGF21 produced by liver and may function as a paracrine or even an autocrine factor. In agreement with this hypothesis, Mashili *et al.* (38) recently showed that FGF21 increases insulin-stimulated glucose uptake in human myotubes. However, it is interesting to notice that *PPARGC1* expression is downregulated in the skeletal muscles after 5 days of HF overfeeding as previously shown in these subjects (26), despite the known stimulatory effect of FGF21 on *PPARGC1* and lipid oxidation in at least liver (3) and adipose tissue (39). In order to elucidate FGF21's role on lipid metabolism in skeletal muscles, further studies are needed.

In conclusion, the marked increase in FGF21 serum levels after short-term HF overfeeding is most likely due to increased production of FGF21 by the liver, as the *FGF21* mRNA levels in muscle at baseline did not increase significantly with diet and were furthermore not at all present in the subcutaneous fat. Additionally, no difference in FGF21 serum protein or tissue mRNA levels was observed between the NBW and LBW subjects, indicating that LBW may confer risk of type 2 diabetes by mechanisms not directly involving FGF21 regulation. Interestingly, insulin infusion increased FGF21 serum levels as well as mRNA expression in both subcutaneous adipose tissue and skeletal muscle, indicating that the additional increase in FGF21 serum levels during acute insulin infusion may be due to transcriptional upregulation in muscle and fat. We propose that the observed increase in FGF21 serum after 5 days of HF overfeeding may represent a compensatory response in order to inhibit lipolysis and to increase fatty acid oxidation, contributing to the modest impairment of *in vivo* insulin action observed in this study during overfeeding.

Declaration of interest

S G Vienberg and B Andersen are employed by Novo Nordisk A/S. S G Vienberg, C Brøns, E Nilsson, A Vaag, and B Andersen are stock holders in Novo Nordisk A/S. A Vaag has received consulting and lecture fees of less than \$10 000. S G Vienberg, C Brøns, E Nilsson, A Astrup, A Vaag, and B Andersen designed and/or supervised the experiments. A Astrup has nothing to disclose.

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Author contribution statement

S G Vienberg, C Brøns, A Vaag, and B Andersen wrote the manuscript.

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