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ORIGINAL ARTICLE

FGF21 is dispensable for hypothermia induced by fasting in mice

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Abstract

BACKGROUND: Fibroblast growth factor 21 (FGF21) is a key metabolic regulator that is induced by peroxisome proliferator-activated receptor α (PPARα) activation in response to fasting. We recently reported that bezafibrate, a pan-agonist of PPARs, decreases body temperature late at night through hypothalamic neuropeptide Y (NPY) activation and others have shown that mice overexpressing FGF21 are prone to torpor.
OBJECTIVES: We examined whether FGF21 is essential for fasting-induced hypothermia using FGF21 knockout (KO) mice.
RESULTS: Acute fasting decreased body temperature late at night accompanied by the induction of hepatic FGF21 and hypothalamic NPY expression in wild-type mice. A deficiency of FGF21 affected neither fasting-induced hypothermia nor hypothalamic NPY induction. Fasting enhanced locomotor activity in both genotypes. On the other hand, a deficiency of FGF21 significantly attenuated chronic hypothermia and hypoactivity induced by a ketogenic diet (KD).
CONCLUSIONS: Our findings suggest that FGF21 is not essential for the hypothermia that is associated with the early stages of fasting, although it might be involved in the adaptive response of body temperature to chronic starvation.

INTRODUCTION

Homeothermic animals maintain body temperature (BT) in a circadian manner from approximately 35 to 38 °C against continuous environmental variations in temperature. Torpor, the controlled lowering of metabolic rate, body temperature and physical activity, is a highly successful adaptation that allows various mammals to cope with periods of low food availability (Melvin

& Andrews 2009). Some mammals periodically turn down their internal thermostat and enter torpor as a means of survival during periods of low food availability and then rewarm to return to a normal level of activity when the environment becomes favourable (Melvin & Andrews 2009). However the mechanisms that integrate the environmental, physiological, metabolic and molecular changes associated with torpor are largely unknown (Melvin & Andrews 2009).

Recent findings imply the involvement of nuclear factors such as peroxisome proliferator-activated receptors (PPARs), farnesoid X receptors (FXRs), liver X receptors (LXRs), retinoid-related orphan receptors (RORs) and REV-ERBs in the regulation of torpor (Nelson *et al.* 2009). Fibroblast growth factor 21 (FGF21) is a member of the endocrine FGF subfamily that lacks the conventional heparin-binding domain (Itoh & Ornitz 2004). It is predominantly expressed in the liver and its expression is remarkably induced via PPARα activation in response to fasting and other ketotic states such as under-feeding with a ketogenic diet (KD). FGF21 plays an important role in the regulation of glucose, lipid, and energy homeostasis (Kharitonenkov & Shanafelt 2008). The overexpression of FGF21 induces torpor-like phenomena such as hypoglycemia, ketosis and hypothermia in mice (Inagaki *et al.* 2007). However, its role in the regulation of BT and metabolism seems to be complex as BT and oxygen consumption increase in obese mice administered with FGF21 (Coskun *et al.* 2008). We previously demonstrated that bezafibrate, a PPAR pan-agonist, decreases BT late at night in concert with the induction of hepatic FGF21 and hypothalamic neuropeptide Y (NPY) expression in mice (Chikahisa *et al.* 2008). We also found that mRNA levels of FGF21 are obviously induced in a circadian manner in mice both under bezafibrate administration (Oishi *et al.* 2008) and KD feeding (Oishi *et al.* 2009). Signaling of the NPY-Y1 receptor should be involved in bezafibrate-induced hypothermia, because the intracerebroventricular injection of a NPY Y1 receptor antagonist diminished this phenomenon (Chikahisa *et al.* 2008). Here, we used FGF21 knockout (KO) mice to determine whether FGF21 is a requirement for the regulation of fasting-induced hypothermia.

METHODS

Animals and experimental design

FGF21 KO mice and wild-type (WT) littermates on a C57BL/6 background were generated as described (Hotta *et al.* 2009). Male mice at 8–14 weeks of age were housed under a 12 h light-12 h dark cycle (LD 12:12; lights on at 0:00 and lights off at 12:00) at a controlled ambient temperature (23.0 ± 1.0 °C). Mice were fed with a normal diet (ND) (CE-2; Clea Japan Inc., Tokyo, Japan) or with a ketogenic diet (KD; 73.9% fat, 8.3% protein and 0.73% carbohydrates, w/w; modified AIN-93G; Oriental Yeast Co. Ltd., Tokyo, Japan) (Oishi *et al.* 2009). A white fluorescent lamp served as a daytime light source. Body temperature and the locomotor activities of mice fed *ad libitum* were recorded as a baseline. On the following day, mice were fasted for 24 h or fed with KD from 0:00. All animal experiments and care proceeded with the approval of our institutional Animal Care and Use Committee (Permission #2009-020).

Monitoring body temperature and locomotor activity

Anesthesia was induced by sevoflurane inhalation. After reaching deep anesthesia, transmitters (TA10TA-F20; Data Sciences Int., USA) were implanted intraperitoneally for telemetrically monitoring BT and locomotion. To continuously collect BT and locomotion data every 5 min, cages containing mice were placed on a receiver plate (RPC-I; Data Sciences International). Data collected through a transmitter were sent to a receiver and analyzed using the Dataquest A.R.T. data acquisition system (version 4.2; Data Sciences International). Both BT and locomotor activity were averaged over intervals of one hour.

Quantitative reverse transcription (RT)-PCR.

A triangular region (each side 2 mm) of the hypothalamus containing the arcuate nucleus was dissected on ice from a coronal brain section 2 mm thick (1 to 3 mm posterior to the bregma) immediately after decapitation, and stored in RNA*later*® (Ambion) at 4 °C until use. Total RNA was extracted using RNAiso (Takara Bio Inc., Otsu, Japan). Single-strand cDNA was synthesized using the PrimeScript™ RT reagent kit (Takara Bio Inc., Otsu, Japan). Real-time RT-PCR proceeded using the SYBR® Premix Ex Taq™ II (Takara Bio Inc., Otsu, Japan) using a LightCycler™ (Roche Diagnostics, Mannheim, Germany). The reaction conditions were 95 °C for 10 s followed by 45 cycles of 95 °C for 5 s, 57 °C for 10 s and 72 °C for 10 s. The amount of mRNA was corrected relative to that of *β-actin*. The forward primer sequence for *NPY* was CTCCGCTCTGCGACACTACA and the reverse primer was AATCAGTGTCTCAGGGCTGGA. The sequences of the primer pairs for *FGF21* and *β-actin* were as reported (Oishi *et al.* 2008).

Statistical analysis

All values are expressed as means ± SEM. Data were statistically evaluated by applying the two-way and one-way analysis of variance (ANOVA). The post-hoc test was Welch's or Student's *t*-test, and values of $p<0.05$ were considered statistically significant.

RESULTS

Circadian fluctuations in BT identified in both WT and FGF21 KO mice were almost identical between the genotypes (Figure 1A). Acute fasting induced moderate hypothermia late during the dark period in both genotypes. Fasting obviously increased physical activity

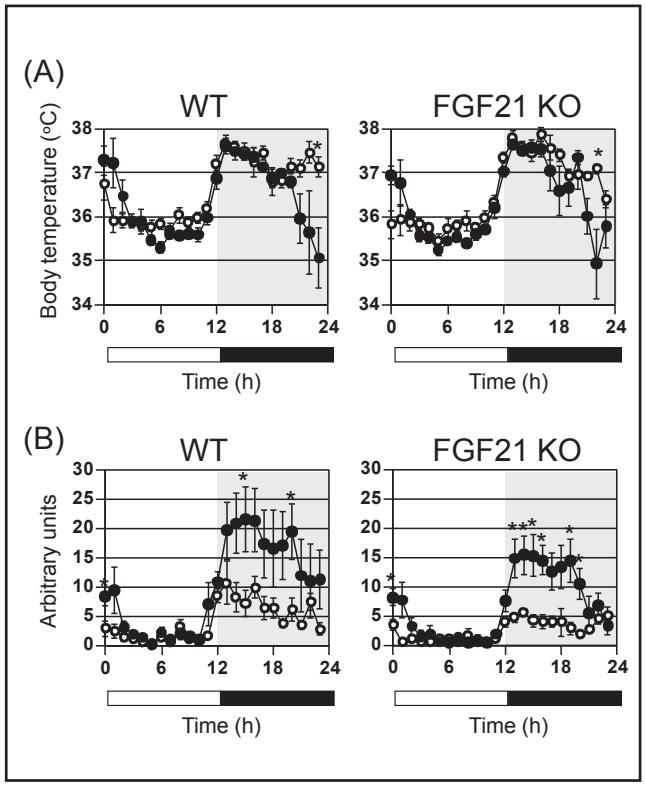

Fig. 1. The effect of acute fasting on body temperature (**A**) and locomotor activity (**B**) in wild-type (WT) and FGF21 knockout (KO) mice.
Mice were housed under LD 12:12 (lights on at 0:00) and deprived of food from 0:00 for 24 h. Open circles, data from day 1 (fed ad libitum); closed circles, data from day 2 (fasting). Dark phase duration is shaded in gray. Horizontal open and solid bars indicate day and night, respectively. Significant differences are indicated as *p<0.05. Values are means ± SEM (n=5).

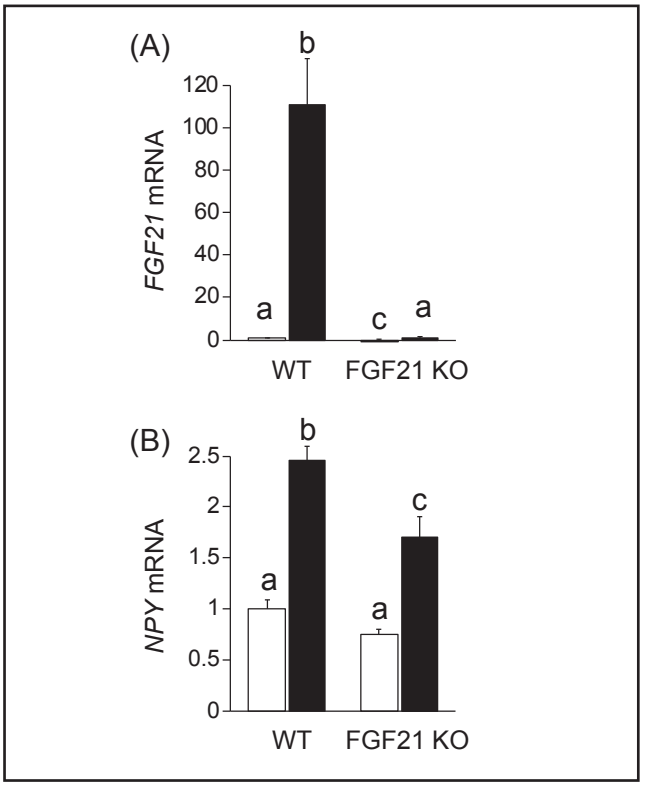

Fig. 2. Expression profiles of hepatic FGF21 (**A**) and hypothermic NPY (**B**) mRNAs after 24 h of fasting in wild-type (WT) and FGF21 knockout (KO) mice.
Mice were housed under LD 12:12 (lights on at 0:00) and deprived of food from 0:00 for 24 h. Tissues were dissected from mice after 24 h of fasting (0:00). Open bars, basal levels of fed mice; closed bars, expression levels after 24 h of fasting. Basal levels of WT mice are set as 1. Different characters indicate statistical significance (p<0.05). Values are means ± SEM (n=6–8).

during the dark period in both genotypes (Figure 1B). Total locomotor activity throughout the day was significantly lower in FGF21 KO mice under conditions of both *ad libitum* feeding and fasting (p<0.05). At the end of the dark period, hypothermia accompanied the disappearance of fasting-induced hyperactivity in both genotypes.

Hepatic *FGF21* mRNA levels were induced > 100-fold by 24 h fasting in WT mice (Figure 2). The expression of *NPY* mRNA in the arcuate nucleus was significantly induced by fasting in both genotypes. Notably the *NPY* mRNA levels were slightly lower in FGF21 KO mice under conditions of both *ad libitum* feeding and fasting compared with those in WT mice, which resembled the observations of physical activity shown in Figure 1.

Chronic KD administration gradually decreased BT especially late in the dark period for the first few days in both genotypes (Figure 3). However, circadian BT levels were significantly higher in FGF21 KO mice after 3 days of KD compared with those in WT mice. Locomotor activity levels were obviously decreased after 4 days of KD in WT compared with FGF21 KO mice. The KD-induced hypoactivity accompanied by hypothermia was obviously attenuated in FGF21 KO mice.

DISCUSSION

The present findings showed that FGF21 is not required for acute fasting-induced hypothermia, although it might be involved in chronic starvation-induced hypothermia accompanied by hypoactivity in mice. We also found that FGF21 is not required for fasting-induced hypothalamic NPY expression.

The physiological role of FGF21 in BT regulation seems complex. Inagaki *et al.* (2007) found considerably enhanced fasting-induced hypothermia in FGF21 transgenic mice. Hypothermia was induced by fasting in WT mice infected with an adenovirus expressing FGF21 (Inagaki *et al.* 2007). These observations and our previous finding of hypothermia induced by PPARα activation accompanied by hepatic FGF21 expression (Chikahisa *et al.* 2008; Oishi *et al.* 2008) suggested that FGF21 induces hypothermia during acute fasting and starvation. However, FGF21 administration elevates BT in mice with diet-induced obesity (Coskun *et al.* 2008).

Our present findings suggest that endogenous FGF21 induction is not essential for acute fasting-induced hypothermia in metabolically intact mice. In accordance with these results, we recently found that PPARα is also dispensable for fasting-induced hypothermia in mice (Shimizu *et al.* in submission). Thus, the PPARα-FGF21 pathway is not apparently required for fasting-induced hypothermia in mice.

Neuropeptide Y is a mediator of fasting-induced torpor in mammals (Nelson *et al.* 2009). We recently found that bezafibrate induces hypothermia accompanied by NPY expression in the arcuate nucleus (Chikahisa *et al.* 2008) as well as fasting (Shimizu *et al.* in submission), and that an NPY Y1 receptor antagonist prevents the associated hypothermia in mice. These findings suggest that the NPY-Y1 pathway mediates hypothermia induced by fasting and PPARα activation in mice. The present findings demonstrated that FGF21 is not required for fasting-induced NPY expression in the arcuate nucleus. However, the mRNA expression levels of hypothalamic NPY were slightly decreased in the FGF21 KO mice under conditions of both *ad libitum* feeding and fasting. These findings might be associated with the lower activity of FGF21 KO, than WT mice, since NPY potently stimulates feeding.

Feeding with KD mimics the metabolic conditions of chronic starvation (Astrup *et al.* 2004). Ketogenic diets have been applied as an approach to weight loss for both obese and non-obese humans (Astrup *et al.* 2004). We found that feeding with KD gradually induced hypothermia accompanied by hypoactivity in WT mice, and that this KD-induced hypothermia was significantly attenuated in FGF21 KO mice. Badman *et al.* (2009) recently demonstrated that body weight and food consumption increases in FGF21 KO mice in contrast to normal weight loss in WT mice under KD feeding. Impaired ketosis in FGF21 KO mice fed with KD (Badman *et al.* 2009) might account for the attenuated hypothermia found in the present study. The overexpression of FGF21 promotes torpor-like metabolic changes such as the activation of lipolysis, a reduction in serum glucose and an increase in serum ketones in mice (Inagaki *et al.* 2007). Lipolysis at low BT is important for mammalian survival during long-term starvation. Attenuation of the hypothermia found in FGF21 KO mice fed with ketogenic diet supports the notion that FGF21 is involved in the physiological response to starvation.

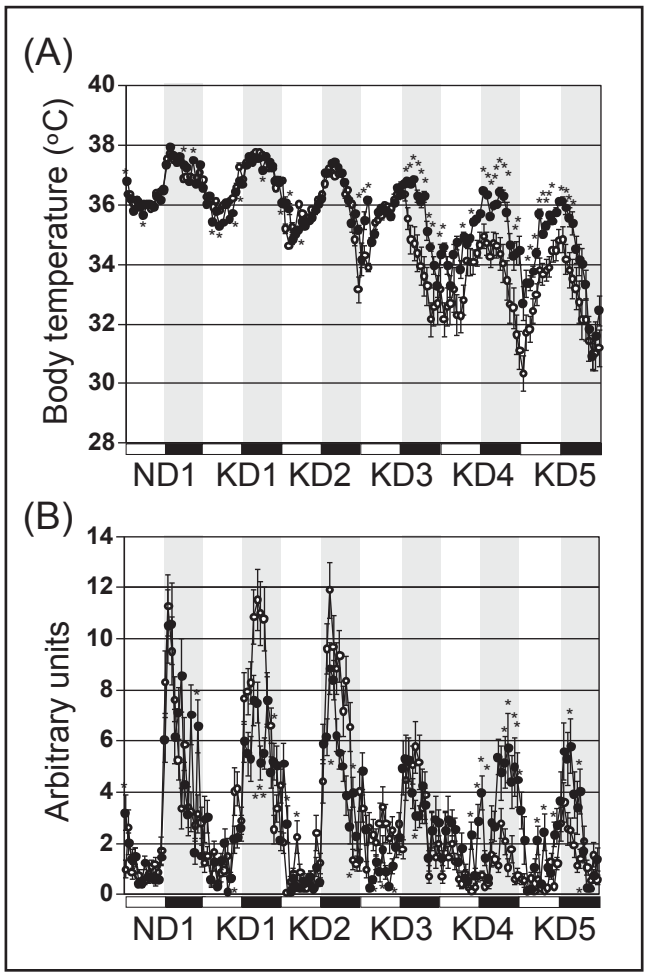

Fig. 3. Effect of feeding with ketogenic diet (KD) on body temperature (A) and locomotor activity (B) in wild-type (WT) and FGF21 knockout (KO) mice.
Mice were housed under LD 12:12 (lights on at 0:00). Food was changed from normal diet (ND) to KD at 0:00. Open and closed circles, data from WT and FGF21 KO mice, respectively. Dark phase duration is shaded in gray. Horizontal open and solid bars indicate day and night, respectively. Significant differences are indicated as $*p<0.05$. Values are means ± SEM (n=4).

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