



Effects of central FGF21 infusion on the hypothalamus–pituitary–thyroid axis and energy metabolism in rats

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Abstract

The aim of this study was to evaluate the impact of intracerebroventricular chronic fibroblast growth factor 21 (FGF21) infusion on hypothalamic–pituitary–thyroid (HPT) axis, energy metabolism, food intake and body weight. Thirty male Wistar albino rats were used and divided into three groups including control, sham (vehicle) and FGF21 infused groups ($n = 10$). Intracerebroventricularly, FGF21 and vehicle groups were infused for 7 days with FGF21 (0.72 $\mu\text{g}/\text{day}$) and artificial cerebrospinal fluid, respectively. During the experimental period, changes in food intake and body weight were recorded daily. Serum thyroid stimulating hormone (TSH), Triiodothyronine (T3) and thyroxine (T4) levels were measured using ELISA. TRH and uncoupling protein 1 (UCP1) gene expressions were analyzed by using RT-PCR in hypothalamus and adipose tissues, respectively. Chronic infusion of FGF21 significantly increased serum TSH ($p < 0.05$), T3 ($p < 0.05$) and T4 ($p < 0.001$) levels. Additionally, hypothalamic TRH ($p < 0.05$) and UCP1 gene expressions ($p < 0.05$) in white adipose tissue were found to be higher than in the vehicle and control groups. While FGF21 infusion did not cause a significant change in food consumption, it caused a reduction in the body weight of rats ($p < 0.05$). Our findings indicate that FGF21 may have an effect on energy metabolism via the HPT axis.

Keywords FGF21 · Intracerebroventricular · Thyroid hormones · UCP1

Introduction

Fibroblast growth factor 21 (FGF21), a protein 208 amino acids long, is predominantly expressed in the liver but is also expressed in multiple metabolic tissues such as skeletal muscle, adipose tissue and pancreas [1, 2], and plays very important roles in glucose uptake and oxidation, lipid metabolism and energy balance [3]. FGF21 administration decreases serum triglyceride, fatty acids and LDL cholesterol levels, suppresses lipogenesis in adipose tissue and promotes use of fatty acids instead of glucose for energy production [4, 5]. Recent studies have shown that FGF21 decreases blood glucose and promotes insulin sensitivity,

energy expenditure and weight loss following a central or systemic administration of FGF21 to rodents or monkeys with obesity or diabetes [6–9]. In order to show any of these effects, FGF21 requires the FGFR1c receptor and β -klotho co-receptor expressed in metabolically active organs, including liver, pancreas and adipose tissue [10, 11]. The β -klotho co-receptor is also expressed in the suprachiasmatic nucleus and paraventricular nucleus in the hypothalamus and the dorsal-vagal complex [12, 13]. In addition, FGFR1c is mainly expressed in the brain; especially in the hypothalamus and pituitary gland, which indicates that FGF21 may play a part in the normal physiological processes in the brain [14].

Thyroid hormones are involved in metabolic processes including energy homeostasis, regulating lipolysis, thermogenesis, and body weight [15, 16]. Thyroid hormones also regulate FGF21 gene expression in liver and adipose tissue, and serum FGF21 levels are positively associated with circulating T3 and T4 levels. On the other hand, peripheral administration of FGF21 decreases serum thyroid hormone levels. Previous studies suggest that FGF21 and thyroid hormones may be closely related, since these two potent

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regulators modulate various similar biological and metabolic functions [17–19]. The white adipose tissue (WAT) and brown adipose tissue (BAT), important target tissues for FGF21 and thyroid hormones, play major roles in energy homeostasis and thermogenesis [20, 21]. FGF21 and thyroid hormones affect the UCP1 levels in both BAT and WAT. While high UCP1 mRNA levels indicate increased mitochondrial activity, using fatty acids as an energy source and consuming more oxygen are also indicators of increasing energy expenditure in WAT, and alterations in UCP1 mRNA levels in BAT give information on the maintenance of body temperature (non-shivering thermogenesis). An elevation of UCP1 expression in WAT implies a decrease in adipogenesis and the “browning” of the white adipocytes [22–25]. FGF21 induces UCP1 expression in adipose tissue that promotes energy dissipation as heat and provides a natural defense against obesity [26].

The hypothalamus is the integration center of the food uptake related signals in the brain, and the hypothalamus–pituitary–thyroid axis plays a key role in the progression of metabolic rate, growth and development as well as energy metabolism [27, 28]. Some studies have indicated that since FGF21 receptors and co-receptors are abundantly localized in the hypothalamus, FGF21 could play a key role in food intake, energy homeostasis and endocrine signals [13, 14, 29]. So far, the effects of FGF21 and thyroid hormones on food consumption, body weight and energy homeostasis in adipose tissues have been reported [30, 31]. Also, several studies have been performed to explain the relationship between thyroid hormones and FGF21, and it has been proposed that these hormones exhibit similar metabolic effects [32–34]. However, the effects of central FGF21 infusion on thyroid hormone levels and energy metabolism in adipose tissues have never been investigated. Therefore, in this study, we investigated whether intracerebroventricular (i.c.v.) FGF21 administration plays a role in food consumption, body weight changes and energy metabolism in adipose tissue through the HPT axis.

Materials and methods

Ethics statement and animal care

The local ethics committee of Inonu University on experimental animal research approved the animal experimental protocols and use of animals in this study (#2014/A-12). The power analysis suggested at least 10 rats in each group with the highest body weight difference of the groups of 20.5 g, assumed standard deviation of 13.5 g, type I error of 0.05 and type II error of 0.20. Thirty male Wistar albino rats 4–6 weeks old and with body weights ranging between 220 and 280 g were used and all animals were randomly divided

into three groups: control, sham [vehicle; artificial cerebrospinal fluid (aCSF)] and FGF21 infused group ($n = 10$, in each group). All rats were individually housed in a temperature-controlled (21 ± 2 °C) environment with a 12 h/12 h light/dark cycle, and they were fed with ad libitum access to a standard laboratory chow diet. The food consumption and body weights were measured every morning between 09:30 and 10:00 for each animal throughout 15 days. The food consumption of the animals was calculated per 24 h.

Animal surgery

Animals were anesthetized with combination of ketamine and xylazine and positioned in a stereotaxic frame (Harvard Apparatus, USA) for i.c.v. infusion. The right lateral ventricle coordinates were determined from the Paxinos and Watson *Rat Brain Atlas* (0.8 mm posterior, 1.4 mm lateral and 4.8 mm vertical from bregma) [35]. As previously described, the brain infusion kits (ALZET, USA) were placed in the right lateral ventricle and fixed by dental cement [36].

After surgery, all rats of sham and FGF21 groups were allowed to recover for 7 days. Aseptically, Alzet osmotic mini pumps were filled with aCSF and FGF21 (dissolved in aCSF; PROSPEC, USA) for sham and FGF21-infused groups, respectively. The animals were again anesthetized after 1 week (after recovery) and osmotic mini pumps (ALZET 2ML1, USA) were subcutaneously implanted under the neck skin and cannulas were connected to osmotic mini pumps for FGF21 (0.72 µg/day) and aCSF (in the same total volumes) infusions [9].

Collection of blood and tissue samples

All animals were sacrificed at the end of 7 days, and blood, hypothalamus, and interscapular white and brown adipose tissues were collected. Serums were separated from the blood samples and stored at -80 °C until thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) hormone assay applications. The removed hypothalamus, and interscapular white and brown adipose tissues, were kept frozen at -80 °C until real-time PCR (RT-PCR) assay.

Total RNA isolation and quantitative real-time PCR

Total RNA was extracted from the hypothalamus and white and brown adipose tissues with a High Pure RNA Tissue kit (Roche, USA; lot no: 10156400). Subsequently, using a spectrophotometer (BioTek, USA) and Gen5 program, isolated and DNase-treated RNAs were quantified by absorbance at 260 and 280 nm. Complementary DNA was synthesized from 77 ng total RNA by reverse transcription with a Transcriptor First Strand cDNA Synthesis

kit (Roche, USA; lot no: 14585924) and random hexamer primers. Relative gene expressions were performed using the Roche Light Cycler 96 RT-PCR, Fast Start Essential DNA Probes Master Kit (Roche, USA; Lot no: 10048800) and β -actin, TRH and UCP1 hydrolysis probes (Roche, USA; β -actin; lot no: 90015222, TRH; lot no: 90015384, UCP1; lot no: 90015220) (Table 1). Relative gene expression was calculated by $2^{-\Delta\Delta C_t}$ relative to β -actin control.

Serum TSH, T3 and T4 analyses

The serum concentrations of TSH, T3 and T4 were measured using commercially available rat TSH, T3 and T4 ELISA kits (Elabscience Biotech, China) according to the manufacturer’s instructions.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (SPSS, USA) and gene expression data distribution was determined for normality using a Shapiro–Wilk test. Since hypothalamic TRH mRNA levels, and white and brown adipose tissue UCP1 mRNA levels were not distributed normally, groups were analyzed by Kruskal–Wallis test, and then a Conover test was used for multiple comparisons. The gene expression data were expressed as median (min–max), and a value of $p < 0.05$ was considered statistically significant. One-way ANOVA with Tamhane’s T2 post hoc test was used for determination of serum TSH, T3 and T4 levels. p values less than 0.05 for T3 levels and p values less than 0.001 for serum TSH and T4 levels were considered statistically significant. Comparisons of food intake and body weight changes among groups were performed with Wilcoxon tests followed by Bonferroni corrections. Statistical significance was defined as values of $p < 0.05$ for change of food intake per 100 g of body weight and body weight changes (%). The results are presented as mean \pm SD.

Table 1 Primers of β -actin, TRH and UCP1 genes

Gene	Primers (F: forward, R: reverse)
β -Actin	F: 5' CTGGCTCCTAGCACCATGA 3' R: 5' TAGAGCCACCAATCCACACA 3'
TRH	F: 5' AGCTCAGCATCTTGGAAAGC 3' R: 5' CCAGCAGCAACCAAGGTC 3'
UCP1	F: 5' GCCTCTACGATACGGTCCAA 3' R: 5' TCTGACCTTCACCACCTCTGT 3'

Results

I.c.v. FGF21 infusion increases thyroid hormones

Following the comparison of control and sham groups, it was determined that no difference was found between the two groups in terms of hypothalamic TRH mRNA expression. When the FGF21 treatment group was compared with the control and sham groups in terms of TRH mRNA levels, TRH mRNA levels of the FGF21 group were significantly higher compared to both control and sham groups ($p < 0.05$; Fig. 1).

Figure 2 shows the TSH levels following FGF21 infusion. TSH hormone levels in the FGF21 infused group

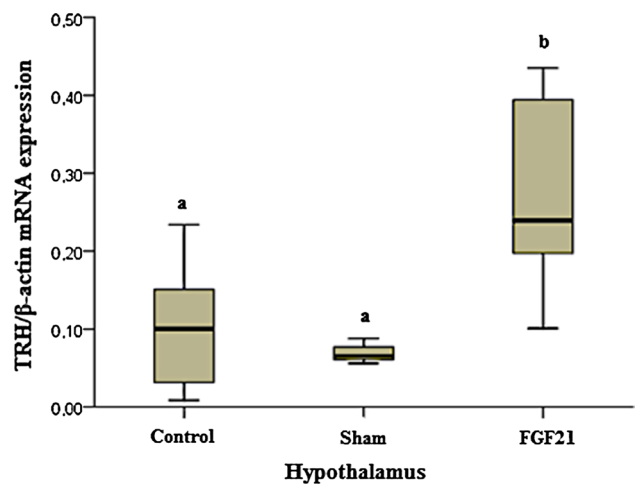


Fig. 1 TRH mRNA levels among groups following FGF21 infusion ($p < 0.05$) ($n = 10$). Groups marked with different letters were statistically different from each other

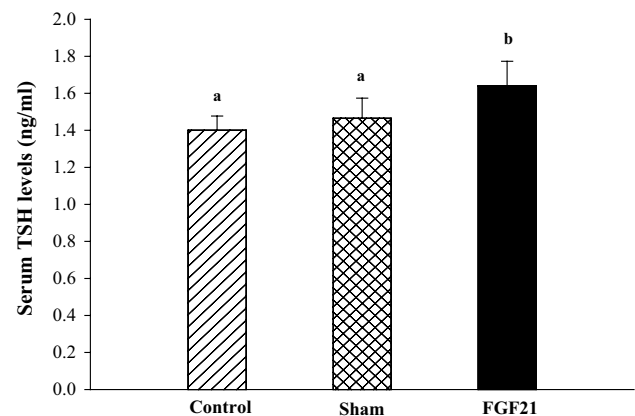


Fig. 2 Serum TSH levels among groups following FGF21 infusion ($p < 0.001$) ($n = 10$). Groups marked with different letters were statistically different from each other

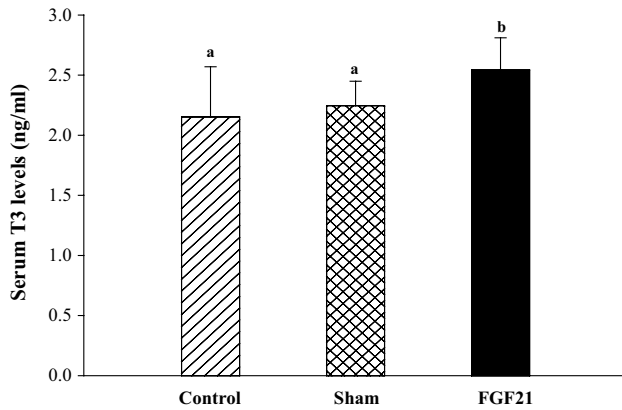


Fig. 3 Serum T3 levels among groups following FGF21 infusion ($p < 0.05$) ($n = 10$). Groups marked with different letters were statistically different from each other

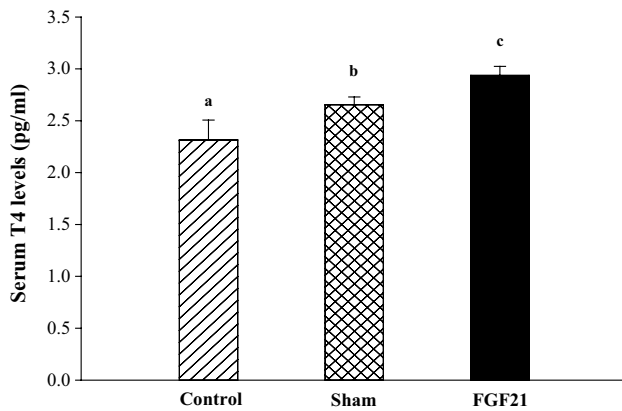


Fig. 4 Serum T4 levels among groups following FGF21 infusion ($p < 0.05$) ($n = 10$). Groups marked with different letters were statistically different from each other

were found to be significantly higher than both sham and control groups ($p < 0.001$).

Serum T3 levels were found to be statistically significantly higher in animals of the FGF21 group according to both control and sham group, but no significant difference was observed between the sham and control groups ($p < 0.05$; Fig. 3).

When the groups used in the study were compared for serum T4 levels, serum T4 levels of the sham group were found to be significantly higher than the control group ($p < 0.001$). The serum T4 levels of rats infused with FGF21 for 7 days were significantly increased when comparing rats in both control and sham groups ($p < 0.001$; Fig. 4).

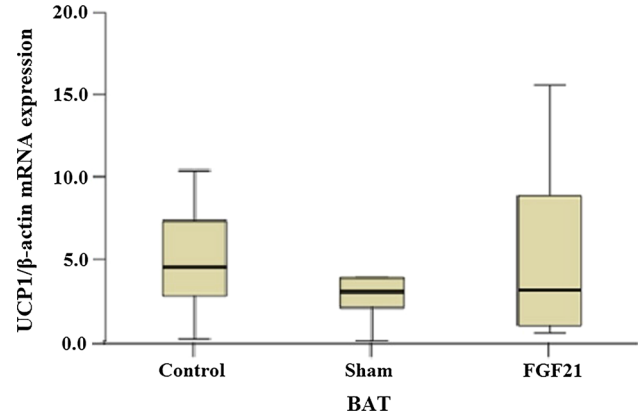


Fig. 5 UCP1 expression levels of groups in BAT following FGF21 infusion

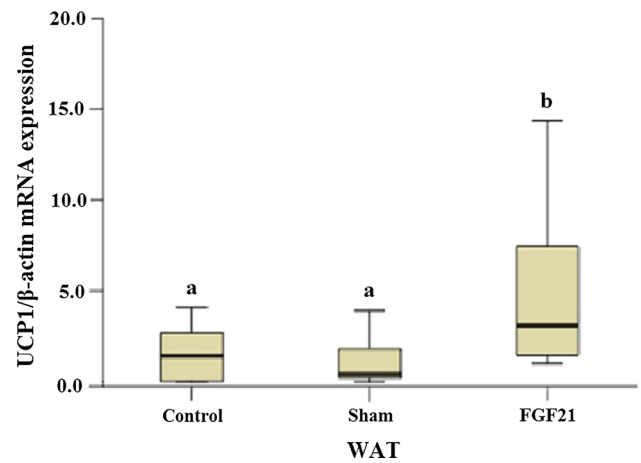


Fig. 6 UCP1 expression levels of groups in WAT following FGF21 infusion ($p < 0.05$) ($n = 10$). Groups marked with different letters were statistically different from each other

I.c.v. FGF21 infusion induces UCP1 gene expression in WAT

When control, sham and FGF21 groups were compared in terms of UCP1 mRNA expression in BAT, no statistically significant difference was observed between the groups (Fig. 5).

In WAT, UCP1 mRNA expression levels were significantly higher in animals of the FGF21 group according to both control and sham groups, while no significant difference was observed between the sham and control groups ($p < 0.05$) (Fig. 6).

I.c.v. FGF21 infusion reduces body weight (%) without changing food intake

Following i.c.v. FGF21 administration to the animals, the percentage changes in food consumption amount per 100 g

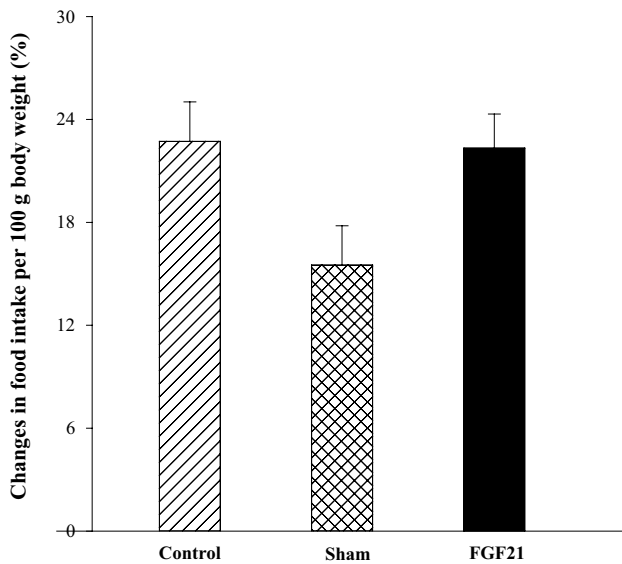


Fig. 7 Food intake among groups per 100 g of body weight ($n = 10$)

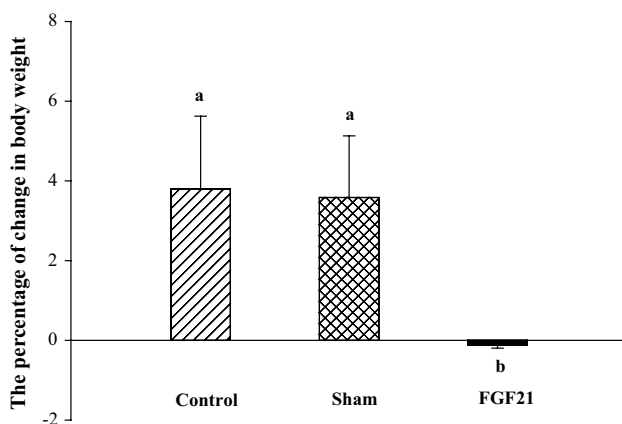


Fig. 8 Body weight changes (%) among groups ($p < 0.05$) ($n = 10$). Groups marked with different letters were statistically different from each other

body weight were calculated. When control, sham and FGF21 groups were compared in terms of food consumption per 100 g body weight, there were no statistically significant differences between the groups (Fig. 7).

The percentage of change in body weight of the group treated with FGF21 was statistically lower than both sham and control groups ($p < 0.05$). There was no statistical significance between control and sham groups (Fig. 8).

Discussion

During fasting, increased amounts of free fatty acids in the circulation induce FGF21 expression in the liver, and FGF21 induces increased lipolysis and adaptation to fasting or starvation. Previous studies have shown that thyroid hormone levels decrease during fasting, while FGF21 levels in circulation increase. In these studies, the reduction in T3 levels was not associated with fasting-mediated increased FGF21 levels or starvation-induced FGF21 levels. FGF21 and thyroid hormones are thought to interact since they regulate similar metabolisms, and are able to regulate each other's circulating levels; however, such possible interactions are still not well understood due to controversial findings [18, 34, 37]. Intraperitoneal (i.p.) T3 administration to mice has been shown to dose-dependently increase FGF21 expression in the liver, while decreasing FGF21 expression in the WAT [19] and also increase FGF21 mRNA expression, FGF21 protein content and serum FGF21 level parameters [38]. It has also been demonstrated that it did not change either the lipid profile or FGF21 expression in the liver of FGF21-KO or wild-type mice, suggesting that T3 was not essential for FGF21 expression in the liver [34]. Furthermore, subcutaneous (s.c.) FGF21 infusion was reported to decrease serum T3 and T4 levels dose-dependently without affecting serum TSH levels [6]. In all of these studies, usually FGF21 levels were examined after s.c. or i.p. administration of thyroid hormones or thyroid hormone levels were evaluated after s.c. or i.p. administration of FGF21. However, our study carries importance since it provides the first evidence related to the effects of i.c.v. FGF21 infusion on the HPT axis. We found that i.c.v. FGF21 infusion has a positive effect on the thyroid axis in central and peripheral systems due to increasing both hypothalamic TRH mRNA expression levels and serum TSH, T3, and T4 hormone levels.

Chronic FGF21 treatment is known to increase blood glucose uptake in adipocytes and induces mitochondrial biogenesis and lipid oxidation in WAT and BAT [39, 40]. Several studies have demonstrated that continuous administration of FGF21 either for short or long durations increases UCP1 expression in the adipose tissue in mice [6, 41]. On the other hand, recently, a single injection of i.c.v. FGF21 (0.5–5 μg) was shown to reduce the level of UCP1 gene expression and energy expenditure in BAT in rats [42] and also administration of s.c. FGF21 (1.6 $\mu\text{g}/\text{day}$) to mice for 5 days did not affect the UCP1 levels in WAT, while lower dose i.c.v. FGF21 (0.4 $\mu\text{g}/\text{day}$) administration for 5 days increased the UCP1 gene expression level in WAT, but not in BAT [43], which is compatible with our findings. Our results have suggested that central

FGF21 infusion can increase thermogenesis and energy expenditure through the HPT axis in WAT by increasing UCP1 gene expression, which is a biomarker of mitochondrial oxidative capacity.

Several studies have shown that FGF21 reduces body weight through sympathetic activation, physical activity or by increasing UCP1 gene expression [43–45]. Following FGF21 infusion to obese or diabetic rodents or monkeys, it was shown that FGF21 did not have a significant effect on food consumption, but reduced body weight and in some studies it was said that FGF21 also induces physical activity [7, 8, 46–49]. Following continuous FGF21 infusion, it was reported that FGF21 decreased food consumption and body weight, but on the other hand, in another study, FGF21 reduced body weight without any decrease in total caloric intake or increased physical activity [6, 50]. A single injection of i.c.v. FGF21 (0.5–5 µg) has been shown to increase food consumption [38], while in another study food uptake was suppressed when blood glucose was increased following a single i.c.v. FGF21 injection [51]. However, in these studies, the food consumption was examined following the injection of a single dose of i.c.v. FGF21. Conversely, in our study, i.c.v. FGF21 was administered with an osmotic mini pump for a week. In a study similar in design to the presented work, i.c.v. administration of FGF21 increased food consumption without changing the body weight [9]. According to the results of our study, i.c.v. FGF21 infusion did not change food intake per 100 g of body weight (%) compared to sham and control groups but reduced the percentage of change in body weight compared to sham and control groups.

In almost all the studies that examine feed consumption and body weight following FGF21 administration, measurements are performed during the light phase. Therefore, we also measured the feed consumptions only in the light phase. However, in recent years it has been reported that FGF21 exhibits a circadian oscillation which can be affected by dietary fat content [52]. Santoso et al. noted that the food intake following i.c.v. FGF21 administration varies among light and dark phases [51]. We suggest that the examination of the relationship between the secretion of FGF21 and the circadian system will enlighten the FGF21 nutrient uptake–energy expenditure association.

Consequently, the physiological effects of FGF21 on certain aspects of energy metabolism are still controversial. In this study, we aimed to contribute to better understanding this complex mechanism. Thus, in light of our findings, it can be said that i.c.v. FGF21 infusion induces energy expenditure by up-regulating UCP1 gene expression in WAT through the hypothalamic-pituitary-thyroid axis, and therefore decreases body weight despite no change in food intake.

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Author contributions Research conception: UY, ST and SS. Experimentation and analysis: UY, MD, and YC. Manuscript draft: UY and SS. Critical review: UY, ST, and SS. All authors approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Ethical Committee of Experimental Animals of the Faculty of Medicine in Inonu University, at which the studies were conducted.

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