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Differential regulation of thyrotropin-releasing hormone mRNA expression in the paraventricular nucleus and dorsomedial hypothalamus in OLETF rats

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Abstract

Thyrotropin-releasing hormone (TRH) plays an important role in the regulation of energy balance. While the regulation of TRH in the paraventricular nucleus (PVN) in response to changes of energy balance has been well studied, how TRH is regulated in the dorsomedial hypothalamus (DMH) in maintaining energy homeostasis remains unclear. Here, we assessed the effects of food restriction and exercise on hypothalamic *Trh* expression using Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Sedentary ad lib fed OLETF rats (OLETF-SED) became hyperphagic and obese. These alterations were prevented in OLETF rats with running wheel access (OLETF-RW) or food restriction in which their food was pair-fed (OLETF-PF) to the intake of lean control rats (LETO-SED). Evaluation of hypothalamic gene expression revealed that *Trh* mRNA expression was increased in the PVN of OLETF-SED rats and normalized in OLETF-RW and OLETF-PF rats compared to LETO-SED rats. In contrast, the expression of *Trh* in the DMH was decreased in OLETF-SED rats relative to LETO-SED rats. This alteration was reversed in OLETF-RW rats as seen in LETO-SED rats, but food restriction resulted in a significant increase in DMH *Trh* expression in OLETF-PF rats compared to LETO-SED rats. Strikingly, while *Trh* mRNA expression was decreased in the PVN of intact rats in response to acute food deprivation, food deprivation resulted in increased expression of *Trh* in the DMH. Together, these results demonstrate the differential regulation of *Trh* expression in the PVN and DMH in OLETF rats and suggest that DMH TRH also contributes to hypothalamic regulation of energy balance.

Keywords

Thyrotropin-releasing hormone; Neuropeptide Y; dorsomedial hypothalamic nucleus; food restriction; exercise; obesity

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Declarations of interest

All authors declare no conflict of interest related to this manuscript.

1. Introduction

Thyrotropin-releasing hormone (TRH) is a tripeptide that plays an important role in the regulation of energy balance [1]. Within the hypothalamus, TRH was originally identified as the hypophysiotropic hormone involved in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis [2]. Hypophysiotropic TRH is produced in the paraventricular nucleus (PVN) of the hypothalamus to control the function of the pituitary-thyroid axis [3], and through which PVN TRH modulates thyroid function to affect energy metabolism and thermogenesis [1]. TRH also exerts nonhypophysiotropic functions including in the control of food intake [4]. Centrally administered TRH suppresses food intake in rodents [5–8], although the precise brain site of this action is undetermined. Consistent with this anorectic effect, *Trh* mRNA expression in the PVN is decreased in response to food deprivation [9] and is up-regulated by leptin, a satiety hormone produced in the adipose tissue [10]. In contrast, a recent report has shown an opposite effect as chemogenetic stimulation of TRH neurons in the PVN activates arcuate orexigenic neurons and induces intense feeding [11]. Furthermore, dense TRH-containing neurons have also been found in the dorsomedial hypothalamus (DMH) and lateral hypothalamus (LH) [12]. It is unclear, however, whether and how these TRH neurons function in the regulation of energy balance.

The Otsuka Long Evans Tokushima Fatty (OLETF) rat is an animal model of non-insulin-dependent diabetes mellitus (NIDDM) with obesity [13] and congenitally lacks cholecystokinin (CCK)-1 receptors [14]. We have examined hypothalamic factors that may contribute to dysregulation of energy balance in OLETF rats and tested them in various conditions. Through these studies, we have identified an important role for neuropeptide Y (NPY) in the DMH in the regulation of food intake and energy expenditure. OLETF rats have elevated expression of *Npy* in the DMH (specifically in the compact subregion) compared to lean Long Evans Tokushima Otsuka (LETO) rats [15, 16], resulting from a lack of CCK-1 receptors in DMH NPY neurons [17], and this overexpression contributes to their hyperphagia and obesity [18]. In the separate studies of the effects of food restriction and physical exercise on obesity, data have shown that both pair feeding (food was pair-fed to the amount of LETO rats) and running wheel activity normalized body weight of OLETF rats [15, 19], implying that both treatments intervene the outcomes of DMH NPY overexpression in OLETF rats [15, 19]. In addition, running activity induces elevated expression of corticotropin-releasing factor (CRF or CRH) in the DMH (specifically in the medial part of the dorsal subregion) of OLETF rats, and we have suggested that this elevation mediates a short term inhibitory effect of exercise on food intake and body weight in OLETF rats [19]. Using the immediate early gene *c-fos* as a neuronal activation marker, a recent study has revealed that running activity induces neuronal activation in the DMH broadly including both dorsal and ventral subregions that do not contain NPY and CRF neurons [20], implicating other non-NPY and non-CRH neurons in the DMH in the regulation of energy balance.

In this study, we aimed at testing the potential relationship between hypothalamic TRH and the feeding and body weight regulation using OLETF rats. We examined the effects of running activity on food intake, body weight and hypothalamic *Trh* expression, and

compared these effects to those produced by food restriction in OLETF rats. Our results demonstrate the differential regulation of *Trh* mRNA expression in the PVN and the DMH, suggesting that DMH TRH may exert a distinct role in the regulation of food intake and body weight.

2. Materials and methods

2.1. Animals

Four weeks old male OLETF and age-matched male lean LETO rats were obtained from Hoshino Laboratory Animals, Inc. in Japan. Rats were individually housed in home cages and maintained on a 12:12-h light-dark cycle (lights on at 0600 h) in a temperature-controlled colony room (22°C) with ad libitum access to standard regular chow and tap water. After one week of adaptation, experimental procedures were carried out. All procedures were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University and in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

2.2 Running wheel access and food restriction

At 5 weeks of age, OLETF rats were assigned randomly into three groups. The first group of eight OLETF rats had ad libitum access to regular chow and without access to running wheels and served as the sedentary normal-fed controls (OLETF-SED). The second group of six OLETF rats was transferred to individual running wheel cages (OLETF-RW) as previously described [19]. Wheels were unlocked at 7 weeks of age and OLETF-RW rats had voluntary access to running wheels. Food intake and running wheel activity were computer monitored 24 h/d. The third group of six OLETF rats was treated as a food restriction group (OLETF-PF) in which OLETF-PF rats were pair-fed to the same amount of chow that was consumed by sedentary normal-fed LETO rats (LETO-SED) as previously described [15]. Chow was provided right before lights off. During the study, eight LETO-SED rats were maintained on regular chow and served as the normal controls. Body weight was recorded daily. At 13 weeks of age, rats were sacrificed in the non-fasted state between 0900 and 1100h. Trunk blood was taken for evaluation of plasma levels of glucose and leptin. Plasma glucose levels were measured using a blood glucose meter (Glucometer Elite, Bayer), and plasma leptin concentration was determined using a rat leptin RIA kit (Linco Research Inc., St. Charles, MO). Brains were removed and rapidly frozen for subsequent analyses of hypothalamic gene expression.

2.3. Food deprivation

Eight male Long-Evans rats (Charles River Laboratories) weighing 250–300g were individually housed in home cages and maintained on a 12:12-h light-dark cycle (lights on at 0700h) in a temperature-controlled colony room. At the beginning of experiments, rats were divided into 2 groups. Ad lib-fed group of rats (n=4) had access to standard chow ad libitum daily. Food deprivation rats (n=4) were deprived food at 1000h for 48h before sacrifice. Their body weights were recorded daily. At the end of experiments, rats were sacrificed between 1000 and 1100h. Brains were removed and rapidly frozen for subsequent analyses of hypothalamic gene expression.

2.4. Cryosections

Coronal sections (14 μ m) through the hypothalamus were cut via a cryostat, mounted on superfrost/plus slides (Fisher Scientific) as series of six slides as previously described [21]. One slide from each series was stained with cresyl violet acetate and used for the selection of sections for the following in situ hybridization determinations. Sections for mRNA determinations of PVN *Trh* (1.8–2.0 mm caudal to bregma) and DMH *Trh* (3.1–3.3 mm caudal to bregma) were selected [21, 22].

2.5. In situ hybridization (ISH) determination

The full length of mouse *preproTrh* cDNA (OriGene Technologies, Inc) was sub-cloned into pGEM-11Zf(+). As previously described [21], ³⁵S-labeled antisense riboprobes of *Trh* and *Npy* were transcribed with *in vitro* transcription systems (Promega, Madison, WI). ISH determinations were carried out using our standard method [21]. Data from OLETF rats were normalized to LETO-SED rats as 100%. Data from food deprivation study were normalized to ad lib-fed Long-Evans rats as 100%.

2.6. RNAscope ISH

Sections containing PVN and DMH regions were selected as described above. Both RNAscope® Probe- Rn-*Trh* (catalog #, 406621) and RNAscope® Probe- Rn-*Npy* (catalog #, 450971-C2) were purchased from Advanced Cell Diagnostics (Hayward, CA). All staining steps were performed following RNAscope manufacturer protocols with minor modification [23, 24]. Stained slides were cover-slipped with fluorescent mounting medium (ProLong Gold Antifade Reagent P36930; Life Technologies), and sections with *Trh* (red) and *Npy* (green) fluorescence were examined on a Zeiss Axio Imager system (Carl Zeiss MicroImaging, Inc.).

2.7. Data analyses

All values are presented as means $\pm$ SEM. Data were analyzed using StatSoft Statistica-7 software. Data for body weight and food intake were analyzed using two-way repeated measures ANOVA. Data for blood glucose, plasma leptin and hypothalamic gene expression were analyzed using one-way ANOVA. All ANOVA's were followed by pairwise multiple Fisher least significant difference (LSD) comparisons. Data from food deprivation study were analyzed using Student *t* test (two-tailed). $P < 0.05$ was interpreted as a significant difference.

3. Results

3.1. Effects of food restriction and running activity on obesity of OLETF rats

As expected, OLETF-SED rats developed obesity from 4 weeks to 13 weeks of age, showing a main effect of strain on body weight compared with LETO-SED rats ($P < 0.001$, Fig. 1A). OLETF-SED rats started to gain significantly more body weight at 5 weeks of age ($P < 0.05$) and became 27% heavier by 13 weeks of age compared with LETO-SED rats (Fig. 1A). OLETF-SED rats were hyperphagic, consuming significantly more chow by an average of 37% than that of LETO-SED rats over the experimental period (Fig 1B). Food restriction via

pair-feeding OLETF rats (OLETF-PF) to the intake of LETO-SED rats normalized body weight of OLETF rats as seen in LETO-SED rats ($P>0.1$, Fig. 1A). Running wheel access and resulting running activity (Fig. 1C) also prevented the obesity of OLETF rats. During the initial week, OLETF-RW rats ate only about 30% of the food consumed by OLETF-SED rats (Fig. 1B). This level of reduction was transient and OLETF-RW rats gradually increased their food intake, but the increase did not compensate for the initial reduction of food intake; the food intake of OLETF-RW rats did not exceed that of OLETF-SED rats during running wheel access (Fig. 1B). Over the experimental period, OLETF-RW rats ate about a total of 17% more than OLETF-PF rats, but still ate 15% less in total than OLETF-SED rats (Fig. 1B). As a result, the body weight of OLETF-RW rats was significantly decreased compared to OLETF-SED rats ($P<0.001$) and down to that of LETO-SED rats ($P>0.05$, Fig. 1A). Together, the body weights among the three groups of LETO-SED, OLETF-RW, and OLETF-PF rats did not differ at sacrifice (Table 1).

3.2. Effects of food restriction and running activity on plasma leptin and glucose levels of OLETF rats

At the end of experiments, analysis of plasma leptin levels revealed a significantly main effect ($P<0.001$, Table 1). OLETF-SED rats had a 3-fold increase in plasma leptin levels relative to LETO-SED controls ($P<0.001$, Table 1). Both OLETF-RW and OLETF-PF rats had significantly decreased plasma leptin levels compared to OLETF-SED rats ($P<0.001$, Table 1). While the plasma leptin levels of OLETF-PF and LETO-SED rats did not differ ($P>0.05$, Table 1), plasma leptin levels in OLETF-RW rats were decreased to 71% and 75% of those in LETOSED ($P<0.05$) and OLETF-PF rats ($P<0.01$) respectively (Table 1), suggesting that running activity itself exerted an additional effect on plasma leptin levels.

As expected, at 13 weeks of age OLETF-SED rats became hyperglycemia. Blood glucose levels were significantly increased in OLETF-SED rats compared with LETO-SED rats ($P<0.01$, Table 1). This increase was significantly reduced in both OLETF-RW and OLETF-PF rats ($P<0.05$) down to the level as seen in LETO-SED rats (Table 1).

3.3. Effects of food restriction and running activity on hypothalamic Trh and Npy mRNA levels

Trh mRNA levels: As previously reported [25], *Trh* was densely expressed in the PVN, the DMH, and the LH (Fig. 2A&B). Analysis of mRNA expression levels revealed a significant group effect on *Trh* expression in the PVN ($P<0.05$). *Trh* expression levels were significantly increased in the PVN of OLETF-SED rats compared with LETO-SED rats ($P<0.01$, Fig. 3A), whereas this increase was reduced in both OLETF-RW and OLETF-PF rats relative to OLETFSED rats (Fig. 3A), down to those of LETO-SED rats ($P>0.05$, Fig. 3A).

Within the DMH, *Trh* expression was primarily localized to the dorsal and ventral subregions (Fig. 2B). The expression of *Trh* in the DMH was significantly different among the groups ($P<0.001$, Fig. 3B). Surprisingly, in contrast to PVN TRH, the expression of *Trh* in the DMH of OLETF-SED rats was down-regulated to about 72% of that in LETO-SED rats ($P<0.05$, Fig. 3B). This alteration was normalized in OLETF-RW rats as seen in LETO-SED rats ($P<0.05$, Fig. 3B). Food restriction caused a significant increase in DMH *Trh*

expression in OLETF-PF rats; their expression levels were 36% higher than those of LETO-SED rats ($P<0.01$, Fig. 3B) and significantly more than those of OLETF-RW rats ($P<0.05$, Fig. 3B). In addition, the expression of *Trh* in the LH did not differ among the groups ($P=0.915$, Fig. 3C).

Npy mRNA levels: *Npy* is mainly expressed in the ARC and the DMH (Fig. 2C) [26]. As we reported previously [15, 19], OLETF-SED rats had decreased expression of *Npy* in the ARC and a slightly but non-significant increase in DMH *Npy* expression compared with LETO-SED rats (Fig. 3D); while decrease expression of ARC *Npy* was reversed in OLETF-RW and OLETF-PF rats (Fig. 3D), both food restriction and running activity resulted in a significant increase in DMH *Npy* expression in OLETF rats relative to LETO-SED rats (Fig. 3E). In addition, we found that although the body weights of OLETF-RW and OLETF-PF rats were similar, DMH *Npy* expression was significantly reduced in OLETF-RW rats compared with OLETF-PF rats ($P<0.001$, Fig. 3E).

3.4. Upregulation of *Trh* expression in the DMH in response to food deprivation

We next examined the effect of acute food deprivation on *Trh* expression in the DMH in intact rats. As expected, food deprivation resulted in significant weight loss (Fig. 4A). While *Trh* expression in the PVN of food deprived rats was reduced by 22% compared with ad lib-fed rats, food deprivation resulted in a 45% increase in *Trh* expression in the DMH of food-deprived rats relative to ad lib-fed rats ($P<0.05$, Fig. 4B). Thus, these results provide additional evidence indicating the differential regulation of *Trh* expression in the PVN and DMH in response to altered energy balance.

3.5. *Trh* and *Npy* were not co-expressed in DMH neurons

Double labeling ISH revealed that *Npy* and *Trh* expression in the DMH were localized into distinct subregions (Fig. 5). NPY-expressing neurons (green) were mainly restricted to the compact subregion of the DMH (Fig. 5A), whereas TRH-expressing neurons (red) were more dispersedly distributed in the DMH; TRH neurons were found at high levels in the dorsal subregion, very few in the compact subregion, and at moderate levels in the ventral subregion (Fig. 5B). Double labeling ISH did not detect dual staining of NPY and TRH in DMH neurons (Fig. 5C).

4. Discussion

In this study, we have determined the effects of food restriction and running activity on the hypothalamic expression of *Trh* in OLETF rats. Both interventions prevented the obesity, hyperglycemia, and hyperleptinemia of OLETF rats. Compared with LETO-SED rats, PVN *Trh* expression was increased in obese OLETF-SED rats and this increase was reversed in normal weight OLETF-PF and OLETF-RW rats. In contrast, OLETF-SED rats had decreased expression of *Trh* in the DMH relative to LETO-SED rats. While this decrease was normalized in OLETF-RW rats, food restriction resulted in a significant increase in DMH *Trh* expression in OLETF-PF rats compared with LETO-SED rats. Overall, these results demonstrate the differential regulation of *Trh* expression in the PVN and the DMH,

suggesting that DMH TRH may exert a distinct role in the regulation of food intake and body weight.

Using OLETF rats, we and other investigators have shown that physical exercise and food restriction ameliorate obesity [15, 19, 27–29], but the neural mechanism underlying these effects remains incompletely understood. PVN TRH plays a central role in the control of HPT function [1] and exercise affects the HPT axis [30]. Evidence has also shown that leptin up-regulates PVN TRH neural signaling, whereas fasting or lowered plasma leptin levels produce opposite effects [10], suggesting that PVN TRH acts to cause negative energy balance. The changes of PVN *Trh* expression in OLETF rats (upregulation in obesity and normalization in normal weight) and decreased expression of PVN *Trh* in food-deprived rats are consistent with this action, implying that PVN TRH is appropriately regulated in response to alterations in body weight or energy balance in OLETF rats.

Although TRH-expressing neurons have long been found in the DMH [25], a role for DMH TRH in the regulation of food intake and energy balance has not been investigated. In this study, we found that in contrast to PVN TRH, the expression of *Trh* in the DMH was significantly decreased in OLETF-SED rats relative to LETO-SED controls, whereas food restriction resulted in a significant increase in DMH *Trh* expression in OLETF-PF rats. These results indicate that DMH *Trh* expression is down-regulated in response to excess energy store as seen in OLETF-SED rats and up-regulated in response to food restriction as seen in OLETF-PF rats, suggesting that DMH TRH may serve as a starvation-associated signal. In support of this point, *Trh* expression was significantly increased in the DMH in response to food deprivation. Such results may also provide the neural basis of the effects of DMH lesions on energy balance [31], i.e., a loss of TRH neurons resulting from lesions of the DMH contributes to their hypophagia and lowered body weight. Furthermore, we found that DMH *Trh* expression was normalized in OLETF-RW rats. Since the body weights of OLETF-PF and OLETF-RW rats did not differ, the different responses of DMH *Trh* expression between food restricted OLETF PF and satiated OLETF-RW rats provide additional evidence that hunger rather than satiation signaling stimulates *Trh* expression in the DMH, also implying that exercise may limit DMH *Trh* overexpression to account for the overall prevention of obesity in OLETF rats.

We have demonstrated that DMH NPY neurons contain CCK-1 receptors but not leptin receptors, and activation of DMH NPY neurons is inhibited by CCK but not leptin [17, 32]. In this study, we found that TRH-expressing neurons were not co-localized with DMH NPY neurons. TRH-expressing neurons were mainly found in the dorsal and ventral DMH and their expression levels were decreased in OLETF-SED rats lacking CCK-1 receptors, suggesting that DMH CCK does not appear to act on TRH neurons. Since leptin receptors are primarily expressed in the ventral DMH of rats [26] where some TRH-expressing neurons were found, whether these TRH neurons contain leptin receptors or are under the control of leptin is unclear, which merits further investigation.

Our previous studies have revealed an etiological role for DMH NPY in the development of obesity in OLETF rats [16, 33]. Overexpression of NPY in the DMH contributes to the hyperphagia and obesity of OLETF rats, whereas knockdown of NPY in the DMH

ameliorates these alterations [15, 16, 18]. In this study, we demonstrated the effects of food restriction and running activity on ARC and DMH *Npy* expression in OLETF rats as we previously reported [15, 19], showing that food restriction and running activity normalized ARC *Npy* expression in OLETF rats and food restriction resulted in a significant elevation of DMH *Npy* expression. In addition, we found that levels of DMH *Npy* expression in OLETF-RW rats were significantly reduced compared with OLETF-PF rats and were no longer different from those of OLETF-SED rats. Overall, these data suggest that running activity prevents the obesity of OLETF rats likely through affecting multiple factors including DMH NPY, DMH TRH and others.

5. Conclusions

Dietary control and physical exercise are two important lifestyle interventions for fighting against obesity and its-associated disorders such as type 2 diabetes. Using OLETF rats, we demonstrated that both treatments prevented obesity, hyperglycemia, and hyperleptinemia. Gene expression determinations revealed that PVN *Trh* expression in OLETF rats was appropriately regulated in response to alterations in energy balance. The pattern of *Trh* expression in the DMH of OLETF rats was opposite to that of PVN *Trh* expression. DMH *Trh* expression was decreased in response to overnutrition but increased in response to negative energy balance. While food restriction prevented the obesity of OLETF rats via limiting energy intake, running activity prevented the obesity via affecting both energy expenditure and intake regulatory aspects. Exercise appears to limit the responses of starvation signals (such as DMH NPY and probably DMH TRH) to increased energy expenditure. Together, these results demonstrate the differential regulation of *Trh* mRNA expression in the PVN and DMH in OLETF rats and suggest that DMH TRH may exert a distinct role in the regulation of food intake and body weight.

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Highlights

- Food restriction and physical exercise prevent the obesity of OLETF rats
- Differential regulation of TRH expression in the PVN and the DMH
- TRH expression is upregulated in the PVN and downregulated in the DMH of OLETF rats
- Exercise normalizes altered expression of TRH in the PVN and the DMH in OLETF rats
- Food restriction results in increased expression of DMH TRH in OLETF rats

Fig. 1.

Effects of food restriction or running activity on body weight in Otsuka Long-Evans Tokushima fatty (OLETF) rats. (A) Body weight in the four groups of rats. OLETF-SED, ad lib-fed sedentary OLETF rats; LETO-SED, ad lib-fed sedentary lean Long-Evans Tokushima rats; OLETF-PF, OLETF rats were pair-fed to the intake of LETO-SED rats; OLETF-RW, OLETF rats had voluntary access to running wheels. (B) Food intake in the four groups of rats. (C) Running activity in OLETF-RW rats. Values are means $\pm$ SEM. $n = 6-8/\text{group}$.

Fig. 2.

Representative images of in situ hybridization determination of thyrotropin-releasing hormone (*Trh*) and neuropeptide Y (*Npy*) mRNA in the hypothalamus with ^{35}S -labeled antisense riboprobes in lean rats. (A-B) *Trh* mRNA expression in the paraventricular nucleus (PVN) and dorsomedial hypothalamus (DMH); (C) *Npy* mRNA expression in the arcuate nucleus (ARC) and the DMH. dDMH: the dorsal subregion of the DMH; cDMH: the compact subregion of the DMH; vDMH: the ventral subregion of the DMH; LH: the lateral hypothalamus.

Fig. 3.

Effects of food restriction or running activity on hypothalamic expression of *Trh* and *Npy*. (A-C) levels of *Trh* mRNA expression in the PVN (A), the DMH (B), and the lateral hypothalamus (LH) (C); (D-E) levels of *Npy* mRNA expression in the ARC (D) and the DMH (E). Values are means $\pm$ SEM. $n = 6-8$ /group. * $P < 0.05$ vs. LETO-SED, # $P < 0.05$ vs. OLETF-SED, § $P < 0.05$ vs. OLETF-PF.

Fig. 4.

Effects of food deprivation on body weight and hypothalamic *Trh* mRNA expression in intact rats. (A) Body weight decreased in food deprived rats (FD) compared with ad lib-fed controls (Ad lib-fed). (B) *Trh* mRNA expression in the PVN and the DMH in response to 48 hours of food deprivation. Values are means $\pm$ SEM. $n = 4/\text{group}$. * $P < 0.05$ vs. Ad lib-fed.

Fig. 5. Representative microimages of NPY and TRH expressing neurons in the DMH using double labeling RNAscope in situ hybridization histochemistry. (A) *Npy* mRNA expression in DMH neurons (green); (B) *Trh* mRNA expression in DMH neurons (red); (C) Merged images of dual staining of NPY and TRH in DMH neurons. 3V: the third ventricle.

Table 1.

Effects of food restriction or running activity on body weight, plasma leptin and glucose levels in OLETF rats at the end of experiments.

	LETO-SED	OLETF-SED	OLETF-RW	OLETF-PF
Body Weight, g	378 ± 8.2	491 ± 14.7 [*]	339 ± 5.2 [#]	359 ± 3.9 [#]
Plasma Leptin, ng/ml	7.5 ± 0.52	22.2 ± 2.58 [*]	2.2 ± 0.63 ^{*,#,\$}	8.8 ± 1.15 [#]
Plasma Glucose, mg/dl	165 ± 11.2	244 ± 21.7 [*]	156 ± 16.4 [#]	163 ± 14.3 [#]

Values are means + SEM. *n* = 6–8/group.

^{*} *P* < 0.05 compared with LETO-SED rats,

[#] *P* < 0.05 compared with OLETF-SED rats, and

^{\$} *P* < 0.05 compared with OLETF-PF rats by one way ANOVA and followed by pairwise multiple Fisher LSD comparisons.