FULL PAPER *Physiology*

Free Access to Running Wheels Abolishes Hyperphagia in Human Growth Hormone Transgenic Rats

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ABSTRACT. Obesity is a major health problem, and increased food intake and decreased physical activity are considered as two major factors causing obesity. Previous studies show that voluntary exercise in a running wheel decreases not only body weight but also food intake of rats. We previously produced human growth hormone transgenic (TG) rats, which are characterized by severe hyperphagia and obesity. To gain more insight into the effects on physical activity to food consumption and obesity, we examined whether voluntary running wheel exercise causes inhibition of hyperphagia and alteration of body composition in TG rats. Free access to running wheels completely abolished hyperphagia in TG rats, and this effect persisted for many weeks as far as the running wheel is accessible. Unexpectedly, though the running distances of TG rats were significantly less than those of wild type rats, it was sufficient to normalize their food consumption. This raises the possibility that rearing environment, which enables them to access to a running wheel freely, rather than the amounts of physical exercises is more important for the maintenance of proper food intake.

KEY WORDS: hyperphagia, obesity, rearing environment, running wheel, voluntary exercise.

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Obesity and its concomitant diseases have been recognized as a major health problem, especially in the developed countries. It is commonly considered that increasing physical activity and reducing food intake are the two main cornerstones in the prevention and treatment of obesity [8, 14]. While exercise provides long-lasting weight loss [25], food restriction is often associated with consequent excessive food intake and weight gain both in humans and animals [16], suggesting the difficulties in controlling dietary limitation. It has been shown that the voluntary exercise in a running wheel decreases food intake of rats [1, 3, 4, 19], indicating that food consumption is under the control of physical activity. However, the effect of voluntary exercise is only transient, and the amount of food intake returns gradually to the level seen in the rats without wheel running. In addition, the effect of wheel running has been examined in the rats with hyperphagia, but the results vary according to the strains used [1, 22]. Thus, elucidating the mechanism in which voluntary running regulates food intake further would be important for establishing novel approaches to prevent obesity.

We have previously generated transgenic (TG) rats expressing human growth hormone (hGH) gene under the control of mouse whey acidic protein (WAP) promoter [12]. The TG rats exhibit unique serum growth hormone (GH) phenotype; serum hGH concentrations are relatively low, while their endogenous pulsatile rat GH secretion was almost completely suppressed [12]. The TG rats show significant hyperphagia from their young stage [5, 6, 9] and severe obesity [5–7, 9, 11], while their body length was almost normal [11, 12]. It is widely accepted that GH has effects on metabolism of carbohydrate and lipid, such as increasing glucose output of liver or increasing free fatty acid levels. Thus, obesity of TG rats is considered to be due to both alteration of these GH functions and hyperphagia.

In the present study, to gain more insight into the relationship between physical activity and food consumption, we employed TG rats as a model for obesity accompanied with hyperphagia and examined whether voluntary running wheel exercise causes an inhibition of their hyperphagia persistently.

MATERIALS AND METHODS

Animals: Generation of the hGH transgenic rats has been described previously [12]. In this study, male transgenic (TG) rats and their male wild type (WT) littermates were used. The number of pups per mother was controlled to seven, and the pups were weaned at 21 days of age. After weaning, 4 male rats were maintained in one cage until used for experiments. They were housed in a room at 23°C with a lightning condition of 12-hr light and 12-hr darkness (lights on at 0800 hr). Laboratory chow (Labo MR Standard, Nihon Nousan Co., Yokohama, Japan) and water were provided *ad libitum*. All animal experiments performed in this study were according to the Guide for the Care and Use of Laboratory Animals, the University of Tokyo, and approved by the Institutional Animal Care and Use Committee of the University of Tokyo.

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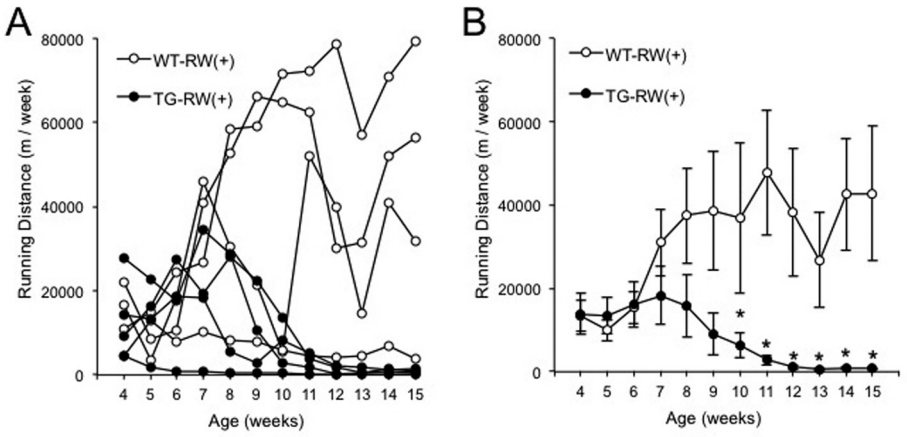

Fig. 1. Running distances in WT-RW (+) and TG-RW (+) rats. (A) Individual running distance (meters per week) in WT and TG rats from 4 weeks of age. (B) The average of running distances in WT-RW (+) and TG-RW (+) rats from 4 weeks of age. Values are means ± SEM (n=4). * $P<0.05$ vs. WT by a Student's *t* test.

Experiment 1: At 4 weeks of age, TG and WT rats were transferred to individual running wheel cages (indicated as TG-RW (+), n=4, and WT-RW (+), n=4, respectively) or normal plastic cages (indicated as TG-RW (−), n=4, and WT-RW (−), n=4, respectively) and maintained for 12 weeks. The size of the normal plastic cage is 28 × 44 × 20.5 cm. The running wheel cage was equipped with a running wheel (diameter 32 cm and width 10 cm) and a home cage (38 × 28 × 38 cm), and the rats were allowed free access to the wheel. The amount of food intake, body weight and running distance were measured every week. Pre-weighed aliquots of food were placed on the lids of the cages, and water was supplied by water bottle. The weight of laboratory chows was measured every same day of the week at the same time 1,200 hr, and the difference of the weight compared to the previous data was regarded as the amount of food intake. Crumbled chows were not collected and counted [23]. Only the running distance in the running wheel was measured by "revolution counter" (Kori seiki, Tokyo, Japan). At the end of the experiment, rats were removed from the cages and killed by decapitation 1–2 hr before the lights were turned off. Pituitary glands, intra-thoracic organs, intra-abdominal organs and leg skeletal muscles were weighed, and femoral lengths were measured. Trunk blood was collected at the time of decapitation, and EDTA·2Na was added (1.25 mg/m*l* blood). The collected blood was kept on ice and centrifuged at 1,200 × *g* at 4°C for 15 min. The plasma was stored at −20°C until use. Plasma leptin concentration was measured by using a leptin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). The intra-assay precisions (as%CV) of this system were less than 10%.

Experiment 2: Based on the results obtained from Experiment 1, we conducted an additional experiment. At 8 weeks of age, TG rats were transferred to individual running wheel cages (indicated as TG-RW (+), n=8) or normal plastic cages (indicated as TG-RW (−), n=5) and maintained as described above. After four weeks of free access to the running wheel, half of TG-RW (+) had their running wheels locked (indicated as TG-RW (+)-locked, n=4). These wheels were locked completely. After four weeks, peripheral blood and cerebrospinal fluid (CSF) were taken for evaluation of human GH levels. Peripheral blood was collected via tail vein and centrifuged at 1,200 × *g* at 4°C for 15 min, and the plasma was stored at −20°C until use. CSF was sampled by the way as described previously [15] and stored at −20°C until use. Human GH concentrations were measured by using a HGH ELSIA kit (Alpha Diagnostic International, San Antonio, TX, U.S.A.). The intra-assay precisions (as%CV) of this system were less than 12%.

Statistics: The data are presented as the means ± SEM for each experimental group. Statistical differences between groups were evaluated using Student's *t* test with Bonferroni corrections or ANOVA followed by Tukey-Kramer's test.

RESULTS

Results 1

Running distances are considerably different between WT and TG rats: To determine whether there are any differences in running activity between WT and TG rats, we measured their running distances under free access to running wheel. Running distances varied among individuals and ages in both WT-RW (+) and TG-RW (+) (Fig. 1A). However, in TG-RW (+), their overall running distances were gradually decreased after 7 weeks of age and showed significantly less distances compared to WT-RW (+) after 11 weeks of age (Fig. 1B, $P<0.05$ vs. WT-RW (+), by a Student's *t* test). These results show that innate running activity is considerably different between WT and TG rats.

Running wheel access decreases body weight gain in both

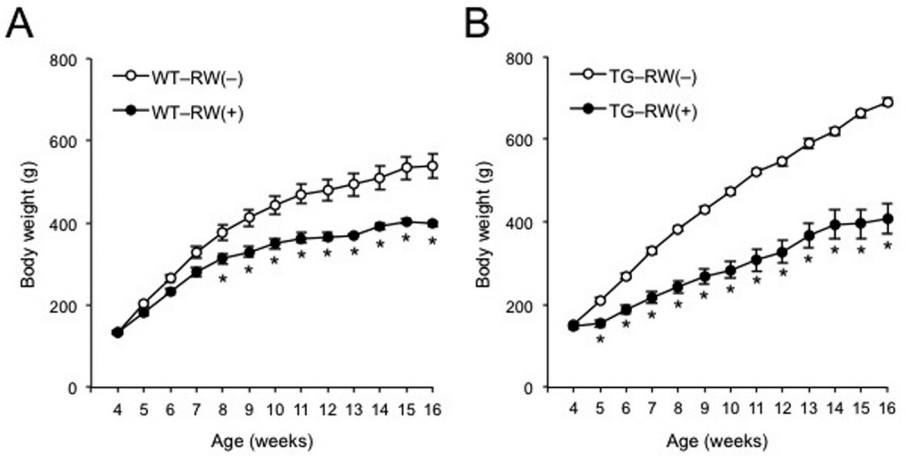

Fig. 2. Changes in body weights in WT and TG rats. The body weight of WT (A) and TG (B) rats with or without a running wheel was measured every week. Values are means ± SEM (n=4). * $P<0.05$ vs. RW (−) by a Student's *t* test.

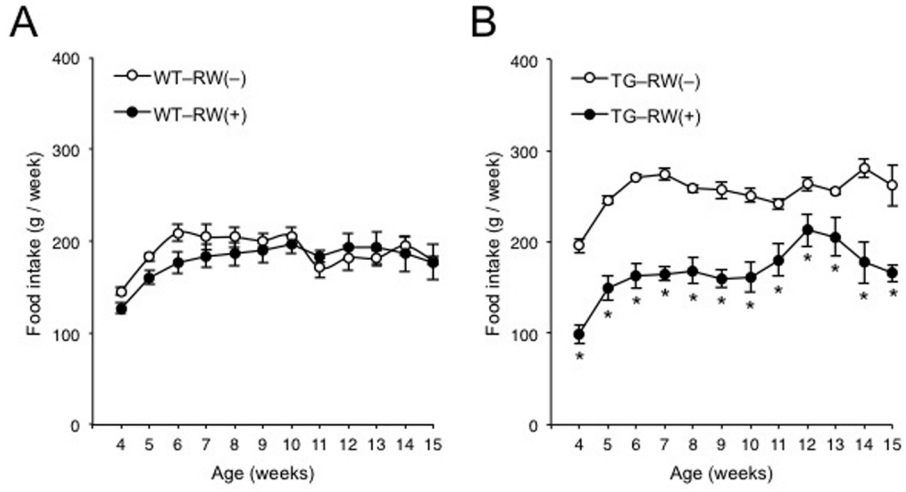

Fig. 3. Effect of running wheel access on food intake in WT and TG rats. (A) The amount of food intake (grams per week) of WT (A) and TG (B) rats with or without running wheel was measured every week. Values are means ± SEM (n=4). * $P<0.05$ vs. TG-RW (−) by a Student's *t* test.

WT and TG rats: We next investigated the changes of body weights in WT and TG rats with or without running wheel (Fig. 2). As reported previously [5–7, 9, 11], body weight gain in TG rats was higher than that in WT rats without running wheel access after 14 weeks of age ($P<0.0167$, by a Student's *t* test and a Bonferroni correction). Running wheel access resulted in decreased body weight gain in both WT and TG rats. Compared to WT rats, access to the running wheel had even greater effects on body weight in TG rats. TG-RW (+) showed significantly less body weight compared to WT-RW (−) from 4 to 12 weeks of age ($P<0.0167$, by a Student's *t* test and a Bonferroni correction) and TG-RW (−) at all time points examined ($P<0.0167$, by a Student's *t* test and a Bonferroni correction), and their body weights were similar to those of WT-RW (+) at the end of the experiment ($P=0.786$, by a Student's *t* test). These results show that free access to a running wheel suppresses body weight gain in both WT and TG rats.

Running wheel access abolishes hyperphagia in TG rats: To examine whether the decreased weight gain in both WT and TG rats with running wheel access is accompanied by an alteration of food consumption, their weekly amounts of food intake were measured. There was no difference in the amounts of food intake between WT-RW (−) and WT-RW (+) throughout the experiment (Fig. 3A, $P>0.0125$, by a Student's *t* test and a Bonferroni correction). In contrast, in TG rats that exhibit innate hyperphagia ($P<0.0125$ vs. WT-RW (−), by a Student's *t* test and a Bonferroni correction), running wheel access significantly decreased their food intakes (Fig. 3B, $P<0.0125$, vs. TG-RW (−) by a Student's

Table 1. Organ weights, femoral lengths and serum leptin levels at 16 weeks of age

	WT-RW (−)	WT-RW (+)	TG-RW (−)	TG-RW (+)
Pituitary gland (mg)	10.28 ± 0.98	9.93 ± 0.84	4.28 ± 0.44[a)]	4.58 ± 0.15[a)]
Heart (g)	1.60 ± 0.06	1.53 ± 0.11	2.06 ± 0.10[a)]	1.46 ± 0.10[b)]
Lung (g)	2.08 ± 0.25	1.71 ± 0.06	2.09 ± 0.17	1.53 ± 0.10
Spleen (g)	1.13 ± 0.06	0.78 ± 0.02	1.09 ± 0.16	0.71 ± 0.08
Kidney (g)	4.36 ± 0.26	3.63 ± 0.07	4.26 ± 0.20	2.94 ± 0.22[a, b)]
Stomach (g)	2.00 ± 0.09	2.16 ± 0.23	2.28 ± 0.14	2.12 ± 0.07
Small intestine (g)	8.46 ± 0.62	7.92 ± 0.90	9.48 ± 0.86	8.70 ± 0.84
Large intestine (g)	3.01 ± 0.11	2.99 ± 0.19	4.12 ± 0.33[a)]	3.26 ± 0.15[b)]
Liver (g)	19.02 ± 1.68	13.57 ± 0.43	22.96 ± 1.74[a)]	14.96 ± 1.63
Cranial tibial (g)	1.39 ± 0.09	1.26 ± 0.07	1.00 ± 0.04[a)]	0.91 ± 0.05[a)]
Extensor digitorum longus (g)	0.41 ± 0.02	0.35 ± 0.01	0.27 ± 0.02[a)]	0.26 ± 0.01[a)]
Soleus (g)	0.33 ± 0.02	0.27 ± 0.01[a)]	0.31 ± 0.01	0.25 ± 0.02[a, b)]
Femoral length (mm)	38.20 ± 0.28	36.67 ± 0.29	35.48 ± 0.45[a)]	34.39 ± 0.78[a)]
Epididymal fat (g)	8.58 ± 1.43	2.27 ± 0.19[a)]	17.49 ± 0.33[a)]	10.79 ± 1.97[b)]
Serum leptin (*n*g/m*l*)	14.52 ± 4.25	1.38 ± 0.47	55.01 ± 6.23[a)]	13.54 ± 3.46[b)]

Values are means ± SEM (n=4). a) $P<0.05$ vs. WT-RW (−) and b) $P<0.05$ vs. TG-RW (−), respectively, by a Tukey-Kramer's test

t test and a Bonferroni correction). Their amounts of food intake were even similar to WT rats (Fig. 3A, $P>0.0125$, by a Student's *t* test and a Bonferroni correction). Furthermore, this decreased food consumption was maintained ($P>0.35$, by one-way ANOVA) until the end of the experiment even after their running distances declined to very low levels (after 11 weeks of age, Figs. 1B and 3B). These results indicate that the condition that allows TG rats to access running wheel freely completely abolishes their hyperphagia.

Body compositions and serum leptin levels are changed by running wheel access: Suppressed body weight gain by free access to a running wheel was observed in both WT and TG rats. To identify which organs are responsible for the decreased body weight gain, the weight of several organs was measured. The results are shown in Table 1. Under the condition without running wheel access, weights of pituitary glands, cranial tibial muscle, extensor digitorum longus muscle and femoral length of TG rats were significantly lower than those of WT rats ($P<0.05$ vs. WT-RW (−), by Tukey-Kramer's test), whereas those of heart, large intestine and liver were higher than those of WT rats ($P<0.05$ vs. WT-RW (−), by Tukey-Kramer's test). Some of our measurements were in accordance with the previous study [20]. In WT rats, only soleus muscle and epididymal fat pad weights decreased after being kept in running wheel cages ($P<0.05$ vs. WT-RW (−), by Tukey-Kramer's test). In TG rats, on the other hand, in addition to soleus muscle and epididymal fat pad, significantly decreased weights were also observed in heart, kidney and large intestine ($P<0.05$ vs. TG-RW (−), by Tukey-Kramer's test). Serum leptin concentrations were in agreement with the weights of epididymal fat pad. These results indicate that free access to a running wheel causes great body composition changes in TG rats.

Results 2

Locking running wheel results in immediate increase of food intake and body weight in TG rats: To examine whether the decreased food intake and body weight gain in TG rats with free access to running wheels persist even after the wheels are fixed, we locked the running wheels and measured the amounts of food intake and body weight. Food intake increased immediately after locking running wheels, and TG-RW (+)-locked rats consumed similar amounts of food compared to TG-RW (−) rats (Fig. 4A, $P>0.05$, by a Tukey-Kramer's test). Body weights of TG-RW (+)-locked rats also increased soon after the locking of running wheels and reached similar levels to those of TG-RW (−) at the end of the experiment (Fig. 4B, $P>0.05$, by a Tukey-Kramer's test). These results revealed that the effect of running wheel access on food intake and body weight in TG rats disappears immediately when their free running becomes impossible.

Concentrations of hGH in TG rats are unaffected by running wheels: It has been demonstrated that GH overexpression in the central nervous system results in hyperphagia and obesity [2]. Our preliminary study showed that hGH concentrations in CSF are over 100-fold higher than the serum concentrations due to the nature of WAP promoter included in transgene in TG rats (data not shown). Given these studies, it is possible that free access to running wheels affected WAP promoter and then induced decreased hGH concentrations in CSF. Thus, we compared hGH levels in CSF and serum between TG rats with and without running wheel access. There was no difference in hGH concentrations in both CSF (1,150 ± 205 *n*g/m*l* in TG-RW (−) vs. 944 ± 55 *n*g/m*l* in TG-RW (+)) and serum (1.92 ± 0.4 *n*g/m*l* in TG-RW (−) vs. 3.9 ± 0.4 *n*g/m*l* in TG-RW (+)) (Means ± SEM, n=5 and 4, respectively). These results indicate that the effects of running wheels on food intake and body weight of TG rats are not due to the changes in the hGH concentrations.

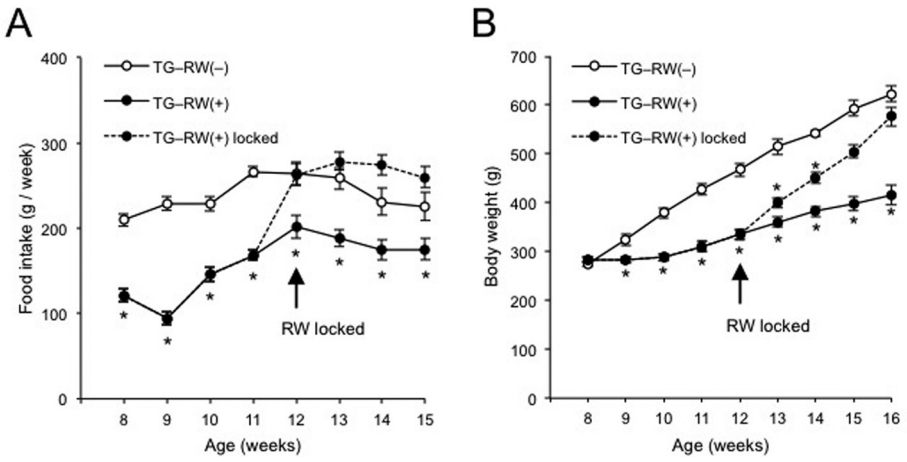

Fig. 4. Effect of locking running wheel on food intake and body weight in TG-RW (+) rats. Half of the running wheels was locked after four weeks of free access to running wheels. The amount of food intake (A) and body weight (B) was measured every week. Values are means ± SEM (n=4 to 5). * $P<0.05$ vs. TG-RW (−) by a Tukey-Kramer's test.

DISCUSSION

In the present study, free access to running wheels completely abolished hyperphagia and obesity in TG rats, and this effect persisted for many weeks as far as the running wheel is accessible.

The present result indicated that free access to running wheel suppresses body weight gain in both WT and TG rats. However, the major factor that mediates the effect of running wheel on body weight gain appears to be different between WT and TG rats, because the running distance of these two rats was significantly different. Increased exercise due to wheel running may account for the weight loss by increasing energy expenditure in WT rats, while the disappearance of hyperphagia caused by wheel running may account for the weight loss by decreasing energy intake in TG rats.

Our findings indicate that free access to running wheels completely abolishes hyperphagia in TG rats. Their declined amount of food intake was similar to that of WT rats, but never exceeded to the level less than the amount of WT rats. And, this effect persists for many weeks as far as the access to the running wheel is possible. It was reported in rats that the amount of food intake decreases when they have access to running wheels [1, 3, 4], however, this decrease is seen only transiently and the amounts of food intake gradually return to similar or even higher level than those of rats without running wheels [1, 3]. Similar temporal decrease of food intake was observed in our TG rats and their WT littermates when they started voluntary running at 10 weeks of age (data not shown). However, after the initial period, food consumption of TG rats did not return to their original amounts and remained at a similar level to those of WT rats, while the amount of food intake returned to the original level in WT rats. We do not have any available data to explain why the temporal decrease of food intake was not observed when we kept rats in running wheel cages from 4 weeks of age. This may depends on the ages when they start running exercise. Thus, our results suggest that the amount of food intake is intrinsically programmed in some unknown mechanism in rats, and free access to running wheels has ability to normalize the feeding behavior of TG rats.

Previous studies investigated the long-term effects of running wheel access on food consumption using hyperphagic rats. However, the results vary among strains of rats. Otsuka Long-Evans Tokushima fatty (OLETF) rats, lacking cholecystokinin receptors, showed temporal decrease of food consumption only in the initial period of wheel access [1], while Zucker fatty rats, which have a leptin receptor missense mutation, did not change their food consumption throughout the experiment [22]. In TG rats, leptin resistance [6], high peripheral ghrelin levels and increased transport of neuropeptide Y (NPY) from the arcuate nucleus (ARC) to paraventricular nucleus (PVN) of the hypothalamus [9] are suspected as the causation of the hyperphagia. Therefore, the different outcomes observed as to the effect of running wheel on food consumption may be attributable to the different causes of hyperphagia. Further studies are needed to explore the principal cause of hyperphagia in TG rats and detailed mechanisms how running wheels change their feeding behavior.

It was rather surprising that hyperphagia in TG rats was suppressed in spite of their significantly less running distances than WT rats. This indicates that in TG rats, even the marginal amounts of running distance are sufficient to normalize their food consumption. This raises the possibility that rearing environment, which enables them to access to a running wheel anytime, is more important rather than the amounts of physical exercises. In recent years, the number of studies on "environmental enrichment" for laboratory rodents is increasing [10, 21]. Environmental enrichment is a

term for exposing laboratory animals to physical and / or social stimulation that is greater than what they would receive under standard housing conditions [18, 21], and running wheels are considered to function as a physical environmental enrichment factor. In addition to improvement of animal well-being, it is shown that environmental enrichment affects many kinds of neurochemistry and resulting behaviors, such as neurogenesis, neural plasticity and drug abuse [21]. Some studies how environmental enrichment affects the eating behavior of not only laboratory animals but also farm or zoo animals are being investigated. For example, the mice from enriched conditions (nest box, plastic tube and wood grawing block) consumed more food than the mice from standard housing conditions [24]. Japanese macaques (*Macaca fuscata*) showed a decreased feeding rate (unit food / min) when they were housed in wide forested enclosures than in narrow nonvegetated enclosures [13]. Piglets reared with vegetal bedding material decreased food neophobia [17]. Considering these reports, it is possible that the running wheel in our condition acted as environmental enrichment, and this feature inhibited hyperphagia in TG rats. Thus, it is worth examining whether any other environmental enrichment factors (e.g., chew woods, tunnel tubes and other toys) can normalize hyperphagia in TG rats.

In conclusion, the present results demonstrate that free access to running wheels completely abolishes hyperphagia in TG rats. Furthermore, it is suggested that rearing environment, which enables them to access to a running wheel anytime, rather than the amount of physical exercises is playing the dominant role in this effect. This may have implications for developing novel therapeutic approach for controlling hyperphagia to prevent obesity.

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