Reduction in circulating ghrelin concentration after maturation does not affect food intake

Hiroyuki Ariyasu1, 2, Go Yamada1, 3, Hiroshi Iwakura1, 4, Sigenobu Matsumura5, Kazuo Inoue5, Kenji Kangawa6, Kazuwa Nakao1, 4 and Takashi Akamizu2

1) Department of Endocrinology and Metabolism, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan
2) The First Department of Internal Medicine, Wakayama Medical University, Wakayama 641-8509, Japan
3) Kurashiki Central Hospital, Kurashiki 710-8602, Japan
4) Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan
5) Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
6) Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka 565-8565, Japan

Abstract. Ghrelin has a potent orexigenic effect and induces adiposity when administered exogenously. Since plasma ghrelin levels rise before meals, ghrelin was thought to play a crucial role in the regulation of appetite. In contrast, mice deficient in the production of ghrelin or the corresponding receptor, GHS-R, do not eat less, throwing the role of ghrelin in the regulation of energy homeostasis into question. Since these mice lack ghrelin or GHS-R from the time of conception, the possibility that compensatory mechanisms may have arisen during development cannot be ruled out. In this study, we used a transgenic mouse model that expresses human diphtheria toxin (DT) receptor cDNA under the control of the ghrelin promoter (GPDTR-Tg mice). As previously reported, an injection of DT into this mouse model ablates ghrelin-secreting cells in the stomach but not in the hypothalamus, resulting in a reduction in circulating ghrelin levels. We used this model system to evaluate the physiological roles of circulating ghrelin in the regulation of food intake. Meal patterns, diurnal and nocturnal meal sizes, and cumulative food intake of DT-treated GPDTR-Tg mice were not affected, although circulating ghrelin levels markedly decreased even after fasting. These mice also displayed normal responses to starvation; however, the use of fat increased and slower weight gain when maintained on a high fat diet was observed. Together, these data suggest that circulating ghrelin does not play a crucial role in feeding behavior, but rather is involved in maintaining body weight.

Key words: Ghrelin, Food intake, Obesity

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thamic area. In addition, these mice may present with developmental adaptations and other compensatory mechanisms that confound the analysis. Therefore, in order to elucidate the physiological roles of circulating ghrelin in feeding behavior and energy homeostasis, we set out to abrogate circulating ghrelin in mice that had reached maturity.

Recently, we generated a transgenic mouse expressing human diphtheria toxin receptor (DTR) cDNA under the control of the ghrelin promoter (termed Ghrelin-Promoter/Diphtheria Toxin Receptor transgenic, GPDTR-Tg, mice), and reported that this mouse expressed the human DTR on ghrelin-secreting cells in the stomach and duodenum, but not in the pancreas, hypothalamus, or pituitary [17]. Therefore, in this mouse only the ghrelin-secreting cells in the stomach and duodenum are ablated by an administration of DT, allowing for the abrogation of circulating ghrelin at any time after birth [17]. Using this model system, we previously reported that circulating ghrelin does not affect either the GH/IGF-I axis or somatic growth during the juvenile period [17]. Here, we used this transgenic mouse model to ablate ghrelin-secreting cells after maturation, which allowed us to evaluate the physiologic significance of circulating ghrelin in feeding behavior and energy homeostasis.

Materials and Methods

Animals (GPDTR-Tg mice)

The generation of transgenic mice expressing human diphtheria toxin receptor (DTR) cDNA under the control of the ghrelin promoter has been previously reported [17]. All animal experiments were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research. Procedures were performed in accordance with the principles and guidelines established by that committee. Mice were housed in an air-conditioned environment, with light between 08:00 and 20:00. Except where noted, animals were fed standard rat chow (CE-2, 352 kcal/100 g, CLEA Japan, Tokyo, Japan) and water ad libitum.

Experiment 1: Physiological roles of circulating ghrelin on food intake

Ablation of ghrelin-secreting cells and measurement of plasma ghrelin levels

Ten-week-old WT and GPDTR-Tg mice were injected intramuscularly (i.m.) with 50 ng/kg of DT (Sigma-Aldrich Japan, Tokyo, Japan) at 09:00 on days 0 and 2. To evaluate changes in plasma ghrelin concentrations after the DT injection, blood samples were drawn from the retro-orbital vein at 10:00 on days 0, 2, 4, and 7 under ad libitum feeding and overnight fasting (16 h) conditions. In addition, to evaluate the diurnal changes in plasma ghrelin levels after DT injection, blood samples were drawn at 09:30, 13:30, 17:30, 19:30, 21:30, and 23:30 on day 4 under ad libitum feeding conditions.

Measurements of plasma ghrelin levels were performed as previously reported [18]. Briefly, blood samples were immediately transferred into chilled siliconized glass tubes containing Na2EDTA (1 mg/mL) and aprotinin (1000 KIU/mL; Ohkura Pharmaceutical, Kyoto, Japan). After centrifugation at 4°C to separate out the plasma, hydrochloric acid was added to samples at a final concentration of 0.1 N. Plasma was immediately frozen and stored at –80°C until assayed. Plasma ghrelin concentrations were determined using a ghrelin ELISA kit (Mitsubishi Kagaku Iatron, Tokyo, Japan).

Monitoring food consumption

Mice were individually housed in cages equipped with BioDAQ food intake monitoring systems (Research Diets Inc., New Brunswick, NJ) [19, 20]. Briefly, hanging wire cages outfitted with spill-proof hoppers were connected to electronics that monitored movement of the food hopper (i.e., feeding activity) and measured its weight. Mice were provided access to the standard diet and water ad libitum, and the time of day, duration, and size of each meal were recorded. Mice were acclimated to individual housing for five days prior to the start of the experiment, and food intake was measured for a week.

Refeeding after starvation

To assess food intake at refeeding after starvation, mice were deprived of food for 16 h from 17:00 on day 3 to 09:00 on day 4 after which they were returned to ad libitum feeding and food intake was measured for 2 h.

Analyses of mRNA expression

Mice were sacrificed by cervical dislocation on day 4. Immediately after decapitation, whole hypothalami were dissected out using the fornix and chiasma opticum as landmarks. Tissues were homogenized with a glass-Teflon homogenizer in a QIAzol reagent (QIAGEN, Hilden, Germany). Total RNA was extracted using an RNeasy mini kit (QIAGEN), according to the manufacturer’s instructions. Total RNA was reverse-transcribed into cDNA using a PrimeScript RT reagent with an oligo deoxymyidine primer (Takara
Roles of ghrelin in food intake

Bio, Otsu, Japan); the reaction was carried out at 42°C for 15 min and terminated by heating at 70°C for 2 min.

Expression levels of neuropeptide Y (NPY), agouti-related protein (AgRP), orexin, melanin-concentrating hormone (MCH), and proopiomelanocortin (POMC) mRNA were quantified by a real-time RT-PCR with an ABI PRISM 7500 Sequence Detection system and a SYBR Premix kit (Applied Biosystems, Carlsbad, CA). The PCR conditions including an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 32 sec. The mRNA level of each gene was normalized using 18s RNA. The gene-specific primers used are shown in Table 1 [21-24].

Restricted feeding schedule

To examine the effects of rapid decreases in circulating ghrelin on the ability to adapt to a negative energy balance, mice were injected with 50 ng/kg of DT at 09:00 on days 0 and 2, and were given a restricted feeding schedule for seven days. During this time, mice were allowed to access food for only 5 h beginning at 13:00.

Experiment 2: The effects of prolonged decreases in circulating ghrelin in adult mice on energy metabolism

Ghrelin sensitivity in ghrelin-ablated mice

To evaluate whether ghrelin sensitivity is up-regulated in ghrelin-ablated mice, ten-week-old WT and GPDTR-Tg mice were placed on a standard diet (SD) and injected with DT (50 ng/kg of DT twice a week) for 4 wk. Then, they were measured 2-h food intake and serum GH levels after an administration of ghrelin (360 µg/kg for food intake and 120 µg/kg for GH).

Diet-induced obesity

Twenty-week-old male WT and GPDTR-Tg mice were treated with DT (50 ng/kg twice a week) for 10 wk. Mice were placed on SD for 5 wk (from 20 to 25 weeks-old) and then divided into two groups. One group was fed SD for 5 more wk, and the other was switched to a high-fat diet (HFD) of 60% fat/kcal (D12493, 5.24 kcal/g [21.92 kJ/g], 60 kcal/kJ% fat; Research diet, New Brunswick, NJ) for another 5 wk (from 25 to 30 weeks-old). Body weights were measured once a week during the experiment, and weekly food consumption was measured during the latter half of the experiment (from 25 to 30 weeks-old).

Fat mass

Fat mass was measured by computed tomography (CT; Laboratory CT, LaTheta LCT-100, Hitachi Aloka, Japan) under pentobarbital anesthesia.

Measuring serum GH, IGF-1, and insulin, and blood glucose levels

Blood samples were collected from tail veins. Serum was isolated by centrifugation and stored at −20°C until assayed. Serum GH, IGF-1, and insulin levels were measured using EIA kits from SPI-BIO (Bonde, France), Diagnostic Systems Laboratories Inc. (Webster, TX), and Morinaga (Tokyo, Japan), respectively, according to the manufacturers’ instructions. Blood glucose was measured using a reflectance glucometer (One Touch II; Lifescan, Milpitas, CA).

Rectal temperature

Rectal temperatures were measured with a digital thermometer (TD-300; Shibaura Electronics, Saitama, Japan) at 10:00 for three consecutive days.

Analyses of UCP-1 mRNA expression

Mice were sacrificed by cervical dislocation at 30 weeks of age. Immediately after decapitation, interscapular brown adipose tissue (BAT) was collected for the analysis of UCP-1 mRNA expression. mRNA extraction, reverse-transcription, and RT-PCR were carried out as described above. Primers used are shown in Table 1.

Locomotion analysis

WT and GPDTR-Tg mice were treated with 50 ng/kg DT twice a week from 17 weeks of age.

Locomotion and respiratory gas analysis

Eighteen-week-old WT and GPDTR-Tg mice were

<table>
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<th>Table 1 Primers used in RT-PCR analysis</th>
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<tbody>
<tr>
<td>mRNA</td>
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</tr>
<tr>
<td>NPY</td>
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<td>AgRP</td>
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<td>Orexin</td>
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<td>POMC</td>
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<td>UCP-1</td>
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<td>18s</td>
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Abbreviations used are: NPY, neuropeptide Y; AgRP, agouti-related protein; MCH, melanin-concentrating hormone; POMC, proopiomelanocortin; UCP, uncoupling protein.
treated with 50 ng/kg DT twice a week until the experiment was over. Twenty-week-old mice were individually placed in ordinary cages, and spontaneous locomotion was measured in a SUPERMEX apparatus with CompACT AMS analysis software (Muromachi Kikai, Tokyo, Japan) [25]. Mice were acclimated to monitoring for 1 h once a day for 3 d before recording began. Another cohort of mice at 20 weeks of age were maintained on HFD and individually placed in air-tight cages (with 72-cm² floor area x 6 cm in height). Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were monitored for 48 h by an open-circuit metabolic gas analysis system connected directly to a mass spectrometer (Arco2000; ArcoSystem, Chiba, Japan). The gas analysis system has been described in detail elsewhere [26, 27]. Briefly, room air was pumped through the chambers at a rate of 0.5 L/min. Expired air was dried in a cotton thin column and then directed to an O₂/CO₂ analyzer for mass spectrometry. Based on the volume of CO₂ production per unit of time (L/min; VCO₂) and VO₂, the total glucose and lipid oxidation were calculated using stoichiometric equations of Frayn [28] as follows: total fatty acid oxidation = 1.67 VCO₂ – 1.67 VO₂, and carbohydrate oxidation = 4.55 VO₂ – 3.21 VCO₂.

Statistical analysis

Results are expressed as the mean ± SEM. Statistical differences between two groups were assessed by an unpaired t-test using the STATVIEW program (SAS Institute, Cary, NC). Differences between genotypes in food intake, body weight, oxygen consumption, respiratory quotient, and locomotion were assessed by repeated-measures ANOVA. p values less than 0.05 were considered to be statistically significant.

Results

Physiological roles of circulating ghrelin in food intake

Plasma ghrelin levels after DT injection

Ten-week-old WT and GPDTR-Tg mice were treated with 50 ng/kg DT on days 0 and 2 and were monitored for a week while eating a standard diet (SD). Plasma ghrelin levels were measured by ELISA on days 0, 2, 4, and 7 under ad libitum feeding and fasting conditions. Plasma ghrelin levels in GPDTR-Tg mice decreased rapidly and markedly, reaching a nadir on day 4. On day 4, plasma ghrelin levels in GPDTR-Tg mice did not increase even after a 16 h fast (Fig. 1a).

We also investigated the diurnal changes in plasma ghrelin levels on day 4. While ghrelin levels in WT mice rose sharply before the onset of the dark phase and dropped just after, ghrelin concentrations in DT-treated GPDTR-Tg mice remained below 6.0 fmol/mL all day (the lower limit of detection was 5.0 fmol/mL; Fig. 1b). DT injections did not induce any noticeable abnormalities in WT mice, which do not possess the DTR and are thus insensitive to DT.

Meal pattern analysis of DT-treated GPDTR-Tg mice

To evaluate the possibility that decreases in circulating ghrelin may affect meal patterns, diurnal and nocturnal meal sizes, and cumulative food intake, we monitored food consumption for a week using an automated food-monitoring system. Despite significant reductions in plasma ghrelin levels in transgenic mice,
the meal patterns of *ad libitum* fed GPDTR-Tg mice were similar to WT (Fig. 2a). The nocturnal and diurnal meal sizes and the cumulative food intake were also similar between the two groups (Fig. 2a, b). Both cohorts of mice consumed the majority of their daily food intake at night (WT vs. GPDTR-Tg: 77.3% vs. 78.0%), and body weights were comparable between the genotypes (Fig. 2c).

**Food intake after an overnight fast**

To elucidate if rapid reductions in circulating ghrelin altered the initiation of food intake after a short-term fast, we measured food intake for 2 h on day 4 after a 16 h fast. No differences were observed between WT and GPDTR-Tg mice (Fig. 2d).

**The mRNA expression levels of hypothalamic neuropeptides**

Real-time PCR analyses could not detect any differences between WT and GPDTR-Tg mice in basal or fasting mRNA expression levels of hypothalamic neuropeptides, including AgRP, NPY, and orexin (Fig. 2e).

**Adaptation of DT-treated GPDTR-Tg mice to a negative energy state**

Next, we evaluated the effect of a rapid reduction in circulating ghrelin on the ability of mice to adapt to a negative energy state. For 7 days, we monitored food consumption in WT and GPDTR-Tg mice kept on a seven-day restricted feeding schedule. Within six days, WT and GPDTR-Tg mice had both adapted to the scheduled food intake, consuming similar amounts of food per day (Fig. 2f). There were no differences in body weight between WT and GPDTR-Tg mice (data not shown).

Together, these observations suggested that rapid reductions in circulating ghrelin levels do not affect feeding behaviors.

**Experiment 2: The effects of prolonged decreases in circulating ghrelin levels in adult mice on energy metabolism**

**Ghrelin sensitivity in DT-treated GPDTR-Tg mice**

An administration of ghrelin increased food intake and GH secretion in DT-treated GPDTR-Tg mice as well as in control mice. Two-hour food intake after an administration of ghrelin at the dose of 360 μg/kg was $0.48 \pm 0.05$ g/2h and $0.47 \pm 0.03$ g/2h in GPDTR-Tg and WT mice, respectively. Serum GH levels after an administration of ghrelin at the dose of 120 μg/kg was $173.1 \pm 27.0$ ng/mL and $166.3 \pm 23.9$ ng/mL in GPDTR-Tg and WT mice, respectively. These results suggest that ghrelin sensitivity is not altered in this model mouse.

**Body weight changes in DT-treated GPDTR-Tg mice fed standard or high-fat diets**

To study the acute effects of reduced plasma ghrelin concentrations on the regulation of energy metabolism in mature mice, 20-week-old male WT and GPDTR-Tg mice were treated with DT for 10 wk, after which time plasma ghrelin levels were undetectable (< 2.5 fmol/mL) and 69.0 ± 6.0 fmol/mL in GPDTR-Tg and WT mice, respectively. Mice were placed on a standard diet (SD) for 5 wk (from 20 to 25 weeks-old), then divided into two groups, one of which received SD for 5 more wk and the other of which was given a high-fat diet (HFD) for 5 wk. On SD, there were no significant differences in average food intake and body weight gain between the two cohorts, but when placed on HFD, GPDTR-Tg mice gained weight at a significantly reduced rate as compared to WT, without altering food intake (Fig. 3a, b). This resulted in a 6.3% lower body weight in GPDTR-Tg as compared to WT mice at 30 weeks of age. The fat masses measured by CT in GPDTR-Tg mice tended to be lower than those in WT mice on HFD (Fig. 3c). In accordance with this finding, insulin sensitivity in GPDTR-Tg mice was higher than that in WT mice on HFD. Although there were no significant differences in blood glucose levels between the two genotypes at 30 weeks of age, in animals on HFD, serum insulin levels were significantly lower in GPDTR-Tg than in WT mice. Since alternations in serum GH levels and in the IGF-I axis affect insulin sensitivity, serum GH and IGF-I levels were evaluated in WT and GPDTR-Tg mice at 30 weeks of age. No significant differences in these parameters were observed in either SD or HFD fed animals (Table 2).

**Energy expenditure**

As the difference in body weight in HFD-fed mice did not appear to result from alternations in the GH/IGF-I axis or energy intake, we investigated whether a reduction in circulating ghrelin levels affected energy expenditure and/or fat utilization. First, we measured rectal body temperatures (*e.g.*, core body temperatures) and evaluated the expression levels of mRNA for UCP-1 in the BAT at 30 weeks of age. No significant differences between WT and GPDTR-Tg mice were observed for these features under either SD or HFD conditions (Fig. 4a, b). Next, we monitored locomotion, and measured oxygen consumption and respiratory quotients. There were no significant differences in locomotion or oxygen consumption between the genotypes (Fig. 4c, d). These results suggested that a
Fig. 2 Decreased circulating ghrelin levels do not affect rate of food intake, body weight, or expression of hypothalamic neuropeptides.

To decrease circulating ghrelin levels, 10-week-old GPDTR-Tg and WT mice were injected with 50 ng/kg DT on days 0 and 2. (a) Nocturnal and diurnal food intake in GPDTR-Tg and WT mice over 7 consecutive days. The dark phases are denoted by gray boxes (20:00-08:00). (b) Average food consumption during the light and dark phases. (c) Changes in body weight after DT injection. (d) One- and two-hour food intake after an overnight fast on day 4. (e) Hypothalamic mRNA levels of NPY, AgRP, orexin (OR), melanin-concentrating hormone (MCH), and proopiomelanocortin (POMC) in ad libitum fed and fasting mice on day 4. mRNA signals are normalized to 18s ribosomal RNA levels. The mean of mRNA levels in WT mice upon ad libitum feeding is set to 1.0 arbitrary units (a.u.). (f) Adaptation to a negative energy state over a 7-day restricted feeding schedule. For all figures, data represent the mean ± SEM (n=12). NS = not significant.
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Table 2  Circulating glucose, insulin, triglycerides, free fatty acids, GH, and IGF-1 levels at 30 weeks of age on an ad libitum diet

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GPDTR-Tg</th>
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<tbody>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG (mg/dL)</td>
<td>159.0±4.7</td>
<td>168.0±10.5</td>
<td>ns</td>
</tr>
<tr>
<td>IRI (ng/mL)</td>
<td>1.10±0.22</td>
<td>1.04±0.24</td>
<td>ns</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>107.1±7.8</td>
<td>120.9±13.1</td>
<td>ns</td>
</tr>
<tr>
<td>FFA (mEq/L)</td>
<td>1.46±0.20</td>
<td>1.44±0.12</td>
<td>ns</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>14.0±1.2</td>
<td>14.3±0.8</td>
<td>ns</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>460.7±17.8</td>
<td>443.2±14.4</td>
<td>ns</td>
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<tr>
<td><strong>HFD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG (mg/dL)</td>
<td>170.6±4.3</td>
<td>179.6±5.8</td>
<td>ns</td>
</tr>
<tr>
<td>IRI (ng/mL)</td>
<td>16.2±2.5</td>
<td>4.2±0.7</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>205.6±10.0</td>
<td>170.7±9.3</td>
<td>ns</td>
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<tr>
<td>FFA (mEq/L)</td>
<td>3.03±0.34</td>
<td>2.45±0.17</td>
<td>ns</td>
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<tr>
<td>GH (ng/mL)</td>
<td>12.7±0.5</td>
<td>14.6±1.0</td>
<td>ns</td>
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<tr>
<td>IGF-I (ng/mL)</td>
<td>506.8±17.3</td>
<td>492.2±13.4</td>
<td>ns</td>
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</table>

BG, blood glucose; IRI, immunoreactive insulin; TG, Triglycerides; FFA, free fatty acids. Data represent the mean ± SEM. ns, not significant.

Fig. 3  A reduction in circulating ghrelin levels leads to slower weight gain in mice on HFD. To decrease circulating ghrelin levels, 20-week-old GPDTR-Tg and WT mice were injected with 50 ng/kg DT twice a week for 10 wk (from 20 to 30 weeks of age). (a) Changes in body weight under standard (SD) and high-fat diet (HFD) conditions. From 20 to 25 weeks of age, all mice were fed on SD. From 25 to 30 weeks of age, mice were divided into two groups (SD and HFD). (b) Average weekly food intake from 25 to 30 weeks of age, (c) Fat masses analyzed by CT at 30 weeks of age. For all figures, data represent the mean ± SEM (n=12). NS = not significant.
Lower circulating ghrelin levels result in a decreased respiratory quotient in GPDTR-Tg mice.

(a and b) Mice were reared under the same conditions shown in Fig. 3, and were analyzed at 30 weeks of age. (a) Rectal temperatures and (b) mRNA expression levels of UCP-1 in the brown adipose tissue. mRNA signals are normalized to 18s ribosomal RNA levels. The mean of mRNA levels in WT mice fed SD is set to 1.0 arbitrary units (a.u.). Data represent the mean ± SEM (n=12). (c) Locomotion recorded at 20 weeks of age. (d) Oxygen consumption recorded at 20 weeks of age on high-fat diet (HFD), (e) Respiratory quotient (RQ) recorded at 25 weeks of age on HFD. Dark phases are denoted by black bars (20:00–08:00). Data represent the mean ± SEM (n=5). NS = not significant.
reduction in circulating ghrelin did not affect energy expenditure. However, we found that the respiratory quotient in GPDTR-Tg mice was less than that in WT mice (Fig. 4e), suggesting that a reduction in circulating ghrelin levels decreased the utilization of carbohydrates and increased the utilization of fat. This finding was compatible with the observed decrease in body weight in GPDTR-Tg mice maintained on HFD.

Discussion

At present, there are two opposing hypotheses about the physiological roles of circulating ghrelin on feeding behavior. One idea suggests that ghrelin is a circulating orexigen [29-31], contributing to preprandial hunger and participating in meal initiation. This concept is based on analysis of the circadian rhythms of ghrelin secretion and the results of pharmacologic ghrelin administration. Plasma ghrelin levels increase before and decrease after meals [2, 12], and peripheral administration of ghrelin stimulates food intake in rodents and humans [11, 32]. Especially in rats, a dose of ghrelin achieving similar circulating levels to those seen after a 24 h fast stimulates significant food intake [11].

The second theory states that ghrelin is not a critical orexigenic factor [13-16]. This idea is based on the observation that mice deficient in either ghrelin or its receptor exhibit normal feeding behavior [13-16]. These mice display normal spontaneous food intake patterns and lack any impairment in food intake in response to re-exposure to food following fasting [11, 13]. Before accepting the latter idea, however, we must consider any confounding factors, such as compensatory mechanisms and/or developmental adaptations that might have emerged in animals completely lacking this receptor-ligand pair during growth and development.

To resolve these conflicts, we employed transgenic mice expressing the diphtheria toxin receptor (DTR) under control of the ghrelin promoter (GPDTR-Tg mice). As shown in our previous report, this transgenic mouse is a useful model to explore the role of circulating ghrelin, because plasma ghrelin concentrations can be abrogated after maturation without altering pituitary and hypothalamic ghrelin mRNA expression levels [17]. In this study, we demonstrated that DT-treated GPDTR-Tg mice had markedly reduced plasma ghrelin levels even after overnight fasting. By using this model system, we evaluated the effects of rapid decreases in circulating ghrelin on feeding behavior in adult mice. We initially predicted that rapid decreases in circulating ghrelin may disrupt feeding behavior in a manner similar to that which occurs when the NPY neuron is ablated in adult mice [33]. Our investigation, however, revealed that mice in which circulating ghrelin was abruptly decreased did not develop abnormalities in feeding behavior. We thought that ghrelin is secreted from the stomach before meal ingestion, but an elevation of circulating ghrelin is not always required for the initiation of food intake. These results were consistent with previous reports of mice lacking either ghrelin or its receptor, and supported the idea that circulating ghrelin is not a critical orexigenic factor [13-16]. However, we did not evaluate the ghrelin concentration in the portal vein in the present study, we therefore cannot exclude the possibility that increased ghrelin in the portal vein may elicit food intake. Moreover, in contrast to the previous reports using mouse models [13-16], orexinergic effect of ghrelin was shown in rat models [11]. Thus, it remains controversial if there are species differences in the stimulating effect of ghrelin on food intake. As hypothalamic ghrelin-secreting cells were preserved in this mouse, we also could not exclude the possibility that the production of ghrelin by the hypothalamus may regulate food intake. Further studies will be needed to elucidate the role of hypothalamic ghrelin in food intake.

Although obesity is closely associated with an increased risk for type 2 diabetes, cardiovascular disease, cancer, and other life-threatening diseases, there are few effective available treatments. Meanwhile, it is reported that ghrelin and growth hormone secretagogue induce adiposity in animals independent of food intake or GH release [10], suggesting that attenuation of ghrelin signals might be an effective treatment for obesity. It is also reported that mice congenitally lacking ghrelin or its receptor in the whole body were or tended to be resistant to diet-induced obesity [34, 35]. Consistent with these reports, our model system, in which circulating ghrelin was abrogated in mice that had reached maturity, demonstrated that mice lacking circulating ghrelin gained weight at a significantly reduced rate on HFD. Judging from our results and the previous reports [10, 34], this phenomenon likely resulted from increased fat utilization. Tschöp and his colleagues demonstrated that peripheral chronic administration of ghrelin not only stimulated food intake but also reduced fat utilization in rodents [10]. By contrast, ghrelin-null mice were reported to exhibit increased fat
utilization and reduced weight gain [34]. Although these findings suggest that circulating ghrelin plays an important role in long-term body weight maintenance by modifying fat utilization, the mechanism of them is still unclear. Davies et al. demonstrated that ghrelin increased abdominal adiposity in rodents via GHS-R-dependent lipid retention [36]. In contrast, Muccioli et al. reported that ghrelin activated phosphoinositide 3-kinase and phosphodiesterase 3B in isolated adipocytes through an unknown subtype of ghrelin receptor, which resulted in an attenuation of isoproterenol-induced lipolysis [37, 38]. Further study is needed to clarify the underlying mechanism by which ghrelin influence fat metabolism. The anti-obesity phenotype observed in our mice appeared to be mild as compared with that seen in GSH-R-null mice. This difference might have resulted from the fact that hypothalamic ghrelin was preserved in our animal model.

Generally, moderate weight loss of 5–10% improves obesity-related disorders, such as impaired glucose tolerance, hypertension, and dyslipidemia, and might reduce mortality [39-41]. In the present study, the body weights observed in DT-treated GPDTR-Tg mice were 6.3% lower than those in WT mice after 5 weeks on HFD. These data suggest that a reduction in ghrelin/GSH-R signaling might be an effective treatment for obesity.

Recently, McFarlane demonstrated that induced ablation of ghrelin cells in adult mice did not decrease food intake by using the transgenic mice that express the diphtheria toxin receptor in ghrelin-secreting cells [42]. In the present study, we investigated the physiological roles of circulating ghrelin by using the similar system. Together, our results also suggest that circulating ghrelin does not play a crucial role in feeding behavior.

Grants

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Disclosures

The authors have nothing to declare.

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