Influence of visceral adiposity on ghrelin secretion and expression in rats during fasting

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Abstract

Although circulating ghrelin levels correlate inversely with adiposity at baseline, little is known about the effect of percent visceral adipose tissue value (PVATV) on ghrelin expression and secretion in response to fasting. Our study demonstrated that ghrelin increased with 24-h fasting in rats with the lowest PVATV (less than 6%), after 3 days in rats with intermediate PVATV (6–9%) and 5 days in rats with the highest PVATV (greater than 9%). Ghrelin mRNA in the stomach was increased after 3 days in low-PVATV (5-8 ± 0-9%) rats but not in high-PVATV (14 ± 1-6%) rats. Therefore, both ghrelin secretion and mRNA were delayed in response to fasting in rats with increased visceral fat. In rats matched for PVATV, but with different body weights, the fasting induced similar levels of increased ghrelin while in rats with different PVATV ghrelin secretion was different in response to fasting, even when body weights were matched in two groups. These data suggested that the initial PVATV, not lean mass, was related to the pattern of plasma ghrelin in response to fasting in rats.

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Introduction

Ghrelin, a 28 amino acid peptide, is originally identified in the rat stomach as an endogenous ligand for the GH secretagogue receptor (Kojima et al. 1999). It strongly stimulates GH release in a dose-dependent manner in animals and humans (Arvat et al. 2000, Date et al. 2000, Seoane et al. 2000). Apart from a potent GH-releasing action, ghrelin has other activities including stimulation of lactotroph and corticotroph functions, influence on the pituitary gonadal axis, the stimulation of appetite, the control of energy balance, influence on sleep and behavior, the control of gastric motility and acid secretion, and influence on pancreatic exocrine and endocrine functions as well as on glucose metabolism (Lely et al. 2004).

Ghrelin administration in rodents causes weight gain (Tschöp et al. 2000, Nakazato et al. 2001, Wren et al. 2001). A growing sum of data generated in rodents have clearly shown that ghrelin-induced weight gain is based on accretion of fat mass without changes in longitudinal skeletal growth and with a decrease, rather than an increase in lean (muscle) mass (Tschöp et al. 2000). Administration of ghrelin causes weight gain by reducing fat utilization or increasing food intake in mice and rats, instead of stimulating GH secretion. In addition to causing weight gain, long-term regulation of circulating ghrelin appears to occur in relation to body weight changes. Plasma ghrelin concentration is low in obese people and rodents (Weyer et al. 1999, Tschöp et al. 2001a,b, Ariyasu et al. 2002, Shiiya et al. 2002, Marzullo et al. 2004, Williams & Cummings 2005) and the reduction of overweight in humans results in increased ghrelin concentration (Hansen et al. 2002, Faraj et al. 2003, Hanusch-Enserer et al. 2004, Soriano–Guillen et al. 2004). All data raise the possibility that ghrelin functions as an adiposity signal, and a potential counterpart to leptin and insulin.

Although circulating ghrelin levels correlate inversely with adiposity at baseline (Tschöp et al. 2001a,b, Shiiya et al. 2002), little is known about the effect of adiposity on ghrelin expression and secretion in response to fasting. Most studies have reported that plasma ghrelin levels are elevated after fasting in lean rodents or human beings with normal body weight. Ariyasu et al. (2002) reported that the short-term secretory regulation of total ghrelin and active form of ghrelin in response to fasting was delayed in obese animals, in which the severity of obesity was assessed by body weight. In the present study, the relationship between visceral adipose tissue (AT) volume and ghrelin production in response to different durations of fasting was investigated, in order to evaluate the role of ghrelin played in the long-term energy balance.
Methods and procedures

Animals

Male Sprague-Dawley (SD) rats, each weighing 250–300 g, were obtained from BK Company, Shanghai, China. Rats were housed in a temperature-controlled room with a ratio of 12h light: 12h darkness. Rats were allowed to acclimatize to the environment for 7 days before the experiment.

Eighty-six male SD rats were randomly divided into two groups, one group was given an ordinary rodent chow diet (LFD, n = 42), while the other group was given a high-fat diet (HFD, n = 44). Chow diet and HFD were described as previously (Yang et al. 2007). Briefly, 100 g chow diet contained 5 g fat and 22 g protein, and produce 1381 kJ/100 g diet, a HFD was created by mixing lard (20%, w/w), sugar (4%, w/w), whole milk powder (2%, w/w), and cholesterol (1%, w/w) into the chow diet, which contained 25-71 g fat and 19-54 g protein, producing 1987 kJ/100 g diet. The rats had free access to their respective food and water throughout the study. Rats were weighed (1000 h) once a week and fed for 4 months, then used for experiment.

Before fasting, all rats used for experiment were measured by CT to evaluate the percent visceral adipose tissue value (PVATV). First, 22 LFD rats and 24 HFD rats with different PVATVs were fasted for 5 days. Blood was extracted from each rat tail from 0900 to 1100 h on fasting 0 day (f0d), 1 day (f1d), 3 days (f3d), and 5 days (f5d), and then frozen at 80 °C until plasma ghrelin level was determined.

Secondly, the remaining 40 rats, including 20 LFD and 20 HFD rats, were used for the following experiment. These 20 rats in LFD group were divided into four groups matching with PVATV values. Meanwhile, in the HFD group, 20 obesity rats were also divided into four groups matching with PVATV values. Rats were anesthetized and then killed in LFD and HFD groups from 0900 to 1100 h respectively, after fasting for 0 day (control fed animals, f0d, n = 5), 1 day (f1d, n = 5), 3 days (f3d, n = 5), and 5 days (f5d, n = 5). When the rats were killed, blood was collected, and kept frozen at 80 °C until plasma ghrelin level was determined. Samples of whole stomach were quickly removed and frozen with liquid nitrogen, then stored at 80 °C. Tissue samples from corpus were used for northern blot analysis. (This study was approved by an ethical committee in Ruijin Hospital.)

Measurement for visceral adiposity

The volume of visceral ATs was measured by computerized tomography (CT; Ross et al. 1991). Briefly, the anesthetized rats underwent helical CT. The CT was performed on a 16 slice multi detector-row CT (MDCT) scanner (LightSpeed; GE Medical Systems, Milwaukee, WI, USA) according to an established protocol. The CT images were routinely obtained with the rats in a prone position. The scanning parameters were as follows: tube potential, 120 kV; tube current, 300 mA; beam pitch, 1-375; gantry rotation time, 1·0 s; section thickness, 1.25 mm; reconstruction interval, 2·5 mm; and field of view, 9·6. Transverse images were reconstructed with a soft-tissue algorithm. The CT images were measured in ADW4.2 workstation of GE Medical Systems. Transverse slices (2·5 mm thickness) were taken at approximately the diaphragmatic dome to anterior superior iliac spine. Threshold selection for AT was based on the analysis of a sample of typical images and the gray-level histograms. The optimal range for AT was between −190 and −15 HU. AT on the inside of the abdominal wall was considered to be visceral AT. The areas (cm²) of the respective AT regions in each slice were computed automatically by summing AT pixels and multiplying by the pixel surface area. The volume (cm³) of AT for each slice was calculated by multiplying the fat area (cm²) by the slice thickness (2·5 mm). Total AT volume was calculated by adding the volumes of AT of all the slices together. The AT mass was calculated assuming the average AT density was 0·90 g/ml. The PVATV were described as the rate of visceral ATs mass to body weight.

Northern blot analysis

Northern blot was performed using non-isotopic DIG Northern Starter Kit (Roche) as described previously (Li et al. 2004). Briefly, target fragments were cloned into PGEM-T easy vector (Promega) and confirmed by sequencing. DIG-labeled probes were generated by transcription with T7 or SP6 RNA polymerase using the DIG Northern starter kit. Total RNA was isolated by Trizol reagent (Gibco). Five microgram samples of total RNA were run in 1.2% formaldehyde–agarose denaturing gels and transferred to positively charged N-membrane in 20× SSC (3 M NaCl and 0·3 M sodium citrate), the membrane was then baked at 80 °C for 2 h. Hybridization was performed at 68 °C with agitation overnight. The membrane was washed twice with 2× SSC and 0·1% SDS for 5 min at room temperature and twice with 0·1× SSC and 0·1% SDS for 15 min at 68 °C. According to the manufacturer’s instructions, the membrane was washed and blocked and then incubated with anti-DIG serum/alkaline phosphatase conjugate. CDP-star was used as the chemiluminescence substrate. Signals were visualized on X-ray film. The relative intensity of the bands was determined by scanning the northern blot and analyzing the band intensity using Quantity One version 4.3.1 software (Bio-Rad). Arbitrary densitometric units were normalized to GAPDH and expressed as a percentage of the control values (f0d).

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Ghrelin and leptin quantitation

Leptin and total ghrelin were measured by RIA using commercially available kits (Linco Research, St Charles, MO, USA) as described by the manufacturer. Briefly, standards and plasma samples were incubated with the corresponding antibodies diluted in assay buffer. The anti-rat leptin serum or antiserum for total ghrelin RIA was used. After 24-h incubation, $^{125}$I-Rat leptin or $^{125}$I-Rat ghrelin was added to all tubes. Bound and free ligands were separated by the second antibody method after further 24-h incubation, and radioactivity in the pellet was counted with a γ-counter. Duplicate standards and duplicate samples were run within a single assay. The respective intra- and interassay coefficients of variation were 6 and 9% for ghrelin RIA and the 5 and 6% for leptin RIA.

Statistical analysis

Results are shown as means ± s.d. A two-way ANOVA was used to see whether there is an interaction between PVATV and duration of fasting on leptin and ghrelin. Statistical comparisons between different groups were performed using the unpaired Student’s t-test and Student’s paired t-test was used when comparing the ghrelin secretion during different fasting periods within the same group. $P<0.05$ was considered significant.

Results

Effects of HFD in rats

The effect of feeding HFD on body weight and PVATV in rats was indicated in Fig. 1A. After 4 months, the average body weight of rats fed with HFDs was higher than that fed with chow diet (824.29 ± 49.6 and 693.11 ± 69.26 respectively, $P<0.05$). The PVATVs showed dramatic variants among rats fed with high fat (Fig. 1B), thus providing the possibility to investigate the effect of PVATV on ghrelin production in response to fasting. It was reasonable to evaluate the effect of obesity on serum ghrelin in rats during short- and long-term fasting by the weight of animal visceral AT. Unexpectedly, although the weight of epididymal fat pad gradually decreased with extension of fasting, the percent of epididymal fat pad value (the ratio of the weight of epididymal fat pad to body weight) was reversely increased during the rat fasting (data not shown). The reason may be that the body weight loss was more rapid than the loss of epididymal fat pad. Therefore, the PVATV before the rat fasting was used to evaluate the severity of obesity in this study.

Effect of PVATV on plasma ghrelin and leptin concentrations in response to fasting

The effect of PVATV on serum ghrelin in rats during short- and long-term fasting was investigated. The rats were divided into three groups according to their PVATVs and as shown in Fig. 2A, baseline plasma leptin level was 8.7 ± 3.7 ng/ml, 17.8 ± 4 ng/ml, and 21.6 ± 8 ng/ml in rats with PVAT < 6%, 6% ≤ PVAT < 9%, and PVAT ≥ 9% respectively. Baseline plasma leptin levels in rats with PVAT 6–9% or more than 9%, were increased when compared with the rats with PVAT less than 6% ($P<0.05$). When compared with f0d, plasma leptin level decreased significantly in response to fasting in three groups in a time-dependent fashion, with a greater decline in rats with low PVAT than those in rats with high PVAT (Fig. 2A). As showed in Fig. 2B, although the baseline plasma ghrelin level did not show significant differences among the three groups, the baseline ghrelin concentration tended to be low in group with high PVAT. It was noted that the changed patterns of the plasma ghrelin concentrations in rats with different PVATs in response to 1-, 3-, and 5-day fasting were different (Fig. 2B). In the group 1, in which PVAT was less than 6%, the average plasma
ghrelin level determined by RIA prior to fasting was 1153 ± 276 pg/ml. After 1, 3, and 5-day fasting, plasma ghrelin levels in this group rose to 2458 ± 436-9 pg/ml, 1818 ± 415-1 pg/ml, and 1979 ± 289-4 pg/ml respectively. The plasma ghrelin level was significantly up-regulated after 1 day fasting ($P<0.05$, compared with f0d), and the level reached 2.16-fold of that before fasting and remained at high levels after 3- and 5-day fasting ($P<0.05$, compared with f0d; Fig. 2B). However, in group 2 with PVATV of 6–9%, the ghrelin level did not show significant increase after 1 day of fasting ($P=5\times10^{-5}$), but not on leptin ($P=1\times10^{-5}$). These data suggested that both the duration of fasting and the initial severity of obesity were related to ghrelin secretion in rats in response to fasting, when PVATV was chosen to assess the severity of obesity.

**Effect of PVAVT on ghrelin mRNA in stomach tissue in response to fasting**

Since both the duration of fasting and the initial content of visceral ATs had an effect on ghrelin secretion in obese rats during fasting, ghrelin mRNA in whole stomach tissue of rats with different PVAVT in response to fasting was further investigated. As shown in Fig. 3B, compared with group 1 with low PVAVT (5.8%–9% PVAVT), ghrelin mRNA in rat stomach tissues was significantly decreased in group 2 with high PVAVT (14%–16%, $P<0.05$). In group 1 with low PVAVT, ghrelin mRNA in rat stomach tissues was significantly increased after 3-day fasting, and the values reached 2.16-fold of those before fasting and remained at high levels after 5-day fasting (Fig. 3C). However, in the group 2 with high PVAVT rats, ghrelin mRNA in the stomach tissues showed no significant difference throughout the period of fasting (Fig. 3D). These findings suggest that ghrelin mRNA in stomach tissues of rats with high PVAVT were delayed in response to fasting, and the duration of fasting periods as well as the initial visceral adiposity may be related to the expression of ghrelin mRNA in rat stomach tissue during fasting.

**Compared with total body weight, initial PVATV had more influence on ghrelin secretion in response to fasting**

Since body weight has certain association with visceral adiposity, the effect of body weight on ghrelin secretion during fasting was further investigated. As shown in Fig. 4, these rats were re-divided into four groups (group 1, group 2, group 3, and group 4). The rats showed similar PVATV (Fig. 4Aa), but different body weight (Fig. 4Ab) between groups 1 and 2. In group 1, plasma ghrelin levels in response to fasting were 1009±740 pg/ml (fed), 2399±758 pg/ml (f1d), 2285±580 pg/ml (f3d), and 1971±544 pg/ml (f5d) respectively (Fig. 4Ac). Meanwhile, ghrelin secretion in group 2 was measured as following: 0d (1186±299 pg/ml), f1d (2309±714 pg/ml), f3d (2096±895 pg/ml), and f5d (2205±493 pg/ml, Fig. 4Ac). In both groups, plasma ghrelin increased after 1 day of fasting ($P<0.05$ compared with f0d) and remained at high levels throughout the entire fasting periods in two groups ($P<0.05$, compared with f0d, Fig. 4Ac). Interestingly, plasma ghrelin level increased after 1 day of fasting in
Ghrelin mRNA in stomach tissues was detected in rats with different PVAVT in response to fasting. SD rats were fed with LFD or HFD for 16 weeks. Then rats with different PVAVT from LFD and HFD groups were kept fasting for 1, 3, and 5 days. Five micrograms of total RNA from rat stomach tissues were used for northern blot analysis. Signals were normalized by GAPDH. (A) The difference of the percent of visceral adipose tissue values (PVATV) between rats in groups 1 and 2. Twenty rats/LFD or HFD, *P < 0.05 compared with group 1. (B) The ghrelin mRNA was down-regulated in stomach tissues of the rats with high PVAVT. Five rats/group, *P < 0.05 compared with group 1. (C) The ghrelin mRNA was up-regulated in stomach tissues of the rats with low PVAVT after 3 days of fasting by northern blot analysis, and remained at high levels after 5-day fasting. Each group contained five rats within low PVAVT, *P < 0.05 compared with f0d. (D) No change in ghrelin mRNA was noted in stomach tissues of rats with high PVAVT during fasting. Each group contained five rats within high PVAVT. LFD, chow diet; HFD, high-fat diet; PVATV, percent of visceral adipose tissue values; f0d, 0 day fasting; f1d, 1 day fasting; f3d, 3-day fasting; and f5d, 5-day fasting.

Evaluating the effect of body weight and percent of visceral adipose tissue values on plasma ghrelin in rats in response to fasting. (A) The effect of body weight on ghrelin secretion. The pattern of ghrelin secretion in response to fasting was similar in two groups, in which rats had similar PVATV (4.4 ± 0.01% and 4.6 ± 0.01%, P = 0.7), but the body weight showed significant difference (643 ± 16 g and 703 ± 32 g, **P < 0.01). Eleven rats/group, *P < 0.05 compared with f0d. (B) The effect of PVATV on ghrelin secretion. When PVATV of rats in two groups was different (4.3 ± 0.008% and 7.4 ± 0.012%, **P < 0.01), the patterns of rat ghrelin secretion were different, even though their weight was similar (679 ± 42 g and 699 ± 43 g, P > 0.05), *P < 0.05 compared with f0d. PVATV, percent of visceral adipose tissue values; f0d, 0 day fasting; f1d, 1 day fasting; f3d, 3-day fasting; and f5d, 5-day fasting.
group 3 (f1d, 2309±704 pg/ml and f0d, 1076±9±
347 pg/ml, P<0.05; Fig. 4Bc) with low PVATV (4.3±
0.8%; Fig. 4Bb) and remained at high levels throughout
5-day fasting (f3d, 2006-9±602 pg/ml, f5d, 2106±
480 pg/ml, P<0.05 compared with f0d; Fig. 4Bc),
however, in group 4 with higher PVATV (7.4±1.2%;
Fig. 4Bb), the plasma ghrelin levels were not changed
after 1 day fasting (1157.8±328 pg/ml and 1250.3±
665 pg/ml, P>0.05; Fig. 4Bc), but significantly
increased after 3-day fasting (2190-6±607 pg/ml,
P<0.05 compared with f0d; Fig. 4Bc). These findings
suggested that the changed pattern of ghrelin secretion
in response to fasting was connected with initial PVATV,
instead of initial body weight.

Discussion

Ghrelin, mainly secreted from stomach, has generated
significant scientific interest over the past few years, as
available evidence indicates that it is an important
peripheral hormone that increases appetite and food
intake. In the present study, we found that both ghrelin
secretion and expression were delayed in rats with
higher PVATV. Compared with total body weight,
PVATV may be more tightly associated with ghrelin
secretion in response to fasting in rats.

Ghrelin secretion has been found to be sensitive to
different nutrient status. Plasma ghrelin concentration
is increased during fasting and decreased after food
intake (Cummings et al. 2001, Tschöp et al. 2001a,b). But
most studies were performed in lean subjects. In the
present study, plasma ghrelin level was increased
significantly after 1 day of fasting in the animal group
with lower PVATV (<6%); while in the rats with median
PVATV of 6–9% and those with high PVATV of >9%,
plasma ghrelin levels were significantly increased by
fasting for 3 and 5 days respectively, suggesting that the
initial PVATV may be associated with ghrelin secretion
in rats in response to fasting. Notably, although the
plasma ghrelin maintained in the high levels during
fasting, the concentration of ghrelin in all groups with
different PVATV increased significantly and reached
the highest level at the initial phase of fasting and then
tended to decrease (Figs 2 and 4). The mechanism of
the phenomena was remained unclearly. We presumed
that plasma ghrelin was up-regulated at a brief period of
fasting to drive rats to take food. If no food was
available, with the persistence of fasting, AT was
oxidized as the primary fuel to provide the required
energy of these animals. Because peripheral daily
administration of ghrelin caused weight gain by
reducing fat utilization in mice and rats (Tschöp et al.
2000), it is reasonable to hypothesize that the decreased
plasma ghrelin in the long-term fasting animals may
have triggered a progressive acceleration of increase in
the relative contribution of lipids to energy production.
However, this was just our speculation; further studies
were needed to elucidate the change of plasma ghrelin
level during the fasting.

Some studies have also reported changed patterns of
ghrelin secretion in obese subjects in response to fasting.
Ariyasu et al. (2002) reported that the short-term
secretory regulation of total ghrelin and the active
form of ghrelin in response to fasting is delayed in
obesity Zucker rats, which was consistent to our findings.
However, in a study by Ariyasu et al., the percent of AT
value did not survey, they used body weight to assess the
obesity. Ghrelin strongly stimulated feeding in rats and
increased body weight gain by reducing fat utilization
in mice and rats (Tschöp et al. 2000, Nakazato et al. 2001).
Analysis of body composition by dual energy X-ray
absorbiometry after 2 weeks of ghrelin treatment
revealed a significant gain in fat mass, but no change
in lean body mass and bone mass, bone area or body
length (Tschöp et al. 2000). Therefore, it is tempting to
presume that percent of AT value contributed to obesity
effect on the ghrelin secretion in response to fasting in
rats. In the present study, the effects of body weight and
PVATV on ghrelin secretion in response to fasting were
evaluated. The results showed that the changed manner
of plasma ghrelin during fasting was similar in animals
with similar PVATV, even though their body weight
differed. However, when body weight of rats revealed no
difference between two groups, the ghrelin secretion in
response to fasting was delayed in the group with high
PVATV (7.4±1.2%) when compared with the group
with low PVATV (4.3±0.8%). These data suggested that
it was the initial PVATV, but not body weight, that was
related to the pattern of plasma ghrelin in response to
fasting in rats. Perreault et al. (2004) reported that 16-h
fasting had no effect on ghrelin secretion in obese mice
induced by HFD. They explained that the result might
be due to different durations of fasting periods; in the
present experiment, we found that ghrelin secretion in
response to fasting was not only related to the initial
PVATV, but also related to the duration of fasting, which
may confirm their speculation. However, in the present
study, we just observed the relationship between visceral
adiposity and ghrelin secretion after fasting; therefore,
we could not exclude the possibility that other fat
depots such as s.c. fat depots may influence the ghrelin
secretion in response to fasting.

Although both circulating ghrelin level and mRNA
in the stomach increased in response to fasting, the
changed patterns were different. Fasting for 8 h readily
increases ghrelin release, whereas even an 18-h fasting
period is not sufficient for up-regulating the expression
of ghrelin mRNA in the rat stomach tissues, suggesting
that regulations of synthesis and release of ghrelin
are dissociated (Moesgaard et al. 2004). Furthermore,
there are still conflicting results regarding the changing
patterns of ghrelin mRNA in stomach tissues during fasting. Moesgaard et al. (2004) found that 18-h fasting did not affect ghrelin expression in mice fed either with low-fat or HFD; however, Toshinai et al. 2001 reported that ghrelin expression was increased in rats fasted for 48 h. Therefore, we further studied the effect of the duration of fasting and the visceral adiposity on ghrelin mRNA in rat stomach tissues in response to fasting. The result showed that ghrelin mRNA was increased significantly after fasting for 3 days in a group of animals with 5-8±0-9% PVATV, while no change of ghrelin mRNA in rat stomach in response to fasting from 1 to 5-day was detected in the group with 14±1-6% PVATV. These results suggest that both the duration of fasting and initial PVATV may be related to ghrelin mRNA level in the stomach in response to fasting. These data may explain why previous studies got different results about ghrelin mRNA expression in the stomach in response to fasting. All the data suggest that the content of visceral fat was an important factor in determining the effect of fasting on ghrelin secretion and expression in rats.

Many data suggest that ghrelin decreased in obese subjects and increased in subjects after weight reduction. In the present study, although the baseline plasma ghrelin levels appeared to be lower in rats with high PVATV (>9%) than those in the rats with PVATV <6%, the difference did not show significance (P>0.05). However, we found when the obesity rats were fed a very low-caloric diet to reduce the PVATV of these obese rats to less than 6%, the plasma ghrelin levels in the rats treated with very low-caloric diet was higher than those in the rats before treatment, the plasma ghrelin levels showed significant difference in these two groups (data not shown). Morpurgo et al. (2003) found that after a 3-week integrated body weight reduction (BWR) program the weight body of obese subjects was decreased by about 5%, leading to the leptin levels in these subjects reduced. However, the ghrelin levels in fasting and in response to the meal showed no significant difference in these obese subjects treated with or without 3-week integrated BWR program. The possible reason was that 5% weight loss obtained after a short-term period of integrated BWR program is not sufficient to normalize fasting ghrelin levels or to restore the normal ghrelin suppression after a meal in severely obese subjects (24). This result was consistent with our findings.

Although initial visceral adiposity was associated with ghrelin secretion in response to fasting in rats, the exact factor to regulate ghrelin secretion and expression after fasting is unclear. A key regulator of plasma ghrelin level is food intake. In the present study, the food weight taken by the rats in HFD group tended to be lower by about 15% than that by the rats in LFD group during the first 2-weeks when feed was changed from the LFD to the HFD. However, when the rats adapted to the HFD, the weight of food intake in the HFD rats was almost the same as that in the LFD group during the left experimental period. Another regulator of plasma ghrelin level is the type of macronutrient present in the ingested diet. Plasma ghrelin levels are low when a HFD is ingested for a long period of time, such as 30-days (Lee et al. 2002) or 14-weeks (Beck et al. 2002), and are increased when the amount of carbohydrates in the diet increases. Moreover, food intake for 20 min after the fast 14 h produced a decrease in ghrelin mRNA expression that was recovered in 45 min in rats that ate the fat diet, whereas levels remained low when rats ate the carbohydrate diet for 45 min. Serum ghrelin followed a similar tendency (Sánchez et al. 2004). However, in the present study, we found that the ghrelin secretion in response to fasting were the similar trends in rats with similar PVATV whether the rats were fed with LFD or HFD. All of these data suggested that diet may have little effect on ghrelin secretion in response to long-term fasting. Leptin, an adipocyte-derived hormone, has opposite effects to ghrelin (Klok et al. 2007). Therefore, it is tempting to speculate that leptin may play a role in regulating ghrelin production upon fasting. In the present study, we found that leptin concentration was decreased in a time-dependent manner after fasting in rats whether visceral adiposity was high or low, indicating that leptin may not implicate in regulating ghrelin level upon fasting. Plasma total ghrelin contains two different forms of ghrelin, acylated and desacylated ghrelin. Acylated ghrelin was considered the active form. Under some physiological and pathological conditions, the change of plasma total ghrelin and the active form of ghrelin may be discrepant. Although the active form of ghrelin was a better marker to evaluate the physiology function of ghrelin when compared with plasma total ghrelin concentration, in Ariyazu et al. study, they measured both total and active forms of ghrelin in the obesity rats in response to fasting and could not detect any discrepancy in their study, which indicated that both total ghrelin and active form of ghrelin were delayed in obese rats. Therefore, in the present work, we only measured the total ghrelin in rats with different PVATV to evaluate the ghrelin secretion in rats in response to fasting.

In conclusion, the present study demonstrated that both ghrelin secretion and expression in stomach were delayed in rats with high percent of visceral ATs values, and that PVATV, but not lean body mass, may be related to ghrelin secretion in response to fasting in rats.

Declaration of interest

There is no conflict of interest that would prejudice the impartiality of the research or a potential conflict of interest that is not fully declared within the text of the article.
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