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ORIGINAL PAPER

Blunted metabolic response to fasting in obese mice

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Abstract The aim of the study was to evaluate metabolic changes in response to fasting in normal and obese mice. C57BL6 and obese (diet-induced obesity (DIO) and ob/ob) mice were used in this study. They were fasted for 24 h and re-fed for 24 h. Body weight was monitored before, after fasting and during re-feeding (2 and 24 h after re-feeding). Food intake was measured 2 and 24 h after re-feeding began. Blood samples were taken before and after 24 h fasting. As metabolic parameters, blood glucose, plasma insulin, ghrelin levels and oxygen consumption were measured. Blood glucose and plasma insulin levels in DIO and ob/ob mice were higher than normal mice, and plasma ghrelin levels were lower in DIO and ob/ob mice. There was reduced body weight loss in DIO mice than in normal mice for 24 h fasting. When they were re-fed, DIO and ob/ob mice consumed less food intake than normal mice. Twenty-four hours food deprivation induced significantly smaller plasma ghrelin elevation in these obese mice. Fasting-induced decrease in oxygen consumption was significantly smaller in DIO and ob/ob mice than normal mice. This data show that obese mice may have decreased sensitivity to fasting-induced increase in circulating ghrelin and their oxygen consumption exhibited a blunted response to fasting.

Keywords Obese mice · Fasting · Ghrelin · Oxygen consumption

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Introduction

Obesity is defined as an excess of fat storage in the body and is one of the risk factors of cardiovascular disease [1]. Recently, a number of hormones that regulate energy regulation have been identified. Ghrelin is one of these hormones secreted by the stomach and regulates energy metabolism [2]. It has been reported that ghrelin is an anabolic hormone and induces body weight gain and adiposity in rodents by increasing food intake and decreasing fat utilization [2, 3]. Circulating ghrelin levels have been reported to be elevated by fasting in lean rodents [4] and lean humans [5]. Ghrelin levels increase before meals in humans, suggesting an important role of ghrelin in energy homeostasis [6].

Starvation is a threat to energy homeostasis that triggers adaptive responses [7] and ghrelin may play a key role in fasting. The aim of the study was to examine metabolic changes and differences, and to evaluate the involvement of ghrelin in normal and obese (DIO and ob/ob) mice when food was deprived for 24 h and re-fed for 24 h. We monitored percent body weight and food intake, and measured metabolic parameters including ghrelin levels before and after food deprivation.

Results

Body weight change and food intake

Characteristics of animals are shown in Table 1. Body weight of DIO mice was larger by 39% and ob/ob mice by 103.5% compared to normal mice. The ob/ob mice had larger daily food consumption than normal mice, while there was no change in DIO mice before the experiment

Table 1 Characteristics of normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice

	Control	DIO mice	Control	ob/ob mice
Body weight (g)	26.2 ± 0.3	36.4 ± 0.6*	25.6 ± 1.3	52.1 ± 1.9*
Daily food intake (kcal)	11.0 ± 0.3	11.3 ± 0.2	10.5 ± 0.2	15.7 ± 0.3*
Blood glucose (mg/dl)	107 ± 4	131 ± 8*	107 ± 3	145 ± 12*
Plasma insulin (ng/ml)	1.2 ± 0.3	5.8 ± 0.1*	1.2 ± 0.2	36.9 ± 1.8*
Plasma leptin (ng/ml)	5.7 ± 0.6	24.4 ± 1.1*	N.D.	N.D.
Plasma ghrelin (ng/ml)	0.9 ± 0.0	0.6 ± 0.1*	0.9 ± 0.1	0.5 ± 0.0*
O_2 consumption (ml/min)	7.1 ± 0.5	5.7 ± 0.5*	6.3 ± 0.1	6.4 ± 0.3
HOMA-R (mg/dl•μU/ml)	0.2 ± 0.0	0.5 ± 0.2*	0.2 ± 0.0	5.9 ± 1.9*

N.D.: not detected. *$P < 0.001$ vs. normal controls

started. The body weight reduction after 24 h fasting was smaller in DIO mice than in control mice (control: −3.5 ± 0.1 g, DIO: −2.3 ± 0.1 g, $P < 0.001$; control: −2.9 ± 0.2 g, ob/ob: −3.6 ± 0.3 g) (Fig. 1a). For 2 h re-feeding, DIO and ob/ob mice had reduced energy intake (control: 1.7 ± 0.3 kcal, DIO:0.6 ± 0.2 kcal, $P < 0.01$; control: 2.7 ± 0.2 kcal, ob/ob: 1.7 ± 0.1 kcal, $P < 0.01$) (Fig. 1b). Body weight increase after 2 h re-feeding in DIO and ob/ob mice was less than in control mice (control: 1.4 ± 0.2 g, DIO: 0.1 ± 0.1 g, $P < 0.001$; control: 1.5 ± 0.2 g, ob/ob: 0.6 ± 0.1 g, $P < 0.001$) (Fig. 1c). DIO mice still had less food intake after 24 h re-feeding while there was no change in 24 h food intake in ob/ob mice (control: 13.9 ± 0.9 kcal, DIO: 10.0 ± 0.7 kcal, $P < 0.05$; control: 17.2 ± 1.4 kcal, ob/ob: 18.0 ± 0.6 kcal) (Fig. 1d).

Blood parameters

Blood glucose and plasma insulin levels in fed state were higher in DIO and ob/ob mice (Table 1 and Fig. 2a, b). Plasma ghrelin levels in fed state were lower in DIO and ob/ob mice (Table 1, Fig. 2c). After 24 h fast, plasma ghrelin levels in DIO and ob/ob mice increased. However, the increase was significantly smaller compared to normal controls (Fig. 2c). Obese mice exhibited insulin resistance with higher HOMA-R than control mice (Table 1).

Oxygen consumption

We examined oxygen consumption to access energy expenditure. Oxygen consumption in normal mice decreased during 24 h fasting. The reduction of oxygen consumption was significantly less in DIO and ob/ob mice than normal mice in fasting (control: −0.7 ± 0.2 ml/min, DIO: −0.1 ± 0.2 ml/min, $P < 0.05$; control: −0.5 ± 0.1 ml/min, ob/ob: −0.1 ± 0.1 ml/min, $P < 0.05$) (Fig. 3).

Discussion

In this study, we observed low levels of circulating ghrelin in both DIO and ob/ob mice compared to normal mice and

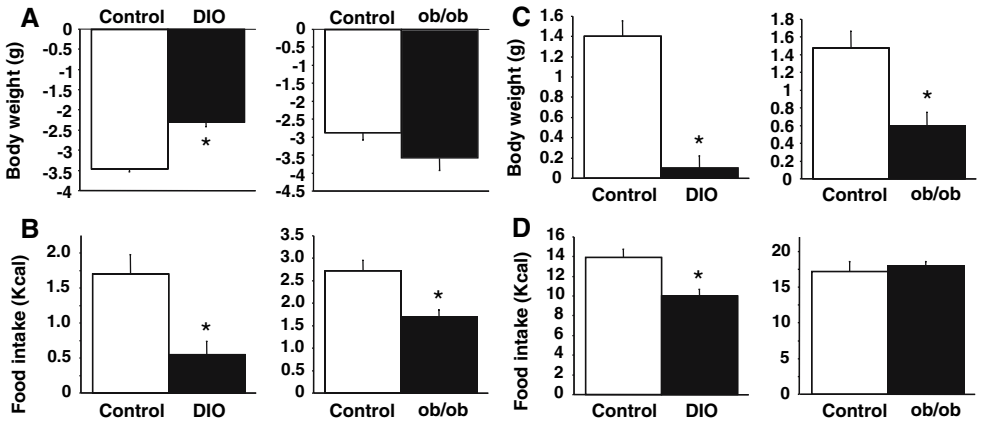

Fig. 1 (**a**) Decrease of body weight in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice after 24 h fasting. *$P < 0.001$ vs. normal controls. (**b**) Food intake (kcal) after first 2 h re-feeding following 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. *$P < 0.01$ vs. normal controls. (**c**) Increase of body weight in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice after 2 h re-feeding. *$P < 0.001$ vs. normal controls. (**d**) Food intake (kcal) during 24 h re-feeding after 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. *$P < 0.05$ vs. normal controls

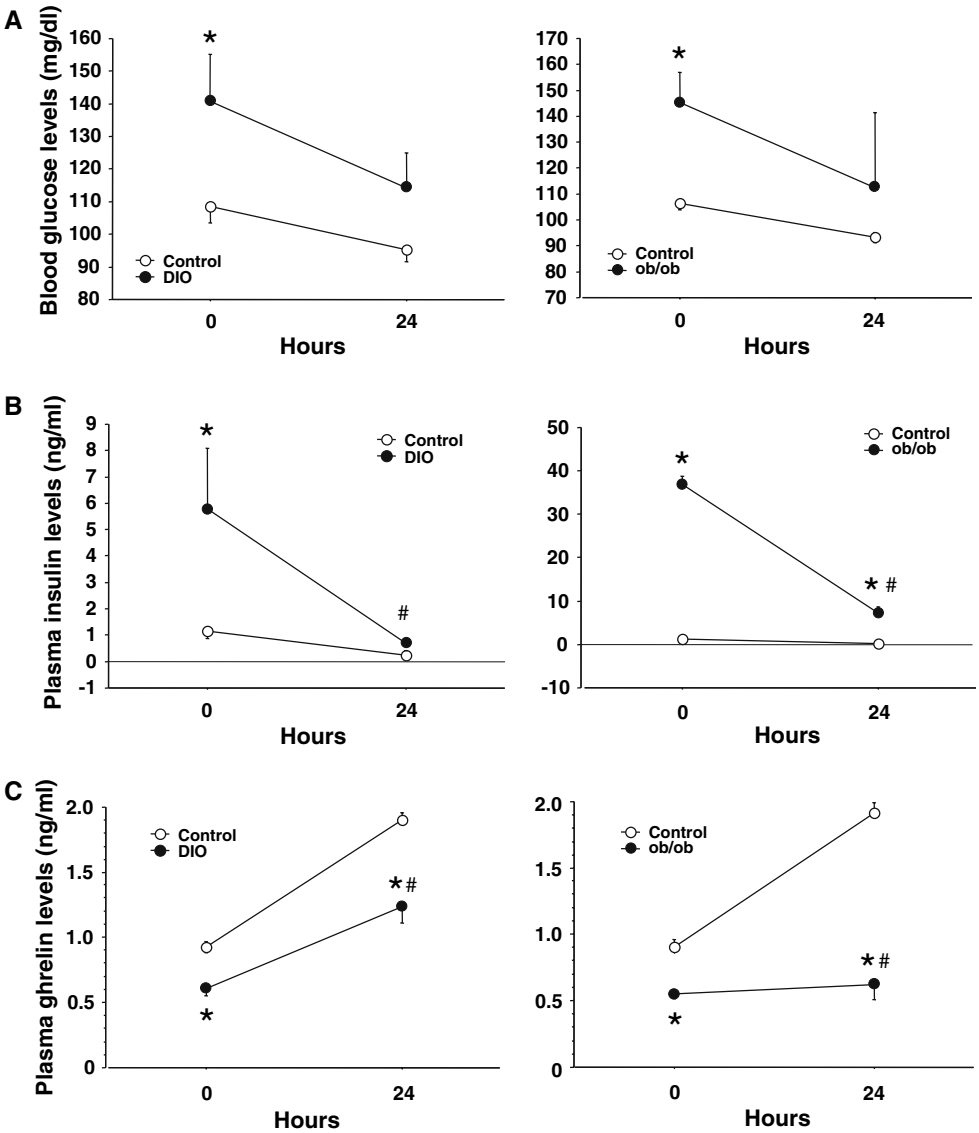

Fig. 2 (**a**) Blood glucose levels before and after 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. $^{*}P < 0.05$ vs. normal controls. (**b**) Plasma insulin levels before and after 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. $^{*}P < 0.05$ vs. normal controls; $^{\#}P < 0.05$ vs. normal controls. (**c**) Plasma ghrelin levels before and after 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. $^{*}P < 0.05$ vs. normal controls; $^{\#}P < 0.05$ vs. normal controls

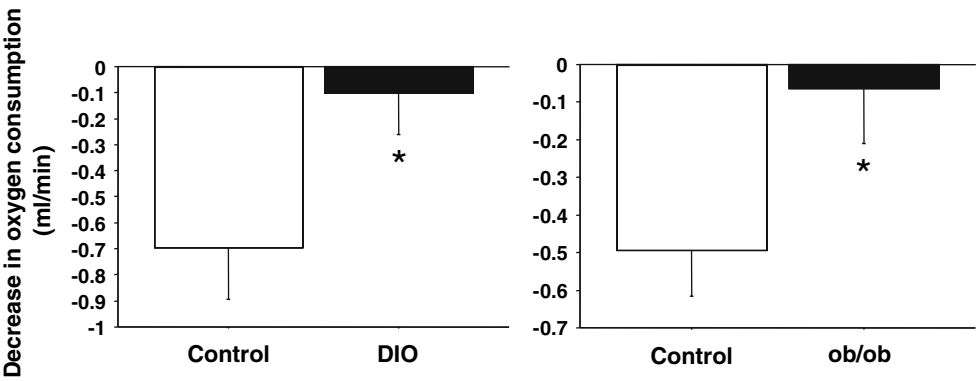

Fig. 3 Decrease in oxygen consumption after 24 h food deprivation in normal ($n = 6$, each group) and DIO ($n = 6$), ob/ob ($n = 6$) mice. $^{*}P < 0.05$ vs. normal controls

ghrelin levels increased during food deprivation for 24 h, which is consistent with previous reports that describe circulating ghrelin levels as negatively correlated with body mass index [8] and circulating ghrelin elevates in fasting [4]. Interestingly, elevation of circulating ghrelin levels was significantly inhibited in DIO and ob/ob mice compared to normal mice when they were fasted for 24 h, and food intake during re-feeding was less in these obese mice. Since circulating ghrelin increases in fasted state and initiates food intake [6], this inhibition of ghrelin elevation

in DIO and ob/ob mice may have resulted in decreased food intake during early phase of re-feeding. It might be possible that higher blood glucose and plasma insulin levels after 24 h fasting cause anorectic effect in DIO and ob/ob mice. We analyzed correlation coefficients between feeding response to fasting and blood glucose levels (DIO mice: $r = -0.207$; ob/ob mice: $r = -0.240$), plasma insulin levels (DIO mice: $r = -0.360$; ob/ob mice: $r = -0.328$), and plasma ghrelin levels (DIO mice: $r = 0.518$, $P \leq 0.05$; ob/ob mice: $r = 0.832$, $P \leq 0.01$). These results show that circulating ghrelin may be more responsible for decreasing food intake after fasting in these obese mice. Ghrelin may be involved in short-term regulatory system in energy homeostasis.

After 2 h re-feeding, the increase of body weight in DIO and ob/ob mice was less than control mice, indicating energy consumption reflected the body weight change. Obese mice may not be sensitive to starvation in terms of energy intake. Food intake during 24 h re-feeding in DIO mice was still reduced compared to normal mice; however, there was no change in ob/ob mice. This difference in energy intake between DIO and ob/ob mice may be attributed to the existence of endogenous leptin.

Perreault et al. and Ariyasu et al. reported that ghrelin secretion after fasting was impaired in obese mice [9] and rats [10]. Perreault et al. reported that diurnal rhythm pattern was lost in DIO mice compared to lean mice, showing impared ghrelin regulation in obese mice. They also observed that obese mice are resistant in feeding behavior to exogenous ghrelin. These data including our results show that the sensitivity of ghrelin at least in the fasted state may be decreased in obese mice. This indicates a possibility of a negative feedback system of ghrelin in developing obesity. It has been speculated that lean mice may have increased sensitivity to ghrelin in fasting.

It has been shown that circulating ghrelin and insulin levels have a negative correlation [8]; low ghrelin levels are associated with increased insulin resistance [11]. In this study, DIO and ob/ob mice exhibited insulin resistance with hyperglycemia, hyperinsulinemia, and higher HOMA-R. DIO and ob/ob mice showed decreased plasma ghrelin levels compared to normal mice. Our results were consistent with previous cited reports. It may be possible that reduced ghrelin levels were due to existence of insulin resistance. Insulin infusion decreased circulating ghrelin levels in clamp studies [12] and glucose infusion lowered circulating ghrelin levels [13]. Hyperinsulinemia and/or hyperglycemia may have blocked an increase in ghrelin levels in obese mice.

Interestingly, decrease in oxygen consumption during fasting was significantly less in DIO and ob/ob mice. This indicates that metabolic response to fasting in energy expenditure was blunted in obese mice. There was a smaller decrease in body weight after 24 h fasting, although decrease in oxygen consumption was smaller in DIO mice, showing conflicting data. There seem to be factors other than oxygen consumption maintaining body weight in fasting. It is likely that obese mice are not able to adapt starvation to keep energy homeostasis. We have previously reported that ghrelin administration reduced oxygen consumption in obese mice [14]. Considering there is blunted increase in circulating ghrelin levels and blunted metabolic reduction in energy expenditure, at least in part, ghrelin may regulate energy expenditure during fasting. Taking another point of view, it may be speculated that less obese mice may be more sensitive to storing energy.

In this study, we found that obese mice had smaller energy intake during early phase of re-feeding after 24 h fasting compared to normal mice. Blunted response of ghrelin to fasting may have resulted in reduced energy intake and blunted fasting-induced decrease in oxygen consumption in obese mice. This data suggest that there may be blunted metabolic response to fasting in obese mice, and this may be attributed to decreased ghrelin sensitivity in obese mice.

Materials and methods

Animal experiments

C57BL/6J mice (4 weeks old, male) were purchased from JAPAN SLC (Shizuoka, Japan) and ob/ob mice (4 weeks old, male) were obtained from Shionogi Co., Ltd. (Shiga, Japan). For generating diet induced obesity (DIO) mice, C57BL/6J mice were divided into two groups. One group ($n = 6$) received standard diet containing 11% of total energy as fat (3.4 kcal/g) and the other group ($n = 6$) received a high fat diet containing 45% of total energy as fat (4.73 kcal/g) (Clea Japan Inc., Tokyo, Japan) for 14 weeks. The ob/ob ($n = 6$) and control (C57BL/6J) mice ($n = 6$) were fed standard diet. Mice were kept individually in cages with a constant environment (22 ± 2°C, 55 ± 10% humidity, 12 h light/dark cycle). Food and water were available ad libitum. Experiments were approved by the University Animal Care Committee. Food was deprived at 18:00 and mice were fasted for 24 h and re-fed for 24 h. Body weight was monitored before, after fasting, and during re-feeding (2 and 24 h after re-feeding). Food intake was measured 2 and 24 h after re-feeding began. Blood samples were obtained from the tail vein before and after 24 h fasting. Blood glucose concentration was measured with a blood glucose meter (MediSense Precision Xtra; Abbott, Japan). Plasma samples were stored at −40°C until insulin, leptin, and ghrelin radioimmunoassay. HOMA-R was calculated as fasting blood glucose

concentration (mg/dl) × fasting insulin concentration (μU/ml)/405 [15].

Hormone measurement

Plasma insulin was measured by using mouse insulin ELISA kits (ALPCO Diagnostics, Windham, NH), plasma leptin by sensitive rat/mouse leptin radioimmunoassay (RIA) kits (LINCO Research St. Charles, MO), plasma ghrelin by Ghrelin RIA kits (Phoenix Pharmaceuticals, Belmont, CA).

Oxygen consumption

Oxygen consumption was determined by using an O_2/CO_2 metabolism measuring system (model MK-5000, Muromachi Ikikai, Tokyo, Japan) at 22°C. The chamber volume was 560 ml, airflow to the chamber was 500 ml/min, samples were taken every 3 min, and a standard gas reference was taken every 30 min. Mice were kept unrestrained in the chamber without food or water during the light cycle. Oxygen consumption was measured before and after 24 h fasting for 2 h each time. Oxygen consumption test was carried out after 1-week recovery from first experiment. HOMA-R was calculated as fasting blood glucose (mg/dl) fasting plasma insulin/405.

Statistical analysis

Results were expressed as mean value ± SE. Blood glucose, plasma insulin, and ghrelin levels were analyzed using two-way repeated-measures ANOVA with time and strain of mice as variables. Comparison between two groups was analyzed by unpaired *t*-test. Significance was set at $P < 0.05$ for all analyses.

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