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Short Communication

Contribution of Leptin to Energy Expenditure and Glucose Metabolism in Fasting

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Abstract

Leptin regulates energy homeostasis and is involved in glucose metabolism. It is known that leptin plays an important role in neuroendocrine response to fasting; therefore, the essential role of leptin may be the response to starvation. In this study, we examined the role of leptin in energy expenditure and glucose metabolism in fasting.

Methods: C57BL/6 and leptin mutant ob/ob mice were fasted for 48 hours. Oxygen consumption and blood glucose levels were examined before and after fasting. Next, leptin was continuously infused (20ng/g/h) by osmotic pump in C57BL/6 and ob/ob mice to blunt the fall of leptin in fasting. These mice were fasted for 48 hours and the same parameters were measured before and after fasting during the infusion of leptin.

Results: After fasting, there was lesser decrease in oxygen consumption in ob/ob mice (C57BL/6: -1162±274ml/kg/h; ob/ob:-150±150ml/kg/h, $p<0.05$). The reduction in blood glucose was greater in ob/ob mice, though it didn't reach the statistical significance (C57BL/6: -24±8mg/dl; ob/ob: -45±6mg/dl). By continuous infusion of leptin in C57BL/6 mice, decrease in oxygen consumption after fasting was blunted (leptin infusion-: -1162±274, leptin infusion+: 250±294ml/kg/h, $p<0.01$) in C57BL/6 mice. Decrease in blood glucose was greater in C57BL/6 mice by infusion of leptin (leptin infusion-: -24±8, leptin infusion+: -56±6mg/dl, $p<0.05$). There were no differences in those parameters after fasting by leptin infusion in ob/ob mice.

Conclusions: Leptin-fall may be, at least in part, a regulatory factor of energy expenditure and glucose metabolism in starvation.

INTRODUCTION

Starvation is a threat for maintaining energy homeostasis and glucose metabolism. There rise physiological systems to defend the threat, decreasing energy expenditure and maintaining blood glucose levels at the minimum normal range. Leptin plays an important role in feeding behavior and energy homeostasis [1]. Leptin levels rapidly fall with energy deprivation independent of fat mass in humans [2,3] and increase after ingestion of meals in human [4,5]. Those changes of leptin levels may be an adaptation also for short term energy balance, or leptin may be a regulator of energy homeostasis in starvation. Administration of leptin increases oxygen consumption in ob/ob mice [6] and decreases food intake and body weight in wild-type and ob/ob mice [1]. Taken together, these findings suggest that leptin may have a role in controlling energy expenditure in starvation to maintain energy homeostasis. As well as modulating energy homeostasis, several lines of evidence show that leptin regulates glucose metabolism. Administration of leptin lowered blood glucose levels of ob/ob mice than those of pair-feeding littermates [7,8] or lowered blood glucose levels without changing body weight [9,10] showing that leptin modulates glucose metabolism independent of energy intake or body weight. Interestingly, leptin treatment can normalize blood glucose levels in insulin-deficient mice [11-13], suggesting that leptin has an insulin-independent mechanism for lowering blood glucose levels. It has been reported that leptin regulates neuroendocrine hormones in fasting [14]. A single injection of leptin blunted the change in gonadal, adrenal and thyroid axes, showing falling leptin concentration is a critical signal that initiates the neuroendocrine response to starvation, and the essential physiological effect of leptin may be exerted in fasting [15]. We hypothesized that the fall of leptin in starvation may be one of the key factors in energy homeostasis and also glucose metabolism since leptin levels decrease in fasting, and leptin regulates some of counter regulatory hormones in fasting.

In this study, we evaluated energy expenditure and blood glucose levels in fasting in normal (C57BL/6) and ob/ob mice which have lack of leptin and leptin-fall. To further examine the effect of leptin in fasting, the fall of leptin in fasting was blunted by continuous infusion of leptin using an osmotic pump, and oxygen consumption and blood glucose levels were examined before and after the infusion.

MATERIALS AND METHODS

Animals and treatments

C57BL/6 mice (8 weeks old, male, n=8) were purchased from JAPAN SLC (Shizuoka, Japan) and leptin mutant ob/ob mice (8 weeks old, male, n=8) were obtained from Shionogi Co., Ltd (Shiga, Japan). Mice were kept individually in cages with a constant environment (22±2° C, 55±10% humidity, 12 hours light/dark cycle). Food and water were available ad libitum. Experiments were approved by the university animal care committee. Characteristics of C57BL/6 and ob/ob mice in fed state are described in Table 1. Mice were fasted for 48 hours, and blood glucose and oxygen consumption were measured before and after fasting. Blood samples were obtained from the tail veins of the mice. One week after the first experiment, Alzet osmotic pumps (DURECT) filled with leptin (Sigma-Aldrich) were implanted intraperitoneally and leptin was infused (20ng/g/h) in the same C57BL/6 and ob/ob mice. The mice were fasted for 48 hours and the same parameters were measured before and after fasting. Pumps were explanted and no residue was confirmed in each pump after the experiment.

Oxygen consumption

Oxygen consumption was determined by using an O2/CO2 metabolism measuring system (model MK-5000, Muromachi Ikikai, Tokyo, Japan) at 22°C. The chamber volume was 560 ml, airflow to the chamber was 500ml/min, samples were taken every 3 minutes, and a standard gas reference was taken every 30 minutes. Mice were kept unrestrained in the chamber without food or water during the light cycle.

Blood glucose measurement

Blood glucose concentration was measured with a blood glucose meter (MediSense Precision Xtra; Abbott, Japan).

Statistical analysis

Results were expressed as mean values. Oxygen consumption and blood glucose levels were analyzed using two-way repeated-measures ANOVA with time and strain of mice as variables. The changes of these parameters were evaluated by unpaired Student's t-test. Comparison of the changes of these parameters before and after the treatment was analyzed by paired Student's t-test. Significance was set at $p<0.05$ for all analyses.

RESULTS

Oxygen consumption and blood glucose levels after fasting

After 48 hours fasting, oxygen consumption was reduced in both groups (C57BL/6: 5962±192 to 4800±265 ml/kg/h; ob/ob: 2962±132 to 2812±151 ml/kg/h (Figure 1A), and the change in oxygen consumption was less in ob/ob mice than in C57BL/6 mice (C57BL6: -1162±274 ml/kg/h, ob/ob: -150±150 ml/kg/h, $p<0.01$ (Figure 1B). The blood glucose levels were lowered in both groups (C57BL/6: 102±9, to 78±2 mg/dl; ob/ob: 208±7, to 163±3mg/dl). The change in blood glucose was larger in ob/ob mice but it didn't reach statistical significance (C57BL/6: -24±8 mg/dl, ob/ob: -45±6 mg/dl, p=0.08).

Table 1: Characteristics of C57BL/6 (n=8) and ob/ob mice (n=8) in fed state.

	C57BL/6J	ob/ob
Body weight (g)	29.2 ± 0.2	66.9 ± 1.7
Blood glucose (mg/dl)	102 ± 9	208 ± 7
Oxygen consumption (ml/h/kg)	5962 ± 192	2962 ± 132
Values are expressed as mean value ± SE.		

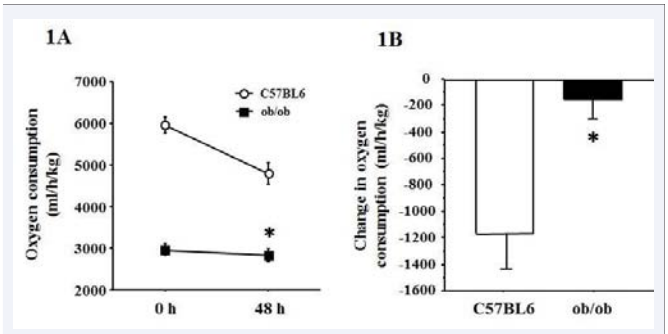

Figure 1 Levels of oxygen consumption (1A) before and after 48 hours fasting in C57BL/6(○) and ob/ob(■) mice. Changes in oxygen consumption (1B) after 48 hours fasting in C57BL/6 and ob/ob mice. *p < 0.05 vs. C57BL/6 mice.

Changes of the parameters before and after the treatment of leptin infusion

In C57BL/6 mice with continuous infusion of leptin, the decrease in oxygen consumption after fasting was blunted (leptin infusion-:-1162±274, leptin infusion+:250±294 ml/kg/h, $p<0.01$ (Figure 2A). The reduction in blood glucose was greater (leptin infusion-: -24±-8, leptin infusion+: -56±-6 mg/dl, $p<0.05$ (Figure 3A). There were no differences in these parameters by continuous infusion of leptin after fasting in ob/ob mice (Figure 2B,3B).

CONCLUSION

The aim of this study was to investigate whether leptin has a role for regulating energy homeostasis and glucose metabolism in starvation. To elucidate the effect of leptin-fall in starvation, first, we simply compared oxygen consumption and blood glucose levels in fasting in normal mice and leptin-deficient ob/ob mice. In the second study, mice were given continuous infusion of leptin by using osmotic pump to blunt the fall of leptin concentration in fasting.

In the first study, there was lesser decrease in oxygen consumption in ob/ob mice than normal mice, suggesting the suppression of energy expenditure in fasting may be blunted in ob/ob mice compared to normal mice. This attenuation of energy

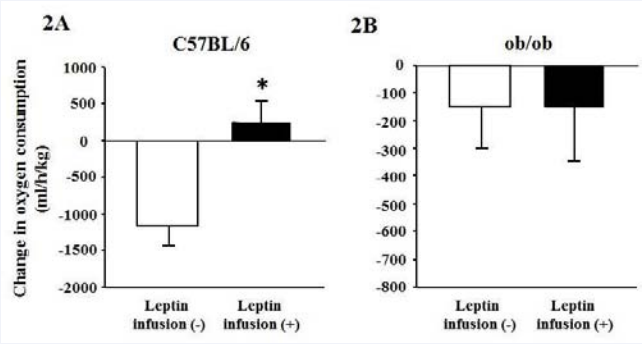

Figure 2 Changes in oxygen consumption after fasting in continuously leptin-infused C57BL/6 (2A) and ob/ob (2B) mice. *p < 0.05 vs. leptin infusion (-).

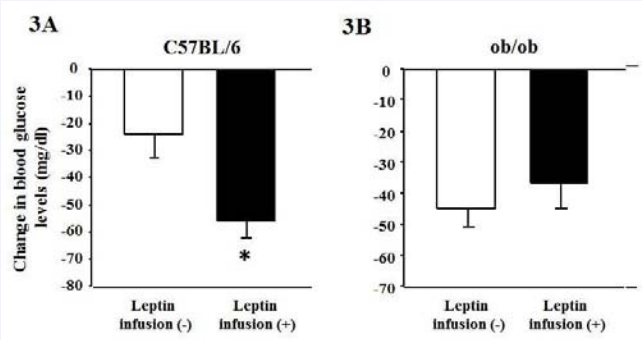

Figure 3 Changes in blood glucose levels after fasting in continuously leptin-infused C57BL/6 (3A) and ob/ob (3B) mice. *p < 0.05 vs. leptin infusion (-).

expenditure may be attributed to the lack of leptin or the lack of leptin-fall in ob/ob mice. Previously, we have reported that there was a blunted response to 24 hours fasting in energy expenditure in obese mice [16], which is compatible with this data. Leptin increases UCP1 expression in brown adipose tissue and reduces body weight and food restriction improves thermogenesis in brown adipose tissue in ob/ob mice [12,17-18]. It might be possible that starvation can cause disruption in brown adipose tissue in terms of maintaining energy balance in the absence of leptin or leptin-fall, leading to the inhibition of decrease in energy expenditure in fasting. The decrease in blood glucose levels in fasting in ob/ob mice was greater than in normal mice in the first study, however; it didn't reach statistical significance. This result spurred the second study, to further examine the involvement of leptin-fall in glucose metabolism in starvation.

In the second study, the decrease in oxygen consumption in fasted normal mice was eliminated by continuous infusion of leptin. Continuous ICV leptin infusion attenuated blunted fasting-induced reductions in heart rate and oxygen consumption [19], which supports our data. However, the same result was not obtained in fasted ob/ob mice even with leptin infusion. This may show that absence of leptin-fall seems more responsible rather than the absence of leptin levels for energy homeostasis. There was a further decrease in blood glucose in fasted normal mice by continuous infusion of leptin, implying that the fall of leptin may be important for maintaining fasted blood glucose levels. This suggests the fall of leptin in fasting may inhibit further decrease in blood glucose levels to prevent hypoglycemia or to maintain the level of blood glucose in the normal range. Corticosterone is one of the counter-regulatory hormones increasing blood glucose levels, and it has been reported that leptin inhibits levels of corticosterone [20]. Leptin-fall may contribute to the elevation of corticosterone in fasting. A single injection of leptin did not alter blood glucose levels, and leptin replacement in fasting blocked the decrease in thyroxine and the increase of corticosterone [14]. In our study, we speculated that continuous infusion of leptin may have blunted leptin-fall in fasting, and possibly blocked the decrease in thyroxine levels and the increase of corticosterone, resulting in the inhibition of the decrease in energy expenditure and resulting in a further decrease in blood glucose levels. Activation of brown adipose tissue induces energy expenditure and glucose uptake into its tissue. Central leptin gene therapy increased UCP1 and GLUT4 expression in brown adipose tissue [12] and continuous leptin infusion resulted in increasing oxygen consumption and GLUT4 expression in brown adipose tissue [21]. Thus, brown adipose tissue plays an important role in energy expenditure and glucose metabolism simultaneously. Given that blunting leptin-fall in fasting influence oxygen consumption and also blood glucose levels, there may be a link between energy expenditure and glucose uptake in brown adipose tissue. Brown adipose tissue may be one of the major target organs of leptin and may have played a major role for the result of this study.

A problem in this study was lack of information about leptin levels in these animals. It has been reported that peripheral leptin infusion with the same concentration (20 ng/body weight gram/hour) as our study using the same osmotic pump resulted in 4.7±0.6 ng/ml [22]. We confirmed that there was no residue in the pumps in all the animals after the experiment. Peripheral ghrelin administration produced positive energy balance by promoting food intake and decreasing energy expenditure [23].

The major effects of ghrelin may be linked as a protective mechanism against starvation [24]. In our previous study, ghrelin elevation in response to fasting was attenuated in obese mice compare to normal mice [16], possibly affecting the lesser decrease in oxygen consumption in ob/ob mice. The decrease in glucose levels in fasting did not differ between leptin-infused ob/ob mice and non-infused ob/ob mice. Taken together with the result of oxygen consumption in ob/ob mice, lack of leptin-fall rather than absence of leptin may contribute to those changes in energy and glucose metabolism in fasting. The fall of leptin may be an important signal for maintaining energy and glucose homeostasis in fasting.

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