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# Evidence of growth hormone effect on plasma leptin in diet-induced obesity and diet-resistant rats

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**Abstract**

**Background:** Plasma leptin is regulated by several factors, including growth hormone (GH), which influences the pathophysiology of obesity.

**Objective:** To demonstrate the short-term effect of GH on plasma leptin levels in 3 conditions *in vivo* with the different amount of body fat mass.

**Methods:** Adult male Wistar rats were fed with standard chow or hypercaloric diet (HC). The HC rats were demonstrated as HC-feeding obese (HC-O) and HC-feeding resistant (HC-R) rats. Then, they were treated with GH or saline for 3 days. Basal plasma leptin levels were measured at 24 and 32 h. For meal-induced condition, all rats were fed for 2 hand plasma leptin was measured. Further 16-h fasting period, plasma leptin, insulin, and insulin sensitivity indexes were determined.

**Results:** The short-term GH treatment decreased basal plasma leptin at 32 h after the first GH injection in HC-O rats. However, GH treatment had no effect on meal-induced plasma leptin in all rats. Furthermore, GH treatment attenuated fasting effect on plasma leptin in control and HC-R rats. The insulin resistance (IR) induced by the short-term GH treatment was demonstrated by higher fasting plasma insulin and the increased homeostasis model of IR in HC-R rats.

**Conclusions:** The study demonstrates the important role of greater fat mass in HC-O rats, which results in decreased basal plasma leptin after short-term GH treatment. For meal-induced condition, GH had no effect on plasma leptin in all rats. Interestingly, GH could attenuate fasting effect on plasma leptin in rats that have lower fat mass.

**Keywords:** adipose tissue; growth hormone; insulin resistance; leptin; obesity

White adipose tissue is considered the major leptin-secreting organ. The mechanisms regulating leptin production and secretion are complex and depend on multiple factors. Plasma leptin is well correlated with fat mass in both lean and obese animals [1, 2]. Plasma leptin and its pattern have been investigated in many animals, including laboratory rodents and humans [3–5]. In adult rats, plasma leptin shows basal level without peak in light period [4, 6–7]. This plasma leptin level could be influenced mainly by nutritional status (i.e., feeding and fasting), glucose and free fatty acids (FFAs), and hormonal control (i.e., insulin and catecholamines). Feeding and fasting models suggested important roles of plasma insulin,

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glucose, and FFA in the diurnal patterns of plasma leptin [3, 4, 8–10]. In addition to these well-known effects of insulin on plasma leptin, growth hormone (GH) has also been demonstrated to influence plasma leptin [11–15]. With long-term GH treatment, lower plasma leptin has been demonstrated in accordance with decreased fat mass [16, 17]. This article aimed to study the short-term GH effect on plasma leptin, which was independently of the altered body adiposity. This point was interesting for 3 main reasons. First, obesity is associated with attenuated plasma GH, in which GH has long been considered as hormonal treatment in obesity and metabolic syndrome [18–20]. Second, short-term GH treatment decreased food intake (FI) in laboratory rodents and goats [14, 15], which may associate with elevated plasma leptin in this period. Finally, the metabolic effects of GH are closely related to and influenced by insulin and leptin [21]. The latter argument appeared to be the major cause of the discrepancy in the short-term GH treatment on plasma leptin [11–15]. To study whether fat mass influenced the effect of GH on plasma leptin, the current experiments investigated the short-term effects of GH treatment on 2 different amounts of adipose tissue mass from control and obese rats. In addition, this study emphasized basal plasma leptin , which refers to the light phase plasma leptin in *ad libitum* rats [4], meal-induced and fasting conditions. The information from the current studies would contribute to the fundamental mechanisms of leptin secretion from adipose tissue and perhaps support the idea of GH as an adjunct, but not main, hormonal treatment in obesity.

# Materials and methods

### Animals

Adult male Wistar rats, aged 12 weeks, were purchased from National Laboratory Animal Center, Mahidol University, and individually housed in conventional hanging cages with stainless steel wire mesh floors (33 cm × 18 cm × 20 cm) under standard condition(12 h/12 h light/dark cycle, 22 ± 1°C). All rats were allowed to acclimatize to the experimental condition for at least 2 weeks before starting the experiment. The rats were fed standard diet rat chow (#082; Perfect Companion Group Ltd., Samutprakarn, Thailand; protein 24%, carbohydrate 42%, fat 4.5%, energy 3.04 kcal/g, energy from fat 13%) and water *ad libitum*. During the 6 weeks of obesity induction, rats were randomly divided into 2 groups and fed with either standard diet rat chow (control) or hypercaloric diet (HC; protein 17.52%, carbohydrate 30.66%, fat 30.3%, energy 4.65 kcal/g, energy from fat 60%). At the end of this period, HC obese (HC-O) rats were separated from HC resistant (HC-R) rats at the lowest tertile of body weight (BW) gain. The HC-R rats were the HC-fed rats that gained less weight than HC-O rats and used as diet control rats for HC feeding. The characteristics of the control, HC-R, and HC-O rats are presented in **Table 1**. BW and FI (23 h FI, ± 0.1 g corrected for spillage) were recorded every day during the experimental period. All procedures conducted on animals were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Science, Chulalongkorn University (approval no. 1531026).

### Experimental procedure

This experiment aimed at determining the effect of short-term exogenous GH treatment on basal, meal-induced and fasting plasma leptin in control (n = 12) and HC-R (n = 9) and HC-O rats (n = 12). All rats from each group were randomly divided into 2 subgroups and treated with either normal saline or GH injection (n = 6; for control and HC-O rats, n = 4 and 5; for saline-treated and GH-treated HC-R rats, respectively). The GH-treated rats were injected subcutaneously with recombinant human GH (GenHeal®; Shanghai United Cell Biotechnology Co., Ltd., Shanghai, China) 1 mg/kg twice per day (at 0800 and 1600 h) for 3 days. The 3-day GH treatment was selected based on the effect of GH on basal plasma leptin from our pilot study. Blood sample (0.3 mL) was collected from the ventral tail artery at 24 and 32 h after the first GH injection for basal plasma leptin measurement, because the results from our pilot study indicated that GH decreased basal plasma leptin at 32–36 h under light period. Meal-induced plasma leptin was

**Table 1.** Characteristics of the control, HC-R, and HC-O rats in the current experiments

| | Control (n = 12) | HC-R (n = 9) | HC-O (n = 12) |
|---|---|---|---|
| Initial BW (g) | 441.02 ± 2.93[a] | 420.19 ± 7.58[b] | 447.33 ± 5.40[a] |
| Final BW (g) | 503.68 ± 3.41[b] | 508.27 ± 7.73[b] | 574.52 ± 7.98[a] |
| BW gain (g) | 62.67 ± 1.93[c] | 88.08 ± 4.68[b] | 127.19 ± 5.80[a] |
| BW gain/day (g/day) | 1.57 ± 0.05[c] | 2.20 ± 0.12[b] | 3.18 ± 0.14[a] |
| Body fat mass (%) | 11.25 ± 0.30[c] | 12.92 ± 0.41[b] | 14.97 ± 0.31[a] |
| AUC-IPGTT | 24667 ± 1303[b] | 24304 ± 1198[b] | 28411 ± 1242[a] |

[a–c]Significant difference between groups, $P < 0.05$

AUC-IPGTT, area under the curve from intraperitoneal glucose tolerance test; BW, body weight; HC-O, hypercaloric diet obese; HC-R, hypercaloric diet resistance

investigated at the beginning of dark phase on day 3 after the first GH injection. Rats were fasted for 2 h (1600 h) before dark onset. At the onset of dark phase (1800 h), food was provided; then, the rats were allowed to access the food for 2 h [20]. Blood samples (0.3 mL) for meal-induced plasma leptin were collected before light off (premeal, 1800 h), which was considered as basal leptin after 32 h from the first injection and after eating for 2 h (post-meal, 2000 h). Meal-induced plasma leptin was calculated from the post- and premeal difference of plasma leptin (leptin difference). After meal-induced plasma leptin experiment, food was removed, and then the rats were fasted for another 16 h. At the mid-light phase of day 3, blood samples were collected for the measurement of fasting plasma glucose ($G_{fasting}$), insulin ($I_{fasting}$), $FFA_{fasting}$, and leptin, as shown in **Figure 1**. All blood samples were centrifuged at 3,000 g for 15 min and stored at –20°C for further analysis. The effect of fasting on plasma leptin was calculated from the difference between average basal plasma leptin (from 24 and 32 h after the first injection) and fasting plasma leptin (leptin difference). Insulin sensitivity index was calculated from plasma insulin and glucose during fasting. In addition, the surrogate indexes for insulin receptor sensitivity were calculated in the present experiment. The homeostasis model of insulin resistance (HOMA-IR = $[I_{fasting} \times G_{fasting}]/2,430$) was used to estimate the hepatic insulin sensitivity [22]. Furthermore, the adipose tissue insulin resistance (Adipo-IR = $I_{fasting} \times FFA_{fasting}$) was also used to estimate the adipose tissue insulin sensitivity [23]. At the end of the experiment, all rats were euthanized with intraperitoneal pentobarbital (150 mg/kg, Nembutal®; Ceva Sante Animale, Libourne, France). The visceral and subcutaneous fat masses were dissected to determine the total body fat mass. Leptin secretion per body fat mass was calculated from the ratio between average basal plasma leptin (from 24 and 32 h after the first injection) and total body fat mass.

### Intraperitoneal glucose tolerance test

Glucose tolerance test was used to determine the representative whole-body insulin sensitivity after obesity induction period. The rats were fasted for 16 h before intraperitoneal glucose tolerance test (IPGTT) evaluation. Blood samples were collected by tail-clipping technique for blood glucose measurement (Accu-Chek® Performa; Roche Diagnostic GmbH, Mannheim, Germany) before glucose administration (time 0). The 50% glucose solution (A.N.B. Laboratories Co., Ltd., Bangkok, Thailand) was injected intraperitoneally (2 g/kg BW). Blood glucose was measured at 15, 30, 60, 90, and 120 min after glucose injection.

### Plasma hormone and FFA analyses

Plasma leptin levels were measured by ELISA kits (EZRL-83K; Merck Millipore, Massachusetts, USA). The intra-assay coefficient of variability (CV) for this measurement was 3.46%, and the inter-assay CV was 6.97%. Plasma insulin was measured with a commercial ELISA kit (EZRMI-13K; Merck Millipore, Massachusetts, USA). The intra-assay CV for this measurement was 2.43%. Plasma FFA was measured with colorimetric assay kit (ab65341; Abcam, Cambridge, UK).

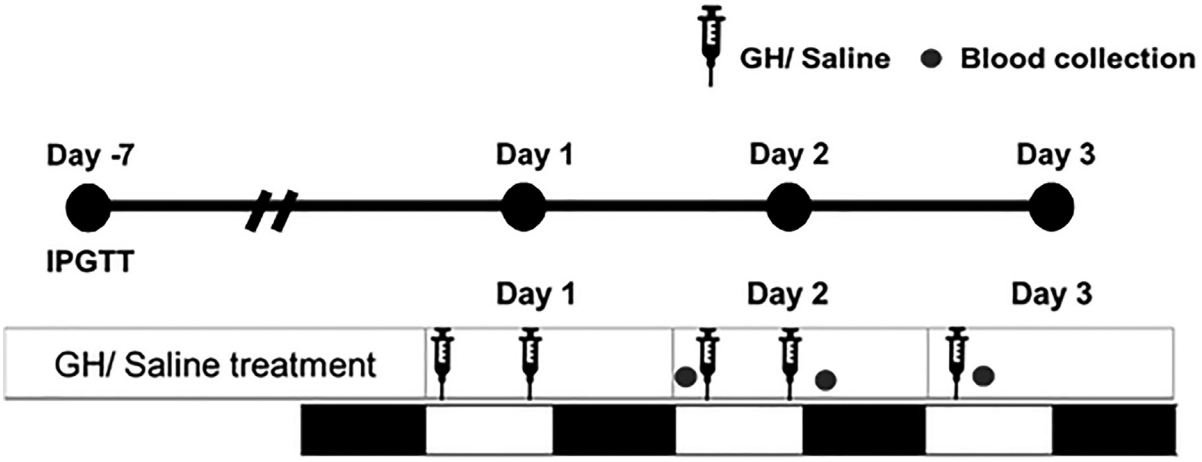


**Figure 1.** The experimental procedure is demonstrated. After the obesity induction period, IPGTT was performed before GH or saline treatment (at day -7). Then, the rats were injected with either GH or saline for 3 days and blood samples were collected for plasma hormones and FFA measurement. The white and black bars indicate light and dark phases, respectively (12h/12h light/dark cycle).
FFA, free fatty acid; GH, growth hormone; IPGTT, intraperitoneal glucose tolerance test

### Statistical analyses

The results were analyzed by 2-way analysis of variance (ANOVA). In addition, for the analysis of basal plasma leptin at 2 different time points either saline or GH, the test was performed by 2-way repeated-measures ANOVA. The significant main effects were followed up using a Bonferroni posttest. A significant mean difference was set at $P < 0.05$. All data are presented as mean ± standard error of the mean (SEM). All the data were analyzed using GraphPad Prism 7.00 (GraphPad Software, California, USA).

## Results

After 24 and 32 h of the first GH injection, Basal plasma leptin levels were not significantly different in control rats ($P > 0.05$; **Table 2**). In addition, there was no significant effect of GH ($P > 0.05$). In HC-R rats, there was no effect of time points (24 and 32 h) and GH on basal plasma leptin levels ($P > 0.05$). However, basal plasma leptin levels were significantly different between 24 and 32 h in both saline and GH-treated HC-O rats ($P < 0.05$). Basal plasma leptin level at 32 h was higher than that of 24 h in both saline and GH-treated HC-O rats ($P < 0.05$). Furthermore, the short-term GH administration had no effect on body adiposity ($P > 0.05$; **Figure 2A**,), while HC-O rats had the greatest fat mass among these 3 groups ($P < 0.001$). For the normalized basal plasma leptin to total adipose mass, GH treatment decreased leptin per fat tissue ($P < 0.05$; **Figure 2B**); this effect was demonstrated only in HC-O rats ($P < 0.05$), except control and HC-R rats ($P > 0.05$). The increased plasma leptin after meal-induced condition was shown as leptin difference. There were no significantly different between groups and no significantly different of GH effect in each group ($P > 0.05$; **Figure 3A**). In addition, the energy intake during 2 h was analyzed in all groups. The energy intake in HC-O rats was significantly higher than that of both control and HC-R rats ($P < 0.05$; **Figure 3B**).

In 16-h fasting condition, fasting effect on plasma leptin level was shown as leptin difference. The effect of GH on the fasting effect on plasma leptin was significantly difference ($P < 0.05$; **Figure 4**).GH attenuated fasting effect on plasma leptin as shown in figure 4 ($P < 0.05$), but not in HC-O rats ($P > 0.05$). Moreover, GH treatment increased fasting plasma insulin in all rats ($P < 0.05$), but there were no significant effects on fasting plasma glucose and FFA in all rats ($P > 0.05$; **Table 3**). The calculated HOMA-IR of the GH-treated group was higher than that of the saline-treated group ($P < 0.05$). The effect of GH was pronounced only in HC-R rats ($P < 0.05$) but not in control and HC-O rats ($P > 0.05$; **Figure 5A**). In addition, the calculated Adipo-IR from saline-treated group was statistically significantly different from the GH-treated group in HC-R rats ($P < 0.05$), but not in control and HC-O rats ($P > 0.05$; **Figure 5B**).

**Table 2.** The effect of short-term GH treatment on basal plasma leptin at 24 and 32 h after GH injection

| Time after the first injection | Basal plasma leptin (ng/mL) | | |
|---|---|---|---|
| | Control | HC-R | HC-O |
| Saline | | | |
| 24 h | 16.98 ± 0.7 | 18.93 ± 2.7 | 21.16 ± 1.0 |
| 32 h | 17.94 ± 1.2 | 18.47 ± 1.9 | 29.20 ± 1.8* |
| GH | | | |
| 24 h | 16.89 ± 0.8 | 15.74 ± 0.9 | 17.00 ± 1.8 |
| 32 h | 17.44 ± 2.5 | 18.38 ± 0.8 | 21.55 ± 2.0*,# |

*Effect of time on basal plasma leptin in HC-O rats, $P < 0.05$

#Effect of GH on basal plasma leptin in saline vs GH treatment at 32 h, $P < 0.05$

GH, growth hormone; HC-O, hypercaloric diet obese; HC-R, hypercaloric diet resistance

## Discussion

Our data showed that the short-term GH treatment decreased basal plasma leptin only in HC-O rats, which suggested that the short-term GH effect on basal plasma leptin partly depends on body adiposity. The results revealed that the short-term GH administration could not alter adipose tissue mass in all rats. Interestingly, GH decreased normalized plasma leptin to body fat mass only in HC-O rats. However, the short-term GH treatment could not influence meal-induced plasma leptin in all rats. In addition, GH administration could attenuate fasting effect on plasma leptin in the control and HC-R groups.

The main objective of the current experiment was to investigate the short-term effect of GH treatment on plasma leptin in different paradigms. By using GH as exogenous hormonal stimulus, the current findings revealed the different mechanisms controlling basal plasma leptin. In the current experiment, we measured basal plasma leptin during light period. After 24 h of the first injection, GH had no effect on basal plasma leptin. However, after 32 h of the first injection, plasma leptin from GH treatment was lower than that from saline injection in the HC-O group. It should be noted that all rats cannot access to food for 1 h before light off during the maintenance period every day. Since all rats were fasted for only 2 h before the light off (at 32 h after the first injection), the premeal plasma

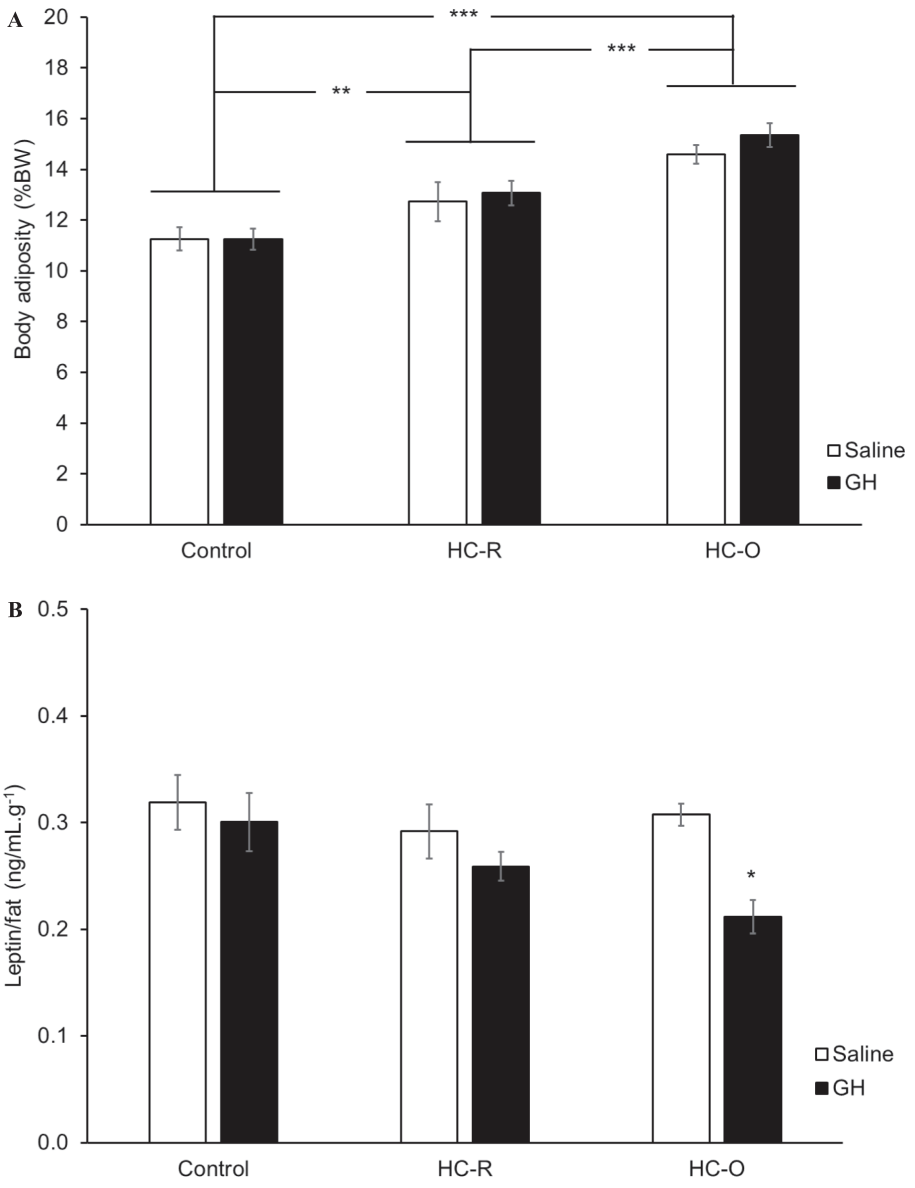


**Figure 2.** The effect of short-term GH treatment on body adiposity and leptin per fat mass. (**A**) There was no effect of GH on body adiposity in all rats, and HC-O rats had the highest fat mass compared to HC-R and control rats (***$P < 0.001$, **$P < 0.01$). (**B**) There was a significant effect of GH on leptin secretion per fat mass in HC-O rats.

*The leptin per fat mass of GH-treated HC-O rats was significantly lower than that from saline treatment, $P < 0.05$

GH, growth hormone; HC-O, hypercaloric diet obese; HC-R, hypercaloric diet resistance

leptin level is considered to be within the range of basal plasma leptin [3, 4]. This interpretation agrees with our pilot study for the GH effect on basal plasma leptin. Our results are consistent with the chronological study of acute GH treatment on basal plasma leptin in healthy humans [12]. Unfortunately, many experiments in rodents were investigated for long-term GH effect on plasma leptin or cross-sectional information [11, 25, 26]. This study suggests that the amount of adipose tissue apparently influences the effect of GH on basal plasma leptin. The previous studies demonstrated that leptin content and secretion rate have been correlated well with the fat mass and the size of adipocyte [27, 28]. However, the reason that GH decreased basal plasma leptin levels and normalized plasma leptin to body fat mass in which specifically occurred in HC-O rats remains unknown (see the following section). In rodents, the diurnal pattern of plasma leptin has been well demonstrated. Plasma leptin is maintained at its basal level during the light phase and gradually increases after the first meal of dark onset [3, 4]. The reason that explained the higher plasma leptin at 32 h than that of 24 h in HC-O rats was unknown. Although many experiments have been performed to study nighttime plasma leptin, the mechanisms that control basal plasma leptin

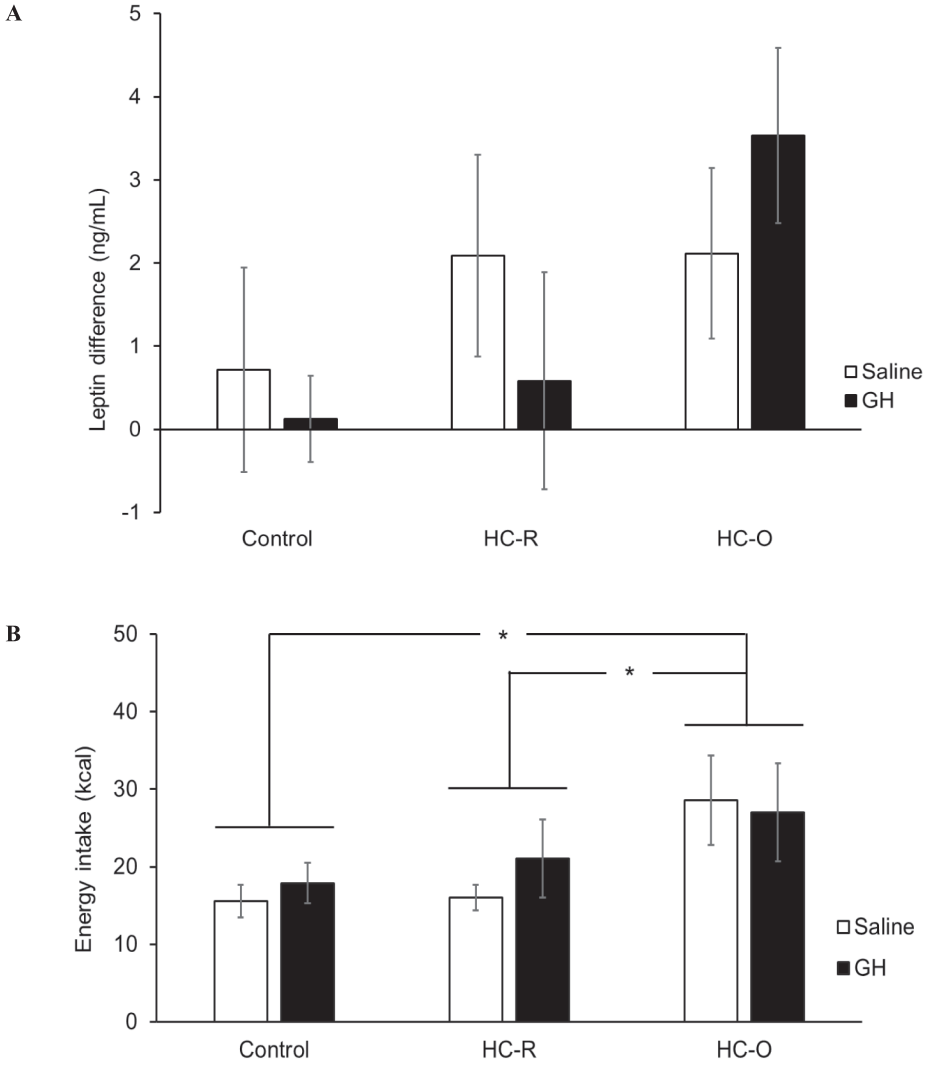


**Figure 3.** The effect of short-term GH treatment on meal-induced plasma leptin. (**A**) There was no effect of GH on meal-induced plasma leptin, which was demonstrated as leptin difference of pre- and post-meal plasma leptin. (**B**) The energy intake from meal-induced plasma leptin experiment revealed that HC-O rats had the highest energy intake compared to control and HC-R rats.
*The energy intake of HC-O rats was significantly different from others, $P < 0.05$
GH, growth hormone; HC-O, hypercaloric diet obese; HC-R, hypercaloric diet resistance

are less studied and remain unclear. Previous studies demonstrated that basal leptin content in adipose tissue depends on new leptin synthesis. The pathway of leptin biosynthesis was apparently regulated by a mechanism independent of insulin action [29]. Taken together, the *in vivo* effect of GH to decrease basal plasma leptin may be mediated by decreasing the leptin content and this action may apparently be regulated by insulin-independent pathway.

Next, we demonstrated that the short-term GH treatment had no effect on meal-induced plasma leptin in all rats. Meal-induced plasma leptin has been well studied and was related to the nighttime plasma leptin in the rodent. The single peak of nighttime plasma leptin in rodents could be influenced strongly by eating behavior [3, 4]. When food was provided only in the light phase, diurnal rhythm of plasma leptin was reversed according to eating activity [4]. Insulin and glucose were important determinants of meal-induced plasma leptin [28, 30]. For instance, plasma leptin after glucose administration was dependent on high plasma insulin [30]. We propose from our previous result that the mechanism of GH effect on basal plasma leptin may be independent on insulin-mediated plasma leptin. The current results support, but not prove, our hypothesis and further inform that GH treatment had no effect on meal-induced plasma leptin (or insulin-stimulating leptin secretion) in HC-O rats during the stage of ample energy. The previous report provided an association between the amount of energy intake and meal-induced plasma leptin [22]. In addition, our results revealed that HC-O rats had the greatest

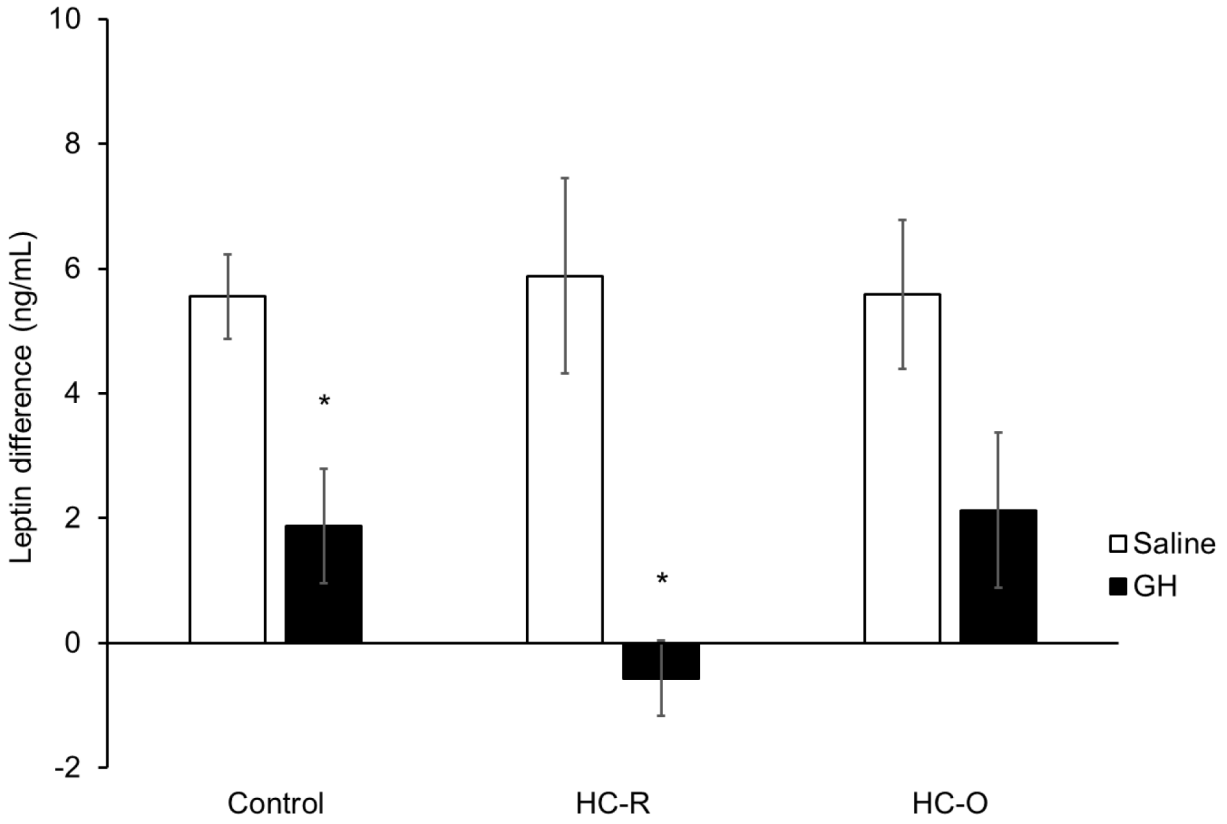


**Figure 4.** The effect of short-term GH treatment on the fasting effect on plasma leptin (leptin difference). There was a significant difference of the effect of GH treatment in the control and HC-R groups.
*GH effect is significantly different from saline treatment, $P < 0.05$.
GH, growth hormone; HC-R, hypercaloric diet resistance

**Table 3.** The effect of short-term GH treatment on fasting plasma insulin, glucose, and FFA

| | Control | HC-R | HC-O |
|---|---|---|---|
| Insulin (ng/mL) | | | |
| Saline | 2.96 ± 0.5 | 2.39 ± 0.7 | 5.12 ± 0.8 |
| GH | 6.09 ± 1.0† | 6.26 ± 1.4† | 6.92 ± 1.2† |
| Glucose (mg/dL) | | | |
| Saline | 131.50 ± 6.8 | 126.00 ± 6.0 | 131.50 ± 4.0 |
| GH | 135.20 ± 3.0 | 126.80 ± 12.8 | 123.30 ± 7.7 |
| FFA (mmol/L) | | | |
| Saline | 0.55 ± 0.13 | 0.29 ± 0.06 | 0.60 ± 0.14 |
| GH | 0.45 ± 0.10 | 0.81 ± 0.12 | 0.67 ± 0.17 |

†GH effect on fasting plasma insulin in all groups, $P < 0.05$
FFA, free fatty acid; GH, growth hormone; HC-O, hypercaloric diet obese; HC-R, hypercaloric diet resistance

energy intake among all groups, which was related to meal-induced plasma leptin.

Fasting plasma leptin appears to share a similar mechanism with basal and meal-induced plasma leptin. First, the leptin secretion during fasting derived mainly from the basal pool of leptin vesicle [29]. Second, decreased plasma leptin during fasting apparently occurred in part from the absence of the signal from insulin-stimulating leptin secretion [30, 31]. During fasting, decreased plasma insulin concentration as well as decreased glucose uptake and adipose tissue oxidation have been well demonstrated [32, 33]. In addition, lipolysis is the main biochemical pathway in adipocytes to provide FFA as an energy source [34]. Although intracellular FFA has been demonstrated to attenuate insulin-stimulated leptin secretion [8], the mechanism apparently fits the stage of ample energy with the presence of insulin and glucose rather than the stage of fasting [8, 29, 31]. With 16-h fasting condition, plasma leptin was decreased in saline-treated control and HC-R rats, which was significantly different from GH-treated control and HC-R rats. This result was apparently due to the GH effect, which could attenuate fasting effect. This attenuation effect may be explained indirectly by the effect of GH on plasma insulin. However, for HC-O rats, the fasting effect was similar as control rats but not statistical significance ($P = 0.067$). The result suggested that adipose mass in HC-O rats may influence fasting effect on plasma leptin. We also demonstrated that

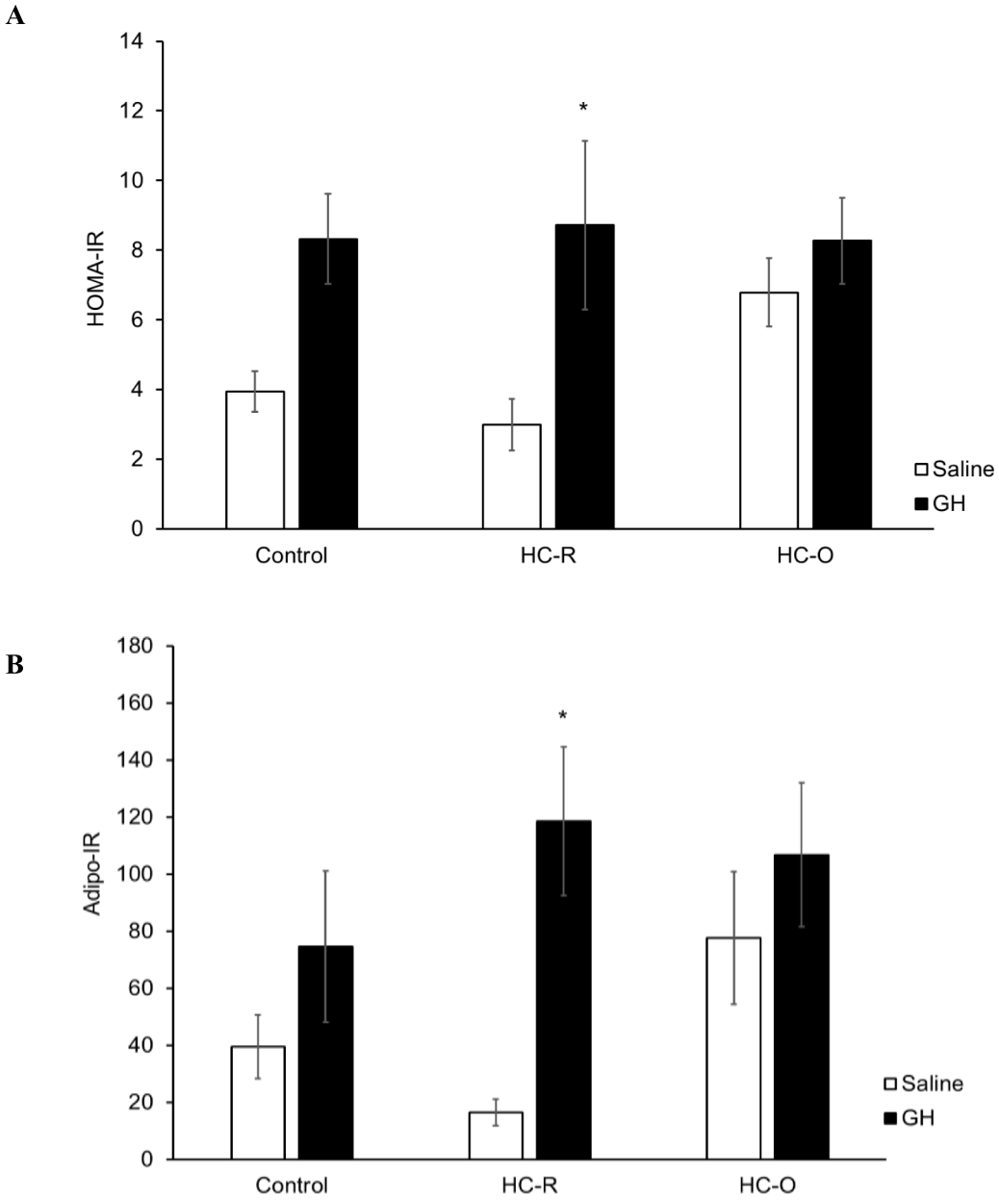


**Figure 5.** The effect of short-term GH treatment on the surrogate indexes for insulin receptor sensitivity. (**A**) The calculated HOMA-IR revealed the significant GH effect in HC-R rats. (**B**) For the Adipo-IR index, GH treatment could increase this index in HC-R rats.
*GH effect is significantly different from saline treatment, $P < 0.05$.
Adipo-IR, adipose tissue insulin resistance; GH, growth hormone; HC-R, hypercaloric diet resistance; HOMA-IR, homeostasis model of insulin resistance.

fasting plasma insulin was increased by GH treatment in all rats. However, the short-term GH treatment had no effect on both fasting plasma glucose and FFA. These results suggested that the higher level of plasma insulin is necessary to maintain plasma glucose and FFA during fasting and GH treatment, and these points agree with previous reports [35, 36]. During the energy deficit of the fasting condition, hepatic glucose output and adipose tissue lipolysis are the major biochemical mechanisms that maintain normal plasma glucose and elevate plasma FFA [34, 37]. In addition, short-term GH administration increased insulin resistance (IR) via HOMA-IR and Adipo-IR in HC-R rats. Since HOMA-IR and Adipo-IR were the surrogate indexes on IR in different organs, this information suggests that the short-term GH treatment could induce IR in systemic glucose metabolism and adipose tissue lipid metabolism [35, 38]. However, it is very important at this stage to keep in mind that the current experiment did not measure directly the organ-specific insulin receptor sensitivity. Nevertheless,

hyperinsulinemia could be able to stimulate leptin secretion from adipose tissue and increase plasma leptin in fasting condition despite the IR [39]. Therefore, the effect of GH on fasting plasma leptin that occurred in the control and HC-R groups apparently mediated in part by insulin-induced leptin secretion. It should be noted that the effect of fasting could promote insulin-mediated glucose uptake [40], which may probably promote leptin secretion.

In conclusion, the current results revealed the short-term effect of GH on plasma leptin levels. GH could decrease basal plasma leptin, which appeared to associate with body adiposity. GH did not produce this effect on meal-induced plasma leptin. In addition, GH could attenuate the fasting effect on plasma leptin.

**Author contributions.** SL, SK-T, and ST contributed substantially to the conception and design of this study. SL and ST substantially acquired the data and analyzed and interpreted it. SL and ST drafted the manuscript. SK-T critically revised it. All the authors approved the final version submitted for publication and take responsibility for statements made in the published article.

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**Conflict of interest statement.** The authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors disclose any conflict of interest.

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