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Growth Hormone (GH)-Independent Stimulation of Adiposity by GH Secretagogues

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Growth hormone secretagogues (GHSs) stimulate growth hormone (GH) secretion, which is lipolytic. Here we compared the effects of twice daily s.c. treatment of GH and the GHS, ipamorelin, on body fat in GH-deficient (*lit/lit*) and in GH-intact (*+/lit* and +/+) mice. In *+/lit* and *lit/lit* mice ipamorelin induced a small (15%) increase in body weight by 2 weeks, that was not further augmented by 9 weeks. GH treatment markedly enhanced body weight in both groups. Ipamorelin also increased fat pad weights relative to body weight in both *lit/lit* and *+/lit* mice. Two weeks GHS treatment (ipamorelin or GHRP-6) also increased relative body fat, quantified by *in vivo* dual energy X-ray absorpiometry (DEXA) in GH-intact mice. GH decreased relative fat mass in *lit/lit* mice and had no effect in GH-intact mice. Treatment with GHS, but not GH, increased serum leptin and food intake in GH-intact mice. Thus, GHSs increase body fat by GH-independent mechanisms that may include increased feeding.

Key Words: **GH secretagogue; ipamorelin; GHRP-6; ghrelin; adiposity; obesity; food intake; body composition; *lit/lit;* dwarf mice.**

Growth hormone secretagogues (GHSs) are orally active synthetic compounds that were initially shown to stimulate growth hormone (GH) secretion by a direct pituitary (1, 2) and a hypothalamic (3, 4) action. These effects are brought about by activation of specific GHS receptors, such as the cloned receptor GHS-R1A, a G-protein coupled receptor (5) that is present at both pituitary and hypothalamic sites (5–7).

Recently, an endogenous ligand for the GHS-R1A receptor was isolated, sequenced and named "ghrelin" (8). Initial pharmacological studies show that ghrelin, like synthetic GHSs, increases GH secretion following systemic injection (1, 8–9) and activates hypothalamic neurones (3–4, 10) and induce food intake (9, 11–13) when given systemically or centrally. Ghrelin is produced by the oxyntic glands of the stomach. Immunoreactive ghrelin has been identified in the circulation and it has been suggested that ghrelin may be an endocrine signal from gut to hypothalamus (8). Although ghrelin's physiological role has not yet been identified, its localization may suggest a role in feeding and nutrition.

Long term treatment with GHSs induces similar anabolic effects to GH and it is largely assumed that these effects are mediated by GH (1, 2). For instance, we reported previously that chronic GHS infusion to female rats for 3 months increases body weight reflecting, in part, growth effects on bone; these effects were similar to GH treatment (14). We also demonstrated that chronic GHS treatment increases lean body mass in humans (15).

Although it has been reported that GHSs enhance lean body mass and bone mass, it is less clear to what extent the short term stimulatory effects of GHSs on food intake and secretion of GH are accompanied by long term effects on body fat mass. Chronic stimulation of food intake by GHSs could result in increased body fat. On the other hand, chronic stimulation of GH secretion by GHSs could decrease body fat, as GH is known to be lipolytic (16). It is not clear which of these two effects that would prevail, and GHS may also affect body fat via other, as yet unknown, mechanisms. Most previous studies of GHS effects on body fat have been performed in obese rats (17–18) and humans (14), as these models are best suited for detection of possible lipolytic effects. Here we investigated the effects of chronic GHS treatment on body fat in normal nonobese mice. In addition, we compared the effects of GHSs in GH-deficient *lit/lit* mice and GH-intact (heterozygous *+/lit* and wild-type +/+) mice to discern the GH-dependent from the GH-independent effects of these compounds.

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MATERIALS AND METHODS

Animals. Female *lit/lit* mice and their non-GH-deficient *+/lit* heterozygous littermates (12 weeks old, mean body weight at onset: *lit/lit* = 12 g; *+/lit* = 20 g; Jackson Labs, ME) and normal female C57 black mice (12 weeks old, mean body weight at onset = 30 g; C57/black, BK Universal, Copenhagen, Denmark) were housed in groups under standardized environmental conditions (21.5–22.5°C; 50–60% relative humidity, artificial lighting at 0500–1900). The mice were given free access to water and to standard rodent chow (BK Universal, Stockholm, Sweden). All experimental procedures were carried out according to ethical guidelines for animal experimentation at the University of Gothenburg, Sweden.

Nine weeks of ipamorelin treatment to GH-deficient lit/lit mice. The *lit/lit* mouse is an appropriate model to investigate the GH-independent effects of GHSs as these mice are profoundly GH deficient and are insensitive to the GH-stimulating effects of GHSs (19) as well as GHRH (20), although the hypothalamus is still responsive to the GHSs (21). Over a 9-week period female *lit/lit* mice and their non-GH-deficient *+/lit* heterozygous littermates (back crossed to the C57/black strain) were injected s.c. twice daily with ipamorelin (500 μg/kg/day; $n = 8$); human GH (GH; 3.5 mg/kg/day Norditropin, Novo Nordisk A/S, Copenhagen, Denmark; $n = 8$); or vehicle (0.9% saline, $n = 8$). Body weight was measured at baseline and thereafter every 3 days for the duration of the study. At the end of the experiment, mice were anaesthetized i.p. with a combination of ketamine hydrochloride (1.8 mg/ml; Ketalar, Park Davies, Morris Plains, NJ; 67 mg/kg) and xylazine (13 mg/ml; Rompun, Bayer AG, Leverkusen, Germany). Blood was collected under anaesthesia by cardiac puncture and serum reserved and stored at −20°C until assay. Brain, heart, liver and kidneys were dissected and weighed. Inguinal, retroperitoneal and parametrial fat pads were dissected bilaterally and weighed.

Two weeks of GHRP-6 and ipamorelin treatment to normal female mice. To further investigate the effects of ipamorelin on adiposity, in a second study, we extended our observations to include not only fat pad dissection but also dual energy X-ray absorpiometry (DEXA) that provides a quantitative analysis of relative total body fat. Also as GHSs other than ipamorelin have reported effects on glucocorticoids (1) that, in turn, increase body fat (22), we also compared effects of ipamorelin (that does not increase glucocorticoids at lower doses; 23) with the most well described peptide GHS, growth hormone-releasing peptide-6 (GHRP-6). As an increase in body weight occurred very early into the 9-week study (within 1–2 weeks) we investigated changes in body fat during 2 weeks of chronic GHS treatment. The protocol described for the 9 week study was repeated over 2 weeks in normal (+/+) C57/black female mice with an additional GHS-treated group, given GHRP-6 (500 μg/kg/day; $n = 8$). *In vivo* DEXA measurements were performed for all mice at baseline and before sacrifice. Prior to DEXA measurements, mice were anaesthetized with a combination of ketamine hydrochloride (7.5 mg/kg) and medetomidine hydrochloride (1 mg/kg Domitor; Orion Pharma AB, Finland) in isotonic saline. Recovery from anaesthesia was induced by an i.p. injection of the antidote Atipamezol hydrochloride (0.5 mg/kg Antisedan; Orion Pharma AB, Orion Corporation, Finland). Mice were housed individually and body weight was noted every 3 days. Food intake was measured during a 3 day period (days 2–5, corresponding to the time point at which fat accumulation occurred). At sacrifice mice were anaesthetized as described for placement in the DEXA machine. At dissection, trunk blood samples and organs were removed and weighed. Organs removed included brain, heart, liver, kidneys and all visceral fat pads (bilateral inguinal, parametrial and retroperitoneal fat pads as well as the intestinal mesenteric fat pads).

Dual energy X-ray absorpiometry: Quantification of total body fat. To assess changes in total body fat area as a percentage of the body area, whole body scans of normal (+/+) female C57/black mice were performed with the Norland pDEXA Sabre (Fort Atkinson, WI) (24). Three mice were included in each scan. In order to avoid inter-scan variations, a mouse was sacrificed at the beginning of the study and included as an internal standard in all scans. The software Sabre Research (version 3.6) was used (scan speed = 15 mm/s). Areas of >50% fat appeared white on the scanned image. The accuracy of this fat setting was evaluated before each scanning procedure by recalibrating with a standard consisting of 0–100% fat (interscan variation <2%). The printed scan was imported to densitometry software (Scion Image, Scion Corporation, Frederick, MD) for analysis of fat area by subtracting areas of fat free mass (A2) from the total area of mouse body (A1). The % fat area relative to body area was then calculated as the ratio [(A1-A2)/A1]*100.

Blood assays. Leptin concentration in mouse serum was determined using a mouse leptin ELISA kit (Crystal Chem Inc., Chicago, IL) with a minimum detection level of 200 pg/ml and a measurable concentration range from 200–800 pg/ml. Corticosterone concentrations in mouse serum were analyzed using a high specificity ImmunoChem Double Antibody ^{125}I radioimmunoassay kit for mice (ICN Biochemicals Inc., Costa Mesa, CA) with a minimum detection level of 25 ng/ml and a measurable concentration range from 25–1000 ng/ml.

Statistics. Results are presented as the mean ± standard error of the mean (SEM). For repeated *in vivo* body weight data, a 2-way analysis of variance (ANOVA) was performed with time and treatment as the independent variables, followed by Student's *t* test with Bonferroni's adjustment on all values obtained after initiation of treatment. In all other measurements, data were analyzed by a 1-way ANOVA, followed by Dunnet's test against a control group. A 2-tailed $P < 0.05$ was considered significant.

RESULTS

Nine weeks of ipamorelin and GH to GH-deficient lit/lit mice. The GH-dependent and independent effects on body weight following chronic GHS treatment were studied in GH-deficient *lit/lit* mice that do not release GH in response to GHSs or GHRH (19–20), as well as in GH-intact *+/lit* mice. After 9 weeks the *lit/lit* mice showed an overall increase in body weight compared to baseline for all 3 treatment groups: ipamorelin-treated increased by 15.3 ± 2.9%, GH-treated by 95.5 ± 6.8% and saline-treated by 1.6 ± 4.3% (Fig. 1A). In the *+/lit* littermates, body weight was also increased for all treatment groups: ipamorelin-treated increased by 16.9 ± 5.3%, GH-treated by 27.5 ± 2.8% and saline-treated by 3.6 ± 1.3% (Fig. 1B). Compared to saline-treated controls, the increase in body weight by GH was significant ($P < 0.01$) in both *lit/lit* and *+/lit* mice. Ipamorelin increased body weight above that of saline-treated controls in the *+/lit* ($P < 0.01$) and *lit/lit* group ($P < 0.05$). After 9 weeks, ipamorelin-treated *lit/lit* and *+/lit* mice demonstrated a very similar overall percentage increase in body weight from baseline values. Most of the increase in body weight by ipamorelin was induced during the first 1–2 weeks of treatment. This was similar to the timing of the first increase in body weight with GH treatment (day 5), but the GH-treated animals continued to grow throughout the 9-week treatment period. To account for the increase in body weight observed in the GHS-injected mice, several organs

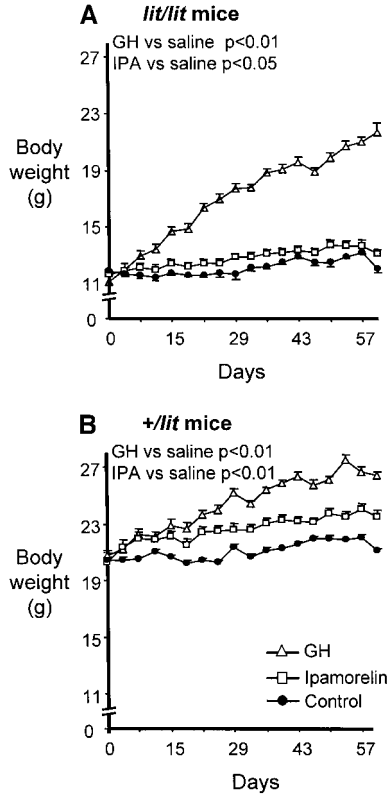

FIG. 1. Body weight (mean ± SEM) during chronic treatment of (A) *lit/lit* and (B) +/*lit* mice injected s.c. twice-daily with saline, ipamorelin or GH ($n = 8$ for all groups). All P values denote significance compared to the saline-treated controls mice during treatment (two-way ANOVA followed by Student's t test with Bonferroni's adjustment).

were removed and weighed at sacrifice. There was no increase in the relative weights of dissected organs (liver, heart, brain or kidneys) of GHS treated *lit/lit* or +/*lit* mice, whilst GH deficient *lit/lit* mice, subject to 9 weeks of GH injections had increased liver weight.

Inguinal, parametrial and retroperitoneal fat pads from *lit/lit* and +/*lit* mice were weighed and expressed as a percentage of body weight. Ipamorelin treatment caused an increase in the relative weights of parametrial fat pads in both *lit/lit* and +/*lit* mice and in the relative weight of inguinal and retroperitoneal fat pads in +/*lit* mice (Figs. 2A–2C). The sum of relative fat pad weights were increased in both *lit/lit* (+42%) and +/*lit* mice (+63%) injected with ipamorelin, compared to saline-treated controls (Fig. 2D). GH treatment decreased relative inguinal fat pad weight in *lit/lit* and +/*lit* mice (Fig. 2A) and also decreased the sum of relative fat pad weight in *lit/lit* mice (Fig. 2D), in line with its characteristic lipolytic effects (16).

Two weeks of GHRP-6, ipamorelin and GH treatment to normal female mice. To investigate whether the observed adipogenic effect is specific for ipamorelin or an effect in common with other GHRP-type of GHSs, we next compared the observed effects of ipamorelin and the prototype GHS, GHRP-6, on adiposity. Moreover, we investigated whether the adipogenic effect could be seen after 2 weeks GHS treatment, as the major effect on body weight was seen within the first 2 weeks of the 9-week study (Fig. 1). After 2 weeks, mice injected with GHRP-6, ipamorelin or GH, all showed an overall increase in body weight (mean ± SEM) compared to baseline values: GHRP-6-treated mice increased by 6.3 ± 1.9%, ipamorelin-treated increased by 7.9 ± 1.5% and GH-treated increased by 18.6 ± 1.2% (all $P < 0.01$). In contrast, normal mice injected with saline vehicle over 2 weeks did not show a significant change in body weight (−1.6 ± 2.2%). The GHSs effects on body weight were similar to those observed with ipamorelin treatment to GH-intact +/*lit* mice during the first 2 weeks of the 9-week study (Fig. 1).

Both ipamorelin and GHRP-6 significantly increased the relative weights of all dissected fat pads (parametrial, mesenteric, retroperitoneal, and inguinal) as compared to saline-treated controls (Table 1). The sum of relative fat pad weights was increased by GHRP-6 and ipamorelin (Table 1). These results indicate that ipamorelin and also the prototype GHS, GHRP-6 in-

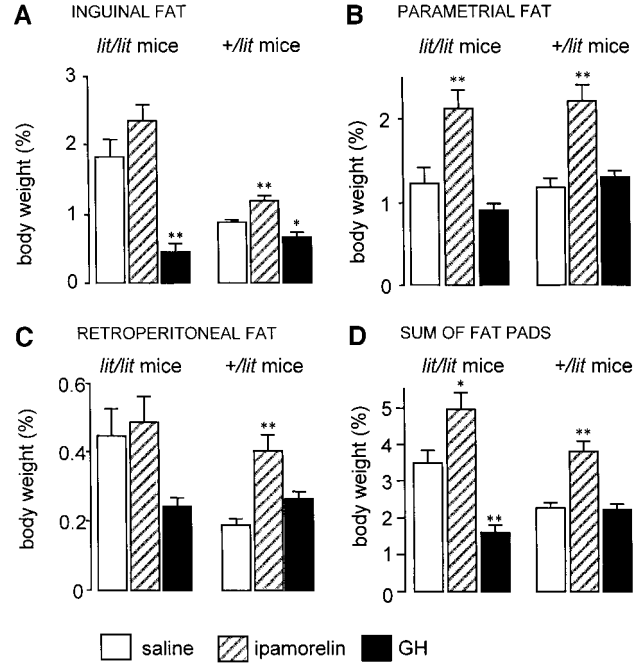

FIG. 2. (A–C) Relative weights (mean ± SEM) of dissected fat pads (i.e., fat pad weight expressed as a percentage of body weight) in GH-deficient *lit/lit* mice and GH-intact +/*lit* mice injected s.c. for 9 weeks with saline, ipamorelin or GH. For each mouse inguinal, retroperitoneal and parametrial fat pads were removed bilaterally and weighed separately. (D) Sum of relative weight (mean ± SEM) of pooled inguinal, parametrial and retroperitoneal fat pads in *lit/lit* and +/*lit* mice. All P values denote significance after 9 weeks treatment compared to saline-treated control mice: one-way ANOVA followed by Dunnet's test against a control group (*$P < 0.05$, **$P < 0.01$). $n = 7–8$ mice for all groups.

TABLE 1

Effects of GH Secretagogues (GHRP-6 and Ipamorelin) or GH on Fat Pad Weights

Fat pads	Saline (n = 8)	GHRP-6 (n = 8)	Ipamorelin (n = 8)	GH (n = 7)
Inguinal	0.59 ± 0.03%	0.81 ± 0.10%**	0.97 ± 0.05%**	0.54 ± 0.05%
Parametrial	0.63 ± 0.04%	1.09 ± 0.12%**	1.27 ± 1.24%**	0.77 ± 0.05%
Retroperitoneal	0.11 ± 0.01%	0.19 ± 0.02%*	0.21 ± 0.01%**	0.14 ± 0.01%
Mesenteric	1.03 ± 0.05%	1.48 ± 0.10%**	1.59 ± 0.07%**	1.34 ± 0.09%
Sum of fat pads	3.78 ± 0.16%	4.86 ± 0.87%**	6.25 ± 0.28%**	3.77 ± 0.20%

Note. Relative weights (mean ± SEM) of dissected fat pads (i.e., fat pad weight expressed as a percentage of body weight) in normal GH-intact +/+ mice injected s.c. for 2 weeks with saline, GHRP-6, ipamorelin, or GH. For each mouse inguinal, parametrial, retroperitoneal, and mesenteric fat pads were removed bilaterally and weighed separately. The sum of these relative fat pad weights is also given. Numbers in parentheses denote the number of individuals in each treatment group. All P values denote significances compared to saline-treated control mice at the end of the study: one-way ANOVA followed by Dunnet's test against a control group (*$P < 0.05$, **$P < 0.01$).

crease body fat. In this 2-week study, the sum of fat pad weight was increased by 65% in ipamorelin-treated GH-intact mice, compared to saline-treated controls. Interestingly, this effect is of similar magnitude as that seen by treatment with ipamorelin for 9 weeks to GH-intact mice (Fig. 2D), indicating that GHSs do not increase adiposity further after 2 weeks treatment. Treatment with GH for 2 weeks did not affect fat pad weights in GH-intact mice (Table 1).

In vivo dual energy X-ray absorpiometry (DEXA) analysis of total body fat. To study the effects of GHSs compared to GH on total body fat, mice were subject to *in vivo* DEXA analysis at baseline and at the end of the 2-week treatment period (Fig. 3). There were no differences in percentage total body fat at baseline between any of the treatment groups as measured by DEXA (saline 6.2 ± 1.0%; ipamorelin 5.8 ± 0.5%; GHRP-6 5.4 ± 0.4%; GH 5.2 ± 0.3%). However, after 2 weeks the ipamorelin and GHRP-6 treated mice had accumulated more fat than saline-treated mice, while there was no effect of GH treatment (representative mice shown in Fig. 3A). Analysis of DEXA scans for each treatment group confirmed that GHRP-6 and ipamorelin, but not GH, enhanced significantly total body fat compared to controls (Fig. 3B). Calculation of changes in total body fat compared to baseline showed that GHRP-6 and ipamorelin, but not GH, enhanced significantly the percentage total body fat (Fig. 3C). These results show that both ipamorelin and GHRP-6 had very marked adipogenic effects in GH-intact mice.

Blood assays. After 2 weeks treatment serum leptin concentration at sacrifice was increased in normal female mice injected with ipamorelin or GHRP-6 ($P <$ 0.01) compared to saline-treated controls while no effect of GH was observed (Fig. 4A). No changes in plasma or liver triglycerides were observed in GHS-treated groups compared to saline-treated controls (data not shown). There was no increase in serum corticosterone concentration in normal mice treated with either ipamorelin or GHRP-6 for 2 weeks: saline 105 ± 24 ng/ml, ipamorelin 90 ± 15 ng/ml, GHRP-6 77 ± 17 ng/ml, GH 23 ± 9 ng/ml. Similarly, in the 9-week study, there was no significant effect of ipamorelin treatment on corticosterone levels in the *lit/lit* or *+/lit* mice.

Food intake. In the 2-week treatment study, daily food intake (expressed as percentage body weight) showed that twice-daily s.c. injections of GHRP-6 and ipamorelin led to an increase in cumulative food intake during days 2–5 compared to saline-treated controls (Fig. 4B).

DISCUSSION

The present findings provide a clear demonstration that the stimulatory effects of chronic GHS treatment on body weight partly reflect, GH-independent, adipogenic effects. As expected there was an increase in body weight in normal GH-intact (*+/lit* or wild-type) mice after 2 weeks of GHS injections. Interestingly, there was a similar increase in body weight in GH-deficient (*lit/lit*) mice that have been shown previously not to release GH in response to GHS (19). The GH-independent effect of GHSs on body weight partly reflect increased fat mass as demonstrated by fat pad dissection and *in vivo* whole body fat analysis (by DEXA) in GH-deficient (*lit/lit*) and wild-type mice.

The adipogenic effects of GHSs are in line with our finding that these compounds enhance levels of serum leptin, a fat-derived hormone that reflects fat mass (25–26). Consistent with this, studies in obese and critically ill humans have shown that GHSs can increase serum leptin (27–28), although GH itself suppresses serum leptin levels in line with its well-known lipolytic effect (16). The fact that GH is lipolytic supports the assumption that the increased adiposity is due to GH-independent effects of the GHSs also in GH-intact mice.

To date, interest on the potential benefits of GHS treatment to decrease fat mass has focused on in-

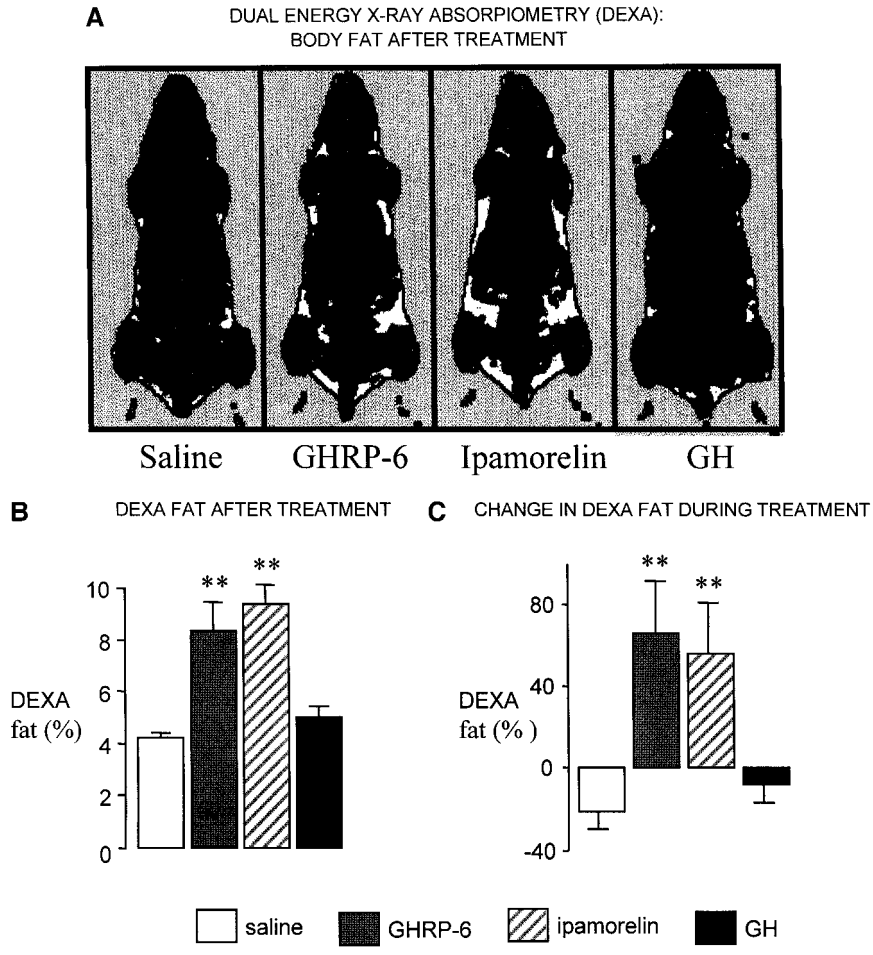

FIG. 3. (A) Adiposity visualised by *in vivo* dual energy X-ray absorpiometry (DEXA) analysis. Each panel displays a representative normal mouse injected s.c.twice daily for 2 weeks with either saline, GHRP-6, ipamorelin or GH. White regions correspond to areas that are >50% fat. (B) Total body fat measured by DEXA after 2 weeks injection s.c. with saline, GHRP-6, ipamorelin, GH. DEXA scans of each mouse were analysed using densitometry software and regions corresponding to >50% fat area on the DEXA scans were included in the calculation of the percentage fat-mass relative to total body area. (C) Percentage change from baseline in total body fat (mean ± SEM) measured by DEXA and analyzed using densitometry software. Numbers in parentheses denote the number of mice in each treatment group. All p values denote significance after 2 weeks treatment compared to saline-treated control mice: one-way ANOVA followed by Dunnet's test against a control group (*$P < 0.05$, **$P < 0.01$). $n = 7–8$ mice for all groups.

creased GH release. Thus, not surprisingly, many chronic GHS studies have been performed in obese humans and animals. However, despite the marked GH releasing capacity of the GHSs, several of these studies have failed to show a decrease in fat mass after GHS treatment to obese humans (15) and rats (17–18). The reason for this may be the direct lipogenic effect of GHSs shown in this study.

The mechanisms mediating the adipogenic effects of GHSs are unknown, but it is likely that the orexigenic effect shown in this and previous studies (11–13) plays a role. We found that 2 different GHSs increased cumulative daily food intake over 72 h during the first week of GHS treatment, that is, at a time when most of the increased in body weight occurred (data not shown). Moreover, GH did not stimulate food intake indicating that the orexigenic effect of GHS, like the adipogenic effect, is GH-independent. However, it cannot be excluded that the adipogenic effects of GHSs are due to changes in energy expenditure as well as food intake. On the other hand, it has been reported that long term GHS treatment in obese men increases rather than decreases energy expenditure (15), an effect that is unlikely to contribute to increased body fat.

The GHS stimulation of food intake may be mediated by neuropeptide Y (NPY) which has very potent orexigenic effects (29). Moreover, it has been shown that the majority of neurones activated by GHSs are also NPY-containing (30) and there is a marked colocalization of GHS-R mRNA and NPY mRNA in the hypothalamic arcuate nucleus (7). It is also possible that other GH-independent actions of the GHSs contribute to the observed adipogenic effects, such as stimulation of the hypothalamo-pituitary-adrenal (HPA) axis (32–33).

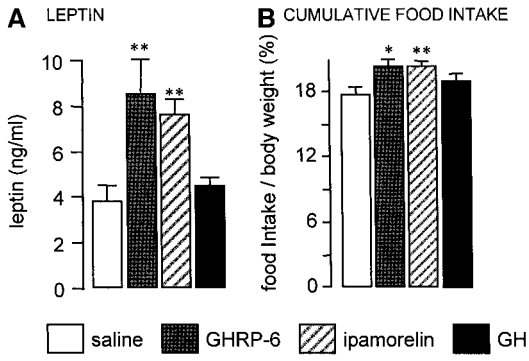

FIG. 4. Two weeks twice daily injection of saline, GHRP-6, ipamorelin or GH to normal female mice: effects on (A) serum leptin concentration in trunk blood and (B) food intake (cumulative intake for days 2–5). Data are expressed as mean ± SEM. All *P* values denote significance compared to saline-treated control mice: one-way ANOVA followed by Dunnet's test against a control group (*$P < 0.05$, **$P < 0.01$). $n = 7–8$ per group.

Hyperactivity of the HPA axis is associated with excess visceral fat accumulation (22). On the other hand, the fact that we saw an adipogenic effect by ipamorelin, a GHS that does not appear to stimulate glucocorticoid release (23), might argue against HPA involvement. Consistent with this, we saw no significant effect of GHSs on corticosterone. From these measurements alone, however, we cannot exclude the possibility that glucocorticoids play a role in GHS-induced adiposity.

During the preparation of this manuscript a report was published by Tschöp and colleagues showing lipogenic and orexigenic effects by 2 weeks treatment with ghrelin, an endogenous GHS ligand, in normal and GH-deficient rats (33). The present report extends this study showing that the lipogenic and orexigenic effects mainly occur during the first 1–2 weeks of treatment, and that an equilibrium is then reached with no further increase in body fat. We also demonstrate that the increase in fat mass is accompanied by an increase in circulating leptin, providing a possible mechanism for the cessation of the adipogenic and orexigenic effects of GHS that occur during chronic exposure. Finally, we show that similar adipogenic effects of GHSs occur in mice as have been reported for ghrelin in rats.

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REFERENCES

1. Bowers, C. Y. (1994) On a peptidomimetic growth hormone-releasing peptide. *J. Clin. Endocrinol. Metab.* **79,** 940–942.
2. Smith, R. G., Feighner, S., Prendergast, K., Guan, X., and Howard, A. (1999) A new orphan receptor involved in pulsatile growth hormone release. *Trends Endocrinol. Metab.* **10,** 128–135.
3. Dickson, S. L., Leng, G., and Robinson, I. C. A. F. (1993) Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons. *Neuroscience* **53,** 303–306.
4. Dickson, S. L., Leng, G., Dyball, R. E. J., and Smith, R. G. (1995) Central actions of peptide and non-peptide growth hormone secretagogues in the rat. *Neuroendocrinology* **61,** 36–43.
5. Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberator, P. A., Rosenblum, C. I., Hamelin, M., Hreniuk, D. L., Palyha, O. C., Anderson, J., Paress, P. S., Diaz, C., Chou, M., Liu, K. K., McKee, K. K., Pong, S. S., Chaung, L. Y., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D. J. S., Dean, D. C., Melillo, D. G., and Van der Ploeg, L. H. T. (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* **273,** 974–977.
6. Bennett, P. A., Thomas, G. B., Howard, A. D., Feighner, S. D., van der Ploeg, L. H., and Smith, R. G., and Robinson, I. C. A. F. (1997) Hypothalamic growth hormone secretagogue-receptor (GHS-R) expression is regulated by growth hormone in the rat. *Endocrinology* **138,** 4552–4557.
7. Willesen, M. G., Kristensen, P., and Rømer, J. (1999) Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* **70,** 306–316.
8. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402,** 656–660.
9. Wren, A. M., Small, C. J., Ward, H. L., Murphy, K. G., Dakin, C. L., Taheri, S., Kennedy, A. R., Roberts, G. H., Morgan, D. G. A., Ghatei, M. A., and Bloom, S. R. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141,** 4325–4328.
10. Hewson, A. K., and Dickson, S. L. (2000) Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J. Neuroendocrinol.* **12,** 1047–1049.
11. Locke, W., Kirgis, H. D., Bowers, C. Y., and Abdoh, A. A. (1995) Intracerebroventricular growth-hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. *Life Sci.* **56,** 1347–1352.
12. Okada, K., Ishii, S., Minami, S., Sugihara, H., Shibasaki, T., and Wakabayashi, I. (1996) Intracerebroventricular administration of the growth hormone-releasing peptide KP-102 increases food intake in free-feeding rats. *Endocrinology* **137,** 5155–5158.
13. Torsello, A., Luoni, M., Schweiger, F., Grilli, R., Guidi, M., Bresciani, E., Deghenghi, R., Muller, E. E., and Locatelli V. (1998) Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release. *Eur. J. Pharmacol.* **360,** 123–129.
14. Svensson, J., Lall, S., Dickson, S. L., Bengtsson, B. A., Rømer, J., Ahnfelt-Rønne, I., Ohlsson, C., and Jansson, J-O. (2000) The GH secretagogues ipamorelin and GH-releasing peptide-6 increase bone mineral content in adult female rats. *J. Endocrinol.* **165,** 569–577.
15. Svensson, J., Lönn, L., Jansson, J-O., Murphy, G., Wyss, D., Krupa, D., Cerchio, K., Polvino, W., Gertz, B., Boseaus, I., Sjöström, L., and Bengtsson, B. Å. (1998) Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure. *J. Clin. Endocrinol. Metab.* **83,** 362–369.
16. Johannsson, G., Marin, P., Lönn, L., Ottosson, M., Stenlöf, K., Björntorp, P., Sjöström, L., and Bengtsson, B. Å. (1997) Growth hormone treatment of abdominally obese men reduces abdomi-

nal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure. *J. Clin. Endocrinol. Metab.* **82,** 727–734.

17. Bercu, B. B., Yang, S. W., Masuda, R., Hu, C. S., and Walker, R. F. (1992) Effects of coadministered growth hormone (GH)-releasing hormone and GH-releasing hexapeptide on maladaptive aspects of obesity in Zucker rats. *Endocrinology* **131,** 2800–2804.
18. Clark, R. G., Thomas, G. B., Mortensen, D. L., Won, W. B., Ma, Y. H., Tomlinson, E. E., Fairhall, K. M., and Robinson, I. C. A. F. (1997) Growth hormone secretagogues stimulate the hypothalamic-pituitary-adrenal axis and are diabetogenic in the Zucker diabetic fatty rat. *Endocrinology* **138,** 4316–4323.
19. Jansson, J-O., Downs, T. R., Beamer, W. G., and Frohman, L. A. (1986) The dwarf "little" (lit/lit) mouse is resistant to growth hormone (GH)-releasing peptide (GHRP-6) as well as to GH-releasing hormone (GRH). *Proc. 68th Ann. Meet. Endo. Soc., Anaheim*, Abstract P397.
20. Jansson, J-O., Downs, T. R., Beamer, W. G., and Frohman, L. A. (1986) Receptor associated resistance to growth hormone-releasing hormone in dwarf "little" mice. *Science* **232,** 511–512.
21. Dickson, S. L., Doutrelant-Viltart, O., and Leng, G. (1995) GH-deficient *dw/dw* rats and *lit/lit* mice show increased Fos expression in the hypothalamic arcuate nucleus following systemic injection of GH-releasing peptide-6. *J. Endocrinol.* **146,** 519–526.
22. Yukimura, Y., Bray, G. A., and Wolfsen, A. R. (1978) Some effects of adrenalectomy in the fatty rat. *Endocrinology* **103,** 1924–1928.
23. Raun, K., Hansen, B. S., Johansen, N. L., Thogersen, H., Madsen, K., Ankersen, M., and Andersen, P. H. (1998) Ipamorelin, the first selective growth hormone secretagogue. *Eur. J. Endocrinol.* **139,** 552–561.
24. Ohlsson, C., Hellberg, N., Parini, P., Vidal, O., Bohlooly, M., Rudling, M., Lindberg, M. K., Warner, M., Angelin, B., and Gustaffson J-Å. (2000) Obesity and disturbed lipoprotein profile in estrogen receptor-deficient male mice. *Biochem. Biophys. Res. Commun.* **278,** 640–645.
25. Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., and Bauer, T. L. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* **334,** 292–295.
26. Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., and Ranganathan, S. (1995) Leptin levels in human and rodent: Measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat. Med.* **1,** 1155–1161.
27. Svensson, J., Carlsson, B., Carlsson, L. M. S., Jansson, J-O., and Bengtsson, B. Å. (1999) Discrepancy between serum leptin values and total body fat in response to the oral growth hormone secretagogue MK-677. *Clin. Endocrinol.* **50,** 451–456.
28. Van den Berghe, G., Wouters, P., Carlsson, L., Baxter, R. C., Bouillon, R., and Bowers, C. Y. (1998) Leptin levels in protracted critical illness: Effects of growth hormone-secretagogues and thyrotropin-releasing hormone. *J. Clin. Endocrinol. Metab.* **83,** 3062–3070.
29. Clark, J. T., Kalra, P. S., Crowley, W. R., and Kalra, S. P. (1984) Neuropeptide Y and human pancreatic polypeptide stimulate feeding in rats. *Endocrinology* **115,** 427–429.
30. Dickson, S. L., and Luckman, S. M. (1997) Induction of c-*fos* messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. *Endocrinology* **138,** 771–777.
31. Thomas, G. B., Fairhall, K. M., and Robinson, I. C. A. F. (1997) Activation of the hypothalamo-pituitary-adrenal axis by the growth hormone (GH) secretagogue, GH-releasing peptide-6, in rats. *Endocrinology* **138,** 1585–1591.
32. Hickey, G. J., Jacks, T., Judith, F., Taylor, J., Schoen, W. R., Krupa, D., Cunningham, P., Clark, J., and Smith, R. G. (1994) Efficacy and specificity of L-692,429, a novel nonpeptidyl growth hormone secretagogue in beagles. *Endocrinology* **134,** 695–701.
33. Tschöp, M., Smiley, D. L., and Heiman, M. L. (2000) Ghrelin induces adiposity in rodents. *Nature* **407,** 908–913.