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ARTICLE

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Ghrelin differentially affects hepatic and peripheral insulin sensitivity in mice

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Abstract *Aims/hypothesis:* This study was conducted to evaluate the effects of ghrelin on insulin's capacity to suppress endogenous glucose production and promote glucose disposal in mice. To establish whether the growth hormone secretagogue (GHS) receptor can mediate the putative effect of ghrelin on the action of insulin, we also determined the metabolic effects of growth hormone releasing peptide 6 (GHRP-6), a specific GHS receptor agonist. In addition, we explored the biological significance of des-ghrelin (unacylated ghrelin) in this experimental context. *Materials and methods:* Vehicle (n=8), ghrelin (n=9), GHRP-6 (n=9), des-ghrelin (n=8) or a combination of des-ghrelin and ghrelin (n=7) were infused i.v. for 3 h. Simultaneously, endogenous glucose production and glucose disposal were measured by ^{14}C-glucose dilution during a hyperinsulinaemic–euglycaemic clamp. Tissue-specific glucose uptake in muscle and adipose tissue was measured using ^{3}H-2-deoxyglucose. *Results:* During hyperinsulinaemia, glucose disposal was 31% higher in mice treated with ghrelin than in those treated with vehicle (77±16 and 59±8 μmol kg^{-1} h^{-1}, respectively, p<0.05). This was in accordance with enhanced 2-deoxyglucose uptake in muscle in ghrelin-treated animals. In contrast, endogenous glucose production was less effectively suppressed by insulin during ghrelin infusion (46±22 vs 71±11% in controls, p<0.05). GHRP-6 did not affect insulin action. Des-ghrelin hampered insulin's capacity to inhibit endogenous glucose production, whereas it did not affect glucose disposal. The restraining effects of des-ghrelin and ghrelin on hepatic insulin action were abolished by simultaneous administration of both peptides. *Conclusions/interpretation:* Ghrelin hampers insulin's capacity to suppress endogenous glucose production, whereas it reinforces the action of insulin on glucose disposal, independently of food intake and body weight. These metabolic effects are unlikely to be mediated by the GHS receptor. Furthermore, simultaneous administration of des-ghrelin abolishes the inhibitory effect of ghrelin on hepatic insulin action.

Keywords Animal models · GHRP-6 · Glucose metabolism · Gut hormone · Hyperinsulinaemic–euglycaemic clamp technique · Insulin resistance · Tracer methodology

Abbreviations des-ghrelin: unacylated ghrelin · 2-DG: 2-deoxyglucose · dpm: disintegrations per minute · α-MSH: α-melanocyte stimulating hormone · EGP: endogenous glucose production · GHRP-6: growth hormone releasing peptide 6 · GHS: growth hormone secretagogue · GHS-R: GHS receptor · NPY: neuropeptide Y

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Introduction

Ghrelin is a small peptide (28 amino acids) that is primarily produced by the stomach and binds to the growth hormone secretagogue (GHS) receptor (GHS-R) [1, 2]. It circulates in two major forms: *n*-octanoyl-ghrelin (ghrelin), which contains an *n*-octanoyl modification at serine-3, and des-octanoyl or unacylated ghrelin (des-ghrelin) [3]. Although des-ghrelin does not bind to the GHS-R [1, 4], it may be biologically active [5].

Ghrelin is a component of the gut-brain axis and is involved in the control of energy balance. It stimulates food intake in rodents and man [5–8], where peak levels in plasma occur just before each meal and fall rapidly after refeeding, suggesting that it serves to initiate food consumption [9]. To enhance appetite, ghrelin acts in the hypothalamus, where it promotes neuropeptide Y (NPY) and orexin gene expression and inhibits the production of pro-opiomelanocortin/α-melanocyte stimulating hormone (-MSH) via activation of the GHS-R [10–16]. We recently showed that intracerebroventricular infusion of NPY hampers insulin's capacity to suppress endogenous glucose production (EGP) [17], whereas central injection of melanotan II, a synthetic analogue of α-MSH, reinforces insulin action on glucose disposal [18]. Also, a number of studies have demonstrated an inverse relationship between fasting plasma ghrelin and insulin concentrations and insulin resistance in adults and children [19–27]. These findings led us to hypothesise that ghrelin might adversely affect insulin sensitivity through activation of the GHS-R.

To test this hypothesis, we intravenously administered vehicle, ghrelin or growth hormone releasing peptide 6 (GHRP-6), which is a specific agonist of the GHS-R, and measured EGP and glucose disposal by ^{14}C-glucose dilution during a hyperinsulinaemic clamp in mice.

Until recently, acylation of the serine-3 residue of the ghrelin molecule was believed to be required for bioactivity. However, recent data suggest that des-ghrelin may counteract ghrelin in the control of energy balance [28], and in vitro experiments have revealed opposing effects of ghrelin and des-ghrelin on glucose production by primary hepatocytes [29]. To further explore the role of des-ghrelin in the regulation of fuel flux, we studied the effects of this peptide in the same experimental context.

Materials and methods

Animals

Male 3-month-old C57BL/6J mice (originating from The Jackson Laboratory, Bar Harbor, ME, USA, and bred in our own animal facility) were housed in a temperature-controlled room on a 12-h light/12-h dark cycle, with free access to water and chow diet. Body weight of the mice is reported in Table 1. All animal experiments were performed in accordance with the regulations of Dutch law on animal welfare, and the institutional ethics committee for animal procedures approved the protocol.

Hyperinsulinaemic–euglycaemic clamp study

Mice were fasted for 4 h, with food withdrawn at 05.00 h. Hyperinsulinaemic–euglycaemic clamp studies were started at 09.00 h as described earlier [30, 31]. During the experiments, mice were sedated with 6.25 mg/kg acepromazine (Sanofi Sante Animale, Libourne Cedex, France), 6.25 mg/kg midazolam (Roche, Mijdrecht, the Netherlands) and 0.3125 mg/kg fentanyl (Janssen–Cilag, Tilburg, the Netherlands).

Vehicle, ghrelin or GHRP-6 (peptides from PhoenixEurope, Karlsruhe, Germany) was administered in a primed (vehicle, 0 μg/nmol; ghrelin, 0.60 μg or 0.18 nmol; GHRP-6, 0.16 μg or 0.18 nmol), continuous (vehicle, 0 μg h^{-1} nmol^{-1} h^{-1}; ghrelin, 1 μg/h or 0.30 nmol/h; GHRP-6, 0.26 μg/h or 0.30 nmol/h) i.v. infusion during the whole experiment (basal and hyperinsulinaemic periods). The doses of ghrelin and GHRP-6 were based on previous reports [6, 7, 32]. First, basal rates of glucose production/uptake were measured by giving a primed continuous infusion of ^{14}C-glucose (primed, 26 kBq; continuous, 44 kBq/h) (Amersham, Little Chalfont, UK) for 80 min. Subsequently, insulin was administered in a primed (4.1 mU), continuous (6.8 mU/h) i.v. infusion for 2–3 h aimed at a steady-state circulating insulin level of approximately 6 ng/ml. A variable infusion of 12.5% D-glucose was used to maintain euglycaemia (measured by tail bleeding; Freestyle TheraSense; Disetronic Medical Systems, Vianen, the Netherlands). Blood samples (75 μl) were taken during the basal period (after 60 and 80 min) and during the clamp period (when glucose levels were stable and 20 and 40 min later) for determination of plasma glucose, NEFA, insulin and ^{14}C-glucose specific activities.

Table 1 Body weight and plasma parameters in mice that received ghrelin (n=9), des-ghrelin (n=8), ghrelin and des-ghrelin together (n=7), GHRP-6 (n=9) or vehicle (n=8)

	Body weight (g)	Glucose (mmol/l)		Insulin (ng/ml)		NEFA (mmol/l)	
		Basal condition	Hyperinsulinaemic condition	Basal condition	Hyperinsulinaemic condition	Basal condition	Hyperinsulinaemic condition
Control	27.1±1.8	5.8±0.73	8.8±1.2	0.93±0.63	6.2±1.5	1.0±0.2	0.5±0.2
Ghrelin	26.6±2.2	5.9±0.84	8.8±0.74	0.91±0.73	5.8±2.1	1.0±0.3	0.4±0.3
GHRP-6	26.3±1.5	4.8±0.48[b]	8.5±1.8	1.0±0.50	6.8±2.6	1.0±0.2	0.3±0.1
Des-ghrelin	26.9±2.2	6.3±1.0	7.8±0.83	1.1±0.38	5.7±1.9	1.0±0.3	0.4±0.1
Des-ghrelin and ghrelin combined	27.6±2.4	4.9±0.90[a]	7.6±1.1	0.87±0.58	6.6±1.8	1.0±0.2	0.4±0.1

Values are mean±SD
[a]p<0.05, [b]p<0.01 compared with control

To assess tissue-specific insulin-mediated glucose uptake, 2-deoxy-D-[^{3}H]glucose (2-DG; Amersham) was administered as a bolus (37 kBq) 40 min before the end of the clamp experiment. At the end of the clamp, mice were killed and skeletal muscle (hindlimb) and adipose tissue were isolated and frozen in liquid nitrogen for subsequent analysis. There were eight (vehicle) or nine (ghrelin, GHRP-6) mice in each group during the hyperinsulinaemic–euglycaemic clamp study. Because of technical problems, data on the tissue-specific, insulin-mediated glucose uptake of three mice were lost in the vehicle group.

Additional studies with des-ghrelin

In addition, a hyperinsulinaemic–euglycaemic clamp study was performed while des-ghrelin or a combination of ghrelin and des-ghrelin was administered. Des-ghrelin or the combination of ghrelin and des-ghrelin were administered in a primed (des-ghrelin, 0.60 μg or 0.19 nmol; combination of ghrelin and des-ghrelin, 0.60 μg or 0.18 nmol ghrelin and 0.60 μg or 0.18 nmol des-ghrelin), continuous (des-ghrelin, 1.0 μg/h or 0.31 nmol/h; combination of ghrelin and des-ghrelin, 1.0 μg/h or 0.31 nmol/h ghrelin and 1.0 μg/h or 0.30 nmol/h des-ghrelin) i.v. infusion during the whole experiment (basal and hyperinsulinaemic periods). The doses of des-ghrelin and the combination of ghrelin and des-ghrelin were based on a previous report [32]. Hyperinsulinaemic–euglycaemic clamps were performed as stated above and randomised with these other groups.

Analytical procedures

Plasma levels of glucose and NEFA were determined using commercially available kits (Instruchemie, Delfzijl, the Netherlands and Wako, Neuss, Germany). Plasma insulin concentration was measured with a mouse insulin ELISA (Mercodia, Uppsala, Sweden). Total plasma ^{14}C-glucose was determined in 7.5 μl plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water. There were seven (combination of ghrelin and des-ghrelin) or eight (des-ghrelin) mice in each group during the hyperinsulinaemic–euglycaemic clamp study.

Tissue analysis

For determination of tissue 2-DG uptake, the homogenate of muscle and adipose tissue was boiled, and the supernatant was subjected to an ion-change column to separate 2-DG-6-phosphate from 2-DG as described previously [30, 33].

Calculations

The turnover rate of glucose (μmol min^{-1} kg^{-1}) was calculated during the basal period and in steady-state clamp conditions as the rate of tracer infusion (disintegrations per minute [dpm] infused per min [dpm/min]) divided by the plasma-specific activity of ^{14}C-glucose (dpm/μmol). The ratio was corrected for body weight. During the hyperinsulinaemic clamp, EGP was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

Tissue-specific glucose uptake in muscle and adipose tissue was calculated from tissue 2-DG content, corrected for plasma-specific activity and expressed as μmol/g tissue.

Statistical analysis

Differences between groups were determined with the Kruskal–Wallis non-parametric test for k independent samples. When significant differences were found, the Mann–Whitney non-parametric test was used as a follow-up test to determine differences between experimental groups and the control group. A p value of <0.05 was considered statistically significant. All values shown represent the mean ± SD.

Results

Plasma parameters

Body weight, plasma glucose, NEFA and insulin concentrations in basal and hyperinsulinaemic conditions are shown in Table 1. Body weight did not differ between the groups of mice. Basal glucose levels were significantly lower in the group that received GHRP-6 than in the

Table 2 Glucose turnover and tissue-specific glucose uptake in mice that received des-ghrelin (n=8), ghrelin and des-ghrelin together (n=7) or vehicle

	Control	Ghrelin	GHRP-6	Des-ghrelin	Ghrelin and des-ghrelin combined
Basal EGP (μmol kg^{-1} min^{-1})	44±9	40±7	33±4[b]	48±9	27±7[b]
Glucose infusion rate (μl/h)	91±18	136±27[b]	117±31	70±30	104±19
Insulin-mediated glucose disposal (μmol kg^{-1} min^{-1})	59±8	77±16[a]	60±9	54±15	50±17
Inhibition of EGP (%)	71±11	46±22[a]	70±22	47±13[b]	75±21
Muscle-specific glucose uptake (μmol/g tissue)	8.6±4.4	26±21[a]	7.1±3.4	11±6.1	10±9.1
Adipose tissue-specific glucose uptake (μmol/g tissue)	2.6±1.7	7.9±11	3.6±1.5	2.6±1.1	2.1±1.3

Values are mean ± SD
[a]p<0.01, [b]p<0.01 compared with control

control group. Basal insulin and NEFA levels were not different between groups. Moreover, in steady-state hyperinsulinaemic conditions, plasma glucose, NEFA and insulin concentrations were not different between groups.

Glucose turnover

Basal EGP, glucose infusion rate, insulin-mediated glucose disposal, inhibition of EGP and tissue-specific glucose uptake are shown in Table 2. In basal conditions, EGP (and thereby whole body glucose disposal) was significantly lower in animals that received GHRP-6 than in control animals. During the hyperinsulinaemic period, the rate of glucose infusion necessary to maintain euglycaemia was significantly higher in animals that received ghrelin than in control mice. Accordingly, the glucose disposal rate was significantly higher in animals that received ghrelin compared with the control group (Fig. 1). In contrast, hyperinsulinaemia suppressed EGP to a significantly lesser degree in animals that received ghrelin than in control animals (Fig. 2).

Tissue-specific glucose uptake

Insulin-mediated 2-DG uptake in muscle tissue was higher in ghrelin-treated animals than in the control group. Insulin-mediated 2-DG uptake in adipose tissue tended to be higher in ghrelin-treated animals than in the control group, although this difference did not reach statistical significance (p=0.09) (Fig. 3a,b).

Additional studies with des-ghrelin

Plasma parameters

Body weight, plasma glucose, NEFA and insulin concentrations in basal and hyperinsulinaemic conditions are shown in Table 1. Body weight did not differ between the groups of mice. Basal glucose levels were significantly lower in the group that received the combination of ghrelin and des-ghrelin compared with the control group. Basal insulin and NEFA levels were not different between groups. Moreover, in steady-state hyperinsulinaemic conditions, plasma glucose, NEFA and insulin concentrations were not different between groups.

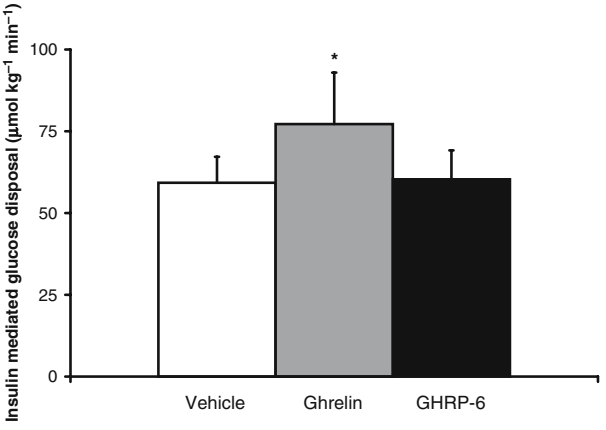

Fig. 1 Insulin-mediated glucose disposal (μmol kg^{-1} min^{-1}) in mice as indicated. Vehicle-treated, n=8; ghrelin-treated, n=9; GHRP-6-treated, n=9. Data are mean and SD. *p<0.05

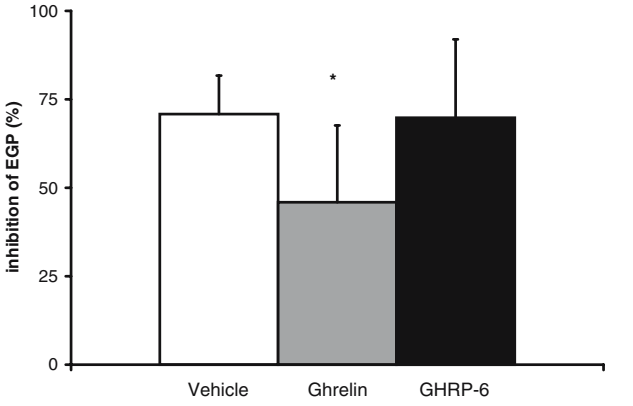

Fig. 2 Inhibition of EGP by insulin in mice as indicated. Vehicle-treated, n=8; ghrelin-treated, n=9; GHRP-6-treated, n=9. Data are mean and SD. *p<0.05

Glucose turnover and tissue-specific glucose uptake

Basal EGP, glucose infusion rate, insulin-mediated glucose disposal, inhibition of EGP, and tissue-specific

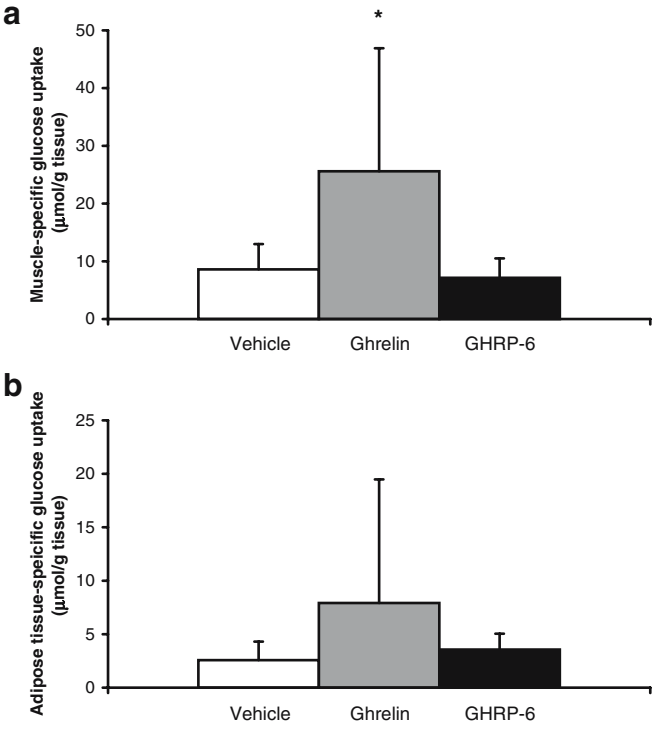

Fig. 3 Muscle-specific glucose uptake (μmol/g) (**a**) and adipose tissue-specific glucose uptake (μmol/g) (**b**) under hyperinsulinaemic conditions in mice as indicated. Vehicle, n=5; ghrelin, n=9; GHRP-6, n=9. Data are mean and SD. *p<0.05

glucose uptake are shown in Table 2. During basal conditions, EGP (and thereby whole body glucose disposal) was significantly lower in animals that received the combination of ghrelin and des-ghrelin compared with the control animals. During the hyperinsulinaemic period, the rate of glucose infusion necessary to maintain euglycaemia, insulin-stimulated glucose disposal and tissue-specific glucose uptake did not differ between the groups. In contrast, hyperinsulinaemia suppressed EGP to a significantly lesser degree in animals that received des-ghrelin than in control animals.

The effects of the combination of ghrelin and des-ghrelin were not identical to the sum of the individual peptides' effects. Glucose infusion rate and the rate of insulin-mediated glucose disposal were significantly increased during ghrelin infusion, whereas des-ghrelin did not affect these parameters. Interestingly, co-administration of the two peptides restored the glucose infusion rate to control values. Although ghrelin and des-ghrelin alone caused hepatic insulin resistance, the combination of the two peptides abolished the individual effects of the peptides on hepatic insulin sensitivity.

Discussion

This study shows that intravenous administration of ghrelin reinforces the action of insulin on glucose disposal, whereas it hampers insulin's capacity to inhibit EGP. In contrast, administration of GHRP-6 did not affect insulin action. Des-ghrelin hampered insulin's capacity to inhibit EGP, whereas it did not affect glucose disposal. The restraining effects of des-ghrelin and ghrelin on hepatic insulin action were abolished by simultaneous administration of both peptides.

The glucose infusion rate required to maintain euglycaemia during insulin infusion was significantly higher in ghrelin-treated animals, indicating that this peptide enhances the whole-body insulin sensitivity of glucose metabolism. In particular, ghrelin clearly stimulated insulin-mediated glucose disposal, as determined by the tracer dilution method, an observation that was consistent with enhanced 2-DG uptake in muscle and adipose tissue during hyperinsulinaemia in ghrelin-treated animals (although statistical significance was reached only for muscle). In contrast, ghrelin hampered inhibition of EGP by insulin. As previous papers have reported that ghrelin stimulates NPY release and inhibits pro-opiomelanocortin/α-MSH neuronal activity in the arcuate nucleus [34], we expected to find that ghrelin would compromise the insulin sensitivity of both liver and muscle/adipose tissue (we recently showed that central NPY hampers insulin's action on EGP, whereas melanocortin pathways promote insulin-mediated glucose disposal [17, 18]). However, the mechanism whereby ghrelin affects insulin action remains to be established. Intravenously administered peptide may act through central pathways, but also through the activation of receptors on peripheral tissues (the GHS-R is present in various tissues, such as pancreas and liver [15, 35]). Indeed, ghrelin blocks insulin's inhibitory effect on the gene expression of key gluconeogenic enzymes in a hepatoma cell line [35]. These data corroborate our own in vivo observation of ghrelin hampering the action of insulin on EGP. In contrast, i.v. administration of ghrelin appears to enhance insulin-mediated glucose uptake, where we had hypothesised a priori that it would block this action (through the down-regulation of hypothalamic α-MSH signalling [18]). It is conceivable that ghrelin acts on myocytes and/or adipocytes directly to stimulate glucose disposal. We are not aware of any in vitro or in vivo data documenting ghrelin's effects on insulin action in muscle or adipose tissue. Thus, further studies are required to establish if ghrelin affects insulin action via central or peripheral mechanisms.

Administration of GHRP-6, an agonist of the GHS-R, did not affect the action of insulin. This observation is in line with other reports indicating that ghrelin stimulates glucose production by primary hepatocytes [29], promotes adipogenesis in bone marrow [36] and inhibits preadipocyte cell proliferation [37], whereas GHS-R agonists do not. Together, these data suggest that ghrelin does not affect insulin action and metabolism via the GHS-R, but via an as-yet unidentified mechanism.

Des-ghrelin blocked the action of insulin on EGP as much as ghrelin did. In contrast, it did not affect insulin-mediated glucose disposal. These data support the emerging view that *n*-octanoyl modification of the serine-3 residue of ghrelin is not absolutely required for bioactivity [28, 29, 32]. However, they apparently contradict a report suggesting that ghrelin and des-ghrelin have opposing (stimulatory and inhibitory, respectively) effects on glucose production by hepatocytes [29]. The contradiction could be explained by the fact that in vitro and in vivo administration of des-ghrelin may have different effects, as des-ghrelin, like ghrelin, can act in the brain as well as in peripheral tissues [28, 36]. Co-administration of des-ghrelin abolished the effect of ghrelin on hepatic insulin sensitivity, which accords with an in vitro study showing similar results in isolated hepatocytes [29]. Our findings corroborate mounting evidence indicating that des-ghrelin has potentially important biological effects. The receptor mediating des-ghrelin bioactivity remains to be identified.

The physiological relevance of the observed effects of ghrelin in decreasing hepatic insulin sensitivity and increasing peripheral insulin sensitivity remains to be established. Ghrelin secretion by (primarily) the stomach is significantly enhanced during fasting to stimulate appetite and initiate meal consumption [9]. In a previous study, we showed that fasting enhances insulin-mediated glucose uptake in mice [38]. The data we present here allow us to hypothesise that the increase in plasma ghrelin levels during fasting is involved in the physiology of this phenomenon. However, des-ghrelin concentrations clearly dominate the plasma profile of ghrelin-like peptides, at least in the human [39, 40]. In view of the current evidence suggesting that des-ghrelin has metabolic effects of its own and indeed appears to interact with ghrelin in the control of metabolism, further studies are required to establish the role of ghrelin peptides in the regulation of energy balance

and fuel flux. Moreover, the receptor(s) mediating the metabolic signals conveyed by (des-)ghrelin need to be identified.

In conclusion, ghrelin differentially affects the tissue-specific action of insulin, hampering its ability to inhibit EGP while reinforcing its effect on glucose disposal. These effects occur acutely and they are not secondary to ghrelin's well-known influence on feeding and body weight. The GHS-R is not likely to mediate ghrelin's metabolic effects. Des-ghrelin also appears to modulate fuel flux and may counteract ghrelin in the control of glucose metabolism.

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References

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
2. Sato T, Fukue Y, Teranishi H, Yoshida Y, Kojima M (2005) Molecular forms of hypothalamic ghrelin and its regulation by fasting and 2-deoxy-D-glucose administration. Endocrinology 146:2510–2516
3. Hosoda H, Kojima M, Matsuo H, Kangawa K (2000) Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 279:909–913
4. Broglio F, Benso A, Gottero C et al (2003) Non-acylated ghrelin does not possess the pituitaric and pancreatic endocrine activity of acylated ghrelin in humans. J Endocrinol Invest 26:192–196
5. Tschop M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908–913
6. Wren AM, Small CJ, Ward HL et al (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141:4325–4328
7. Wren AM, Seal LJ, Cohen MA et al (2001) Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992
8. Nakazato M, Murakami N, Date Y et al (2001) A role for ghrelin in the central regulation of feeding. Nature 409:194–198
9. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
10. Shrestha YB, Wickwire K, Giraudo SQ (2004) Action of MT-II on ghrelin-induced feeding in the paraventricular nucleus of the hypothalamus. Neuroreport 15:1365–1367
11. Lawrence CB, Snape AC, Baudoin FM, Luckman SM (2002) Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 143:155–162
12. Shintani M, Ogawa Y, Ebihara K et al (2001) Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50:227–232
13. Howard AD, Feighner SD, Cully DF et al (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977
14. Muccioli G, Ghe C, Ghigo MC et al (1998) Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland. J Endocrinol 157:99–106
15. Guan XM, Yu H, Palyha OC et al (1997) Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res 48:23–29
16. Date Y, Murakami N, Toshinai K et al (2002) The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 123:1120–1128
17. van den Hoek AM, Voshol PJ et al (2004) Intracerebroventricular neuropeptide Y infusion precludes inhibition of glucose and VLDL production by insulin. Diabetes 53:2529–2534
18. Heijboer AC, van den Hoek AM, Pijl H et al (2005) Intracerebroventricular administration of melanotan II increases insulin sensitivity of glucose disposal in mice. Diabetologia 48:1621–1626
19. Purnell JQ, Weigle DS, Breen P, Cummings DE (2003) Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab 88:5747–5752
20. Schofl C, Horn R, Schill T, Schlosser HW, Muller MJ, Brabant G (2002) Circulating ghrelin levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab 87:4607–4610
21. McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE (2004) Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. J Clin Endocrinol Metab 89:1630–1635
22. Ikezaki A, Hosoda H, Ito K et al (2002) Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 51:3408–3411
23. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML (2001) Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707–709
24. Bunt JC, Salbe AD, Tschop MH, DelParigi A, Daychild P, Tataranni PA (2003) Cross-sectional and prospective relationships of fasting plasma ghrelin concentrations with anthropometric measures in pima Indian children. J Clin Endocrinol Metab 88:3756–3761
25. Bacha F, Arslanian SA (2005) Ghrelin suppression in overweight children: a manifestation of insulin resistance? J Clin Endocrinol Metab 90:2725–2730
26. Gimenez-Palop O, Gimenez-Perez G, Mauricio D et al (2005) Circulating ghrelin in thyroid dysfunction is related to insulin resistance and not to hunger, food intake or anthropometric changes. Eur J Endocrinol 153:73–79
27. Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O (2003) Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 52:2546–2553
28. Asakawa A, Inui A, Fujimiya M et al (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut 54:18–24
29. Gauna C, Delhanty PJ, Hofland LJ et al (2005) Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. J Clin Endocrinol Metab 90:1055–1060
30. Voshol PJ, Jong MC, Dahlmans VE et al (2001) In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. Diabetes 50:2585–2590
31. van den Hoek AM, Heijboer AC, Corssmit EP et al (2004) PYY3-36 reinforces insulin action on glucose disposal in mice fed a high-fat diet. Diabetes 53:1949–1952
32. Gauna C, Meyler FM, Janssen JA et al (2004) Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. J Clin Endocrinol Metab 89:5035–5042

33. Rossetti L, Giaccari A (1990) Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. J Clin Invest 85:1785–1792
34. Riediger T, Traebert M, Schmid HA, Scheel C, Lutz TA, Scharrer E (2003) Site-specific effects of ghrelin on the neuronal activity in the hypothalamic arcuate nucleus. Neurosci Lett 341:151–155
35. Murata M, Okimura Y, Iida K et al (2002) Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. J Biol Chem 277:5667–5674
36. Thompson NM, Gill DA, Davies R et al (2004) Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology 145:234–242
37. Zhang W, Zhao L, Lin TR et al (2004) Inhibition of adipogenesis by ghrelin. Mol Biol Cell 15:2484–2491
38. Heijboer AC, Donga E, Voshol PJ et al (2005) Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. J Lipid Res 46:582–588
39. Yoshimoto A, Mori K, Sugawara A et al (2002) Plasma ghrelin and desacyl ghrelin concentrations in renal failure. J Am Soc Nephrol 13:2748–2752
40. Hotta M, Ohwada R, Katakami H, Shibasaki T, Hizuka N, Takano K (2004) Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa. J Clin Endocrinol Metab 89:5707–5712