



PAPER

Increase of fat oxidation and weight loss in obese mice caused by chronic treatment with human growth hormone or a modified C-terminal fragment

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OBJECTIVE: To observe the chronic effects of human growth hormone (hGH) and AOD9604 (a C-terminal fragment of hGH) on body weight, energy balance, and substrate oxidation rates in obese (*ob/ob*) and lean C57BL/6J mice. *In vitro* assays were used to confirm whether the effects of AOD9604 are mediated through the hGH receptor, and if this peptide is capable of cell proliferation via the hGH receptor.

METHOD: Obese and lean mice were treated with hGH, AOD or saline for 14 days using mini-osmotic pumps. Body weight, caloric intake, resting energy expenditure, fat oxidation, glucose oxidation, and plasma glucose, insulin and glycerol were measured before and after treatment. BaF-BO3 cells transfected with the hGH receptor were used to measure *in vitro* ¹²⁵I-hGH receptor binding and cell proliferation.

RESULTS: Both hGH and AOD significantly reduced body weight gain in obese mice. This was associated with increased *in vivo* fat oxidation and increased plasma glycerol levels (an index of lipolysis). Unlike hGH, however, AOD9604 did not induce hyperglycaemia or reduce insulin secretion. AOD9604 does not compete for the hGH receptor and nor does it induce cell proliferation, unlike hGH.

CONCLUSIONS: Both hGH and its C-terminal fragment reduce body weight gain, increase fat oxidation, and stimulate lipolysis in obese mice, yet AOD9604 does not interact with the hGH receptor. Thus, the concept of hGH behaving as a pro-hormone is further confirmed. This data shows that fragments of hGH can act in a manner novel to traditional hGH-stimulated pathways. *International Journal of Obesity* (2001) 25, 1442–1449

Keywords: human growth hormone fragment; body weight; energy expenditure; lipolysis; growth hormone receptor

Introduction

Human growth hormone (hGH) can mobilize lipid¹ and inhibit the synthesis of triglycerides. For example, hGH can decrease the expression of lipoprotein lipase, increase hormone sensitive lipase² and uncoupling proteins,³ as well as enhancing the sensitivity of adipose tissue to adrenergic stimulus⁴ and decreasing glucose transport.⁵ hGH has limited potential as a useful weight-loss agent, however, because

of its undesirable effects on glucose metabolism. Although hGH can have early 'insulin-like' effects in adipocytes, it is well documented that chronic administration of hGH can produce 'diabetogenic' effects⁶ resulting in hyperglycaemia. hGH has many other actions unrelated to lipid and carbohydrate metabolism, including effects on cell proliferation, calcium metabolism, and growth.⁷ Various isoforms and fragments of hGH exist in plasma.⁸ It has been suggested that these may contribute to the mitogenic,⁹ diabetogenic¹⁰ and insulin-potentiating¹¹ actions of hGH. Here we present evidence that a modified C-terminal fragment of hGH, not yet identified in plasma, may account for the lipid mobilizing effect of hGH without the diabetogenic or mitogenic effects.

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Received 31 October 2000; revised 21 February 2001;
accepted 28 March 2001

We have synthetically modified a 15 amino acid region of hGH (hGH177-191) by adding a tyrosine to help stabilize the molecule, and named this peptide fragment AOD9604. AOD9604 has been extensively studied in our laboratory. We have previously shown that it acutely stimulates fatty acid oxidation, increases *in vitro* lipolysis via the stimulation of hormone sensitive lipase, and reduces *in vitro* lipogenesis by inhibiting the activity of the rate-limiting enzyme acetyl-CoA carboxylase.^{12,13} We have also shown that chronic administration of AOD9604 in *ob/ob* mice results in a marked decrease in fat accumulation, decreased body weight gain, and an improvement in circulating metabolites, such as triglycerides and cholesterol.^{12,14}

This paper extends our previous work by comparing the chronic effect of the parent hormone (hGH) and AOD9604 on energy balance and substrate oxidation rates in both obese *ob/ob* and lean C57BL/6J mice. In addition, we have examined whether chronic treatment with hGH and AOD9604 have different effects on circulating plasma glucose and insulin levels. Given the size of AOD9604 (16 amino acids), it is likely that its effects are not mediated through the hGH receptor. This was investigated using competition binding in cells transfected with the hGH receptor, and by assessing whether AOD9604 interacts with the hGH receptor to increase cell proliferation.

Materials and methods

Hormones

AOD9604 consists of amino acid residues 177–191 of hGH with an additional tyrosine at the C-terminus. It was prepared by solid-phase synthesis and purified by reverse-phase HPLC.¹⁴ The structure of the peptide was verified by mass spectrophotometry and amino-acid analysis. Recombinant hGH was a gift of Bresagen (Adelaide, Australia).

Animals

Male C57BL/6J (lean) and *ob/ob* (obese) mice aged 10–12 weeks were housed in a 12 h light/dark cycle at a constant room temperature of 23°C in the departmental animal house at Monash University. Animals were fed *ad libitum* with a standard laboratory diet, consisting of 11.92 kJ/g (Clark King, Melbourne, Australia) and allowed free access to water at all times.

Experimental groups and design

Both lean and obese mice were age matched into three treatment groups—lean animals (saline ($n=3$); AOD9604 ($n=4$); hGH ($n=6$)) and obese animals (saline ($n=6$); AOD9604 ($n=4$); hGH ($n=5$)). Measurements of body weight, resting energy expenditure rates, and substrate oxidation rates were undertaken in each animal prior to drug treatment. In addition, mice were fasted for 2 h, anaesthetized with sodium pentobarbitone (Nembutal, Boehringer

Ingelheim, NSW, 35 mg/kg), rested for 30 min, and then eye-bled for plasma analysis. Blood samples were collected in heparinized tubes (CapijectT-MLHG, Terumo, USA) and centrifuged at 1000 g for 5 min to obtain plasma. Animals were treated with either saline (control), AOD9604 (250 µg/kg/day) or hGH (1 mg/kg/day) for 14 days via mini-osmotic pumps (no. 1002, Alza Corp, Cupertino, USA). The pump was inserted under the skin between the scapular of the animals after they had been anaesthetized with sodium pentobarbitone. Pumps contained a total volume of 100 µl of either saline, AOD9604 or hGH, prepared under sterile conditions. Animals were housed individually after surgery and body weight and caloric intake were measured every 2 days for 14 days thereafter. Ethics was approved by the Standing Committee on Animals Ethics, Monash University.

On day 14, resting energy expenditure and substrate oxidation measurements were repeated and blood was collected from anaesthetized animals after a 2 h fast by heart-puncture before sacrifice. Plasma was collected as described above. The pumps were removed and checked for adequate drug infusion. Less than 10% of the initial volume was found to be within the pumps, indicating efficient perfusion.

Indirect calorimetry

Resting energy expenditure, fat oxidation, and glucose oxidation rates were measured after a 2 h fast using an indirect calorimeter (Columbus Instruments, Columbus, USA), as previously described.¹² Mice were placed into a 20×13×11 cm Perspex box through which fresh air was drawn at a rate of 0.65 l/min. Mean rates of CO₂ produced and oxygen consumed were calculated every 3 min over a 30 min period. Rates of energy expenditure (kJ/kg/min) were calculated after assuming a urinary nitrogen excretion of 0.84 mg/min/kg body weight, as previously described.¹²

Plasma glucose, insulin and glycerol

Glucose was measured using a glucose analyser (YSI23AM; YellowSprings Instrument Co, Ohio, USA). Insulin was determined using a radioimmunoassay kit (no. RI-13K; Linco, St. Charles, USA). Glycerol was measured colorimetrically using a commercial kit (no. GPO-337-40A; Sigma, St Louis, USA).

Cell culture

BaF3 cells transfected with the hGH receptor gene¹⁵ were grown in suspension in RPMI1640 media (GibcoBRL, New York, USA) containing 10% Serum Supreme (Biowhittaker, Walkersville, USA) and supplemented with hGH (100 ng/l) in 5%CO₂.

Competition binding with the human growth hormone receptor

Cells (BaF3-hGH) were incubated for 6 h in RPMI1640 without hGH and sera to up-regulate receptor expression. Cells were washed twice in PBS and resuspended in 20 mM MgCl₂ binding buffer (IGBBM)¹⁶ to give a cell density of approximately 30–60 × 10⁶ cells/ml.

Both AOD9604 and hGH were dissolved in IGBBM at various concentrations. Drugs were then added to appropriately labelled 5 ml polypropylene tubes, followed by 3–6 × 10⁶ cells (suspended in IGBBM) and I¹²⁵-hGH (200 000 cpm), according to Gobius.¹⁶ Cells were incubated in the presence of drugs for 18 h at 4°C to prevent internalization.

Following incubation, ice-cold PBS (2 ml) was added to stop the reaction. Tubes were then spun at 2200 g for 20 min at 4°C and the supernatant was removed by aspiration. The pellets were counted in a gamma counter (Minaxi Auto-gamma counter 5000 series; Packard Instruments, Meriden, USA).

Cell proliferation assay

Proliferative responses of hGH and AOD9604 were measured by assessing the incorporation of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (no. M2128; Sigma, St Louis, USA). Cells were suspended in RPMI1640 media supplemented with 0.5% Serum Supreme and plated in rows at a variety of cell densities in a 96-well plate.¹⁷ Quadruplicate wells were incubated with defined concentrations of AOD9604 or hGH in 0.1 ml, or with 4.5 nM IL-3 (maximal dose). After 18–24 h of incubation 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (final concentration, 1 mg/ml) was added to the medium and the incubation was continued for a further 3 h. Assays for 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were performed as previously described.¹⁷

Statistical analysis

The Student's *t*-test was used to analyse the results, where a non-parametric Mann–Whitney *U*-test was used for body weight analysis. All data are expressed as the mean ± standard error of the mean (s.e.). *P*-values of <0.05 were accepted as statistically significant.

Results

Pre-treatment parameters

Pre-treatment measures of body weight, plasma glucose, insulin and glycerol, resting energy expenditure, and rates of fat and glucose oxidation are shown in Table 1. As expected, the obese *ob/ob* mice were twice as heavy as lean mice, and had marked hyperglycaemia, hyperinsulinaemia and hyperglycerolaemia. These obese mice also had lower rates of fat oxidation and a higher rate of glucose oxidation

Table 1 Pre-treatment measures of body weight, plasma glycerol, insulin and glucose, resting energy expenditure and fat and glucose oxidation rates in lean C57BL/6J and obese *ob/ob* mice

Parameter	Lean C57BL/6J	<i>ob/ob</i>
Body Weight (g)	28.6 ± 0.5	57.6 ± 1.1*
Glucose (mM)	11.0 ± 0.5	21.6 ± 0.7*
Insulin (pM)	95.6 ± 21.1	3767.9 ± 253.3 [†]
Glycerol (mM)	0.4 ± 0.06	1.5 ± 0.09*
Resting energy expenditure (kJ/kg/min)	0.0238 ± 0.0010	0.0230 ± 0.0010
Fat oxidation (nkat)	19.0 ± 0.91	9.0 ± 1.0 [†]
Glucose oxidation (nkat)	37.0 ± 3.0	64.0 ± 3.0 [†]

**P* < 0.0025, [†]*P* < 0.0005 ; lean vs obese; 1 kat = 1 mol/s

than lean mice. No significant difference was observed in the rate of resting energy expenditure between the two groups.

Body weight and caloric intake

Figure 1A demonstrates the effect of 14 days of AOD9604 and hGH treatment on body weight in lean mice. There were no significant differences in body weight in the lean animals when treated with AOD9604 compared to the saline-treated controls. However, hGH induced a significant increase in body weight by day 6 of treatment in the lean animals when compared to the controls. In contrast, Figure 1B shows that both AOD9604 and hGH reduce body weight in obese mice after 8 and 12 days of treatment, respectively. It has previously been shown that this decrease in body weight is attributable to a decrease in total body fat.^{18,19} Caloric intake was measured during treatment in all groups of mice and no differences were noted between any of the treatment groups, in agreement with our previous studies (data not shown).

Plasma glucose, insulin and glycerol

Chronic administration of hGH has been associated with a hyperglycaemic effect in rodents and humans.^{6,20} This is demonstrated in Figure 2A by a 28 mmol (*P* < 0.01) increase in plasma glucose from basal values after hGH treatment, but this was observed in the obese animals only. Therefore hGH is capable of further promoting hyperglycaemia in this strain of mouse. In contrast, there was no change in circulating plasma glucose levels in the AOD9604-treated mice compared to controls in both the obese and lean strains of mice.

Similarly, as shown in Figure 2B, AOD9604 had no effect on circulating insulin levels in either lean or obese mice. There was also no effect of hGH on plasma insulin in lean mice. However, in obese mice, hGH caused a dramatic 66% fall in the levels of circulating insulin from pre-treatment values.

The lipolytic actions of both hGH and AOD9604 have been well documented in the literature²¹ and in our laboratory.¹² Consistent with this, Figure 2C shows a marked increase in circulating glycerol levels with AOD9604

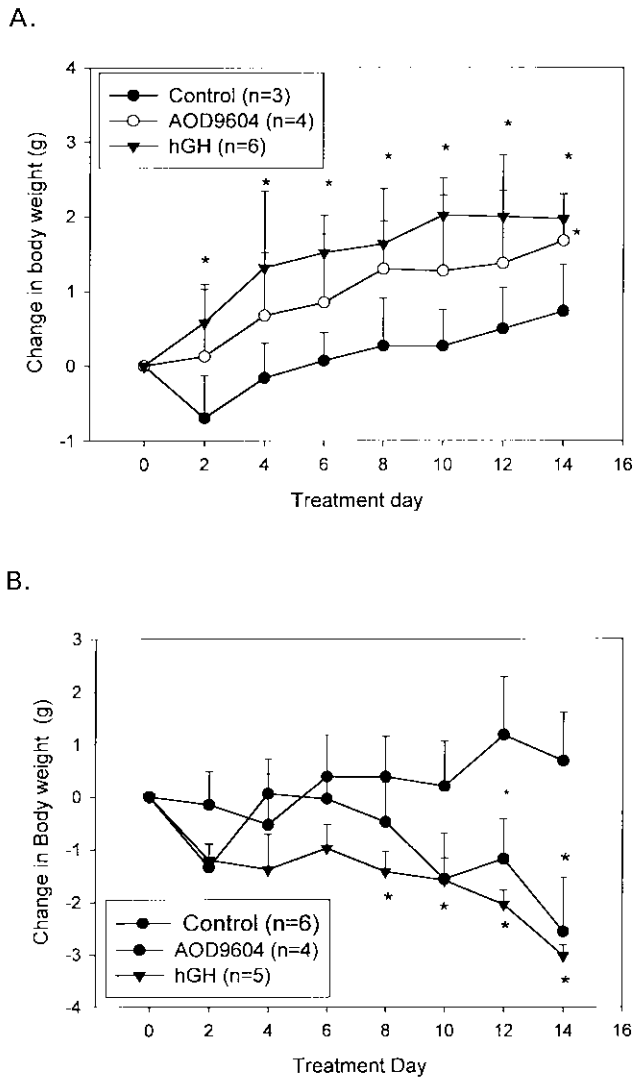


Figure 1 The effects of 14 day infusion of saline (control), AOD9604 (250 µg/kg/day) or hGH (1 mg/kg/day) on body weight accumulation in lean C57BL/6j mice (A) or obese (*ob/ob*) mice (B). Results are expressed as the change in body weight from day 0 in the form of mean ± s.e. (**P* < 0.05, hGH vs treated control).

treatment in both lean and obese mice, and a further increase in glycerol levels with hGH treatment in both strains of mice when compared to the saline-treated control mice.

Rates of fat oxidation, glucose oxidation and energy expenditure

In lean mice, neither AOD or hGH induced significant changes in the oxidation of fat. In obese mice, the stimulation in fat oxidation was more dramatic with a 216% (*P* < 0.005) and 230% (*P* < 0.02) increase following both

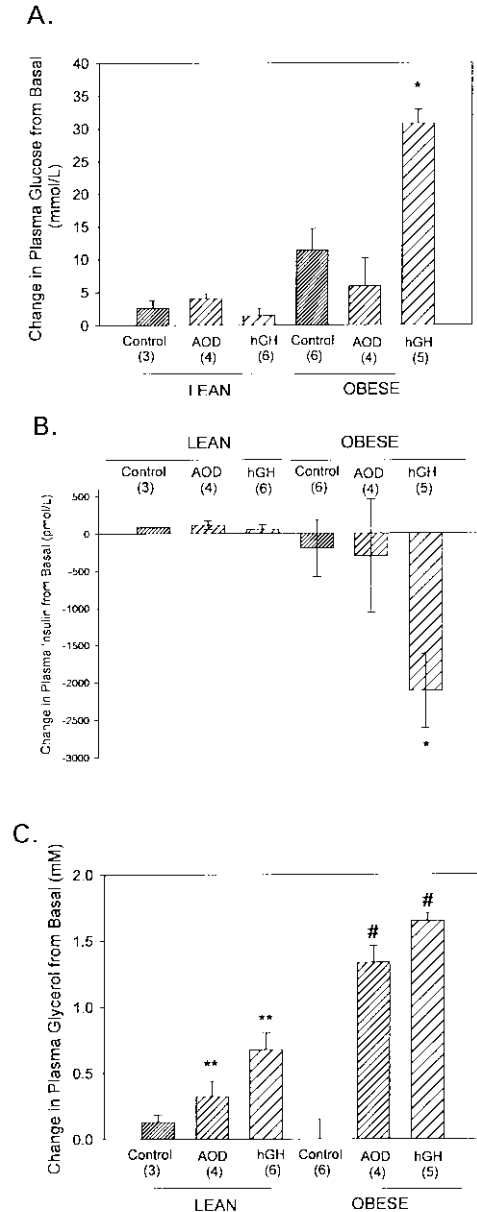


Figure 2 Change in plasma glucose (A), insulin (B) and glycerol levels (C) from day 0 after 14 days of treatment in lean C57BL/6j mice or obese (*ob/ob*) mice. Results are expressed as the mean ± s.e. Numbers in each group are indicated in brackets on the x-axis legends. **P* < 0.02, treated vs control; ***P* < 0.025; #*P* < 0.001.

AOD9604 and hGH treatment compared to pre-treatment values, respectively (Figure 3A).

AOD9604 did not significantly alter glucose oxidation rates in lean and obese mice (Figure 3B). However, chronic hGH treatment was capable of lowering rates of glucose oxidation in hGH treated lean (37 to 10 nkat; *P* < 0.02) and obese animals (64 to 0.6 nkat; *P* < 0.001). Changes in resting energy expenditure after AOD9604 and hGH treatment were

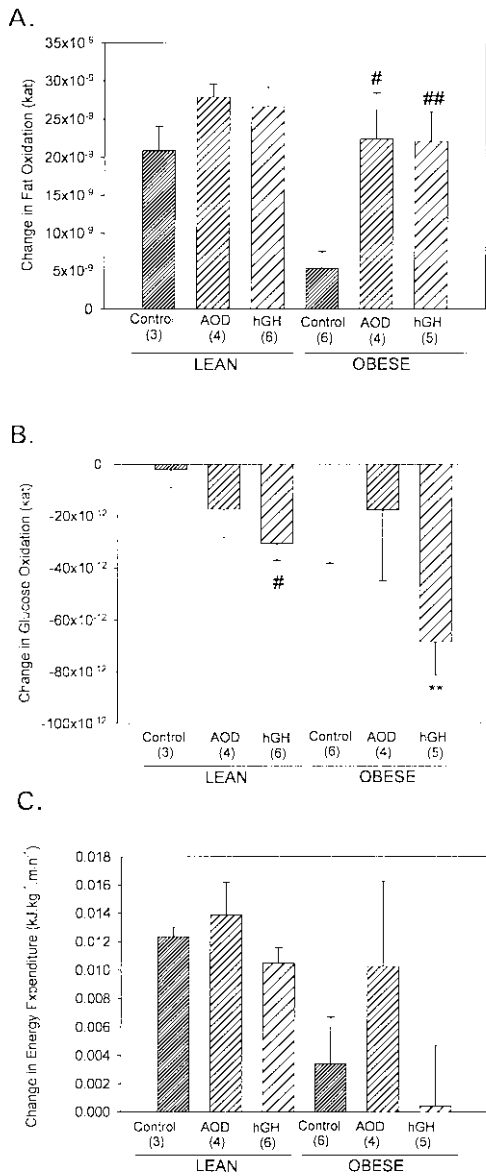


Figure 3 The effects of a 14 day infusion of saline (control), AOD9604 or hGH on rates of fat oxidation (A), glucose oxidation (B), and resting energy expenditure (C) in lean C57BL/6J mice or obese (*ob/ob*) mice. Results are expressed as a change from day 0 in the form of mean ± s.e. Numbers in each group are indicated in brackets on the x-axis legends. **P* < 0.03; #*P* < 0.01; ##*P* < 0.005; treated vs control; 1 kat = 1 mol/s.

not significantly different from changes in resting energy expenditure after saline treatment in either lean or obese mice (Figure 3C).

hGH competition binding

A competition binding assay in hGH receptor transfected cells using ¹²⁵I-hGH was established to test for AOD9604's

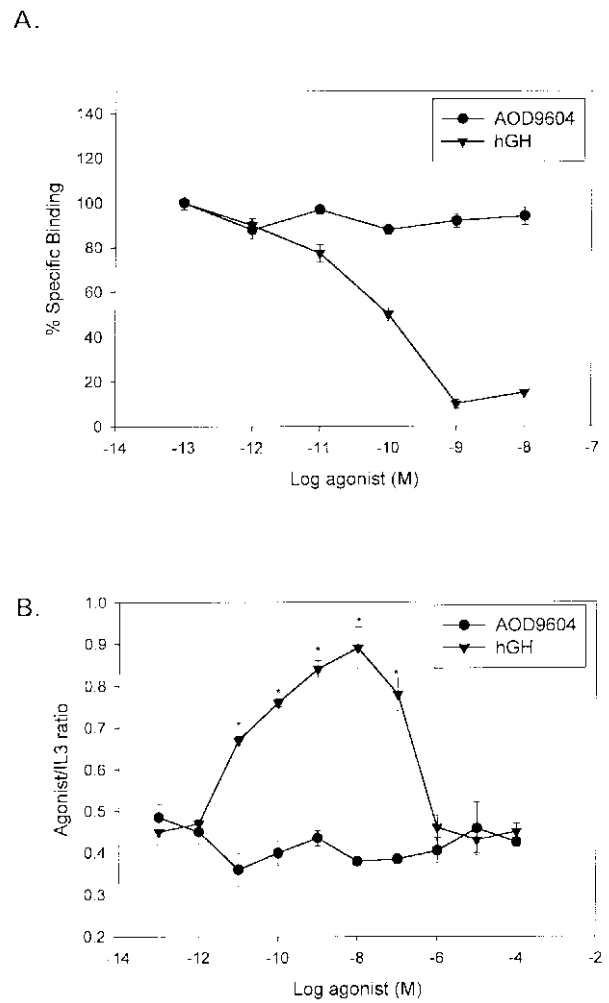


Figure 4 AOD9604 and hGH competing for ¹²⁵I-hGH (A) and cell proliferation (B) in BaF3-hGHR cells. Results in (A) are expressed as the mean ± s.e. of four experiments with two determinations in each, (**P* < 0.002). Results in (B) are expressed as the mean ± s.e. of five separate experiments in quadruplicate (**P* < 0.001).

ability to compete for and recognize the receptor. Figure 4A clearly demonstrates that hGH is capable of competing for the binding site of the hGH receptor with ¹²⁵I-hGH, at picomolar concentrations, but AOD9604 is incapable of competition, even at the concentrations of hGH needed for total displacement.

We then tested whether AOD9604 acts through the hGH receptor to increase cell proliferation using a highly sensitive BaF3 cell proliferation assay. As shown in Figure 4B, hGH shows the classical bell-shaped response curve, whereas AOD9604 is without effect even at 7 log doses above the minimal effective dose for hGH.

Discussion

Fragments of hGH are known to exist *in vivo* and appear to be involved in a variety of biological processes. We have isolated an enzymatically digested fragment derived from the C-terminus of hGH (AOD9604), which is capable of inducing some, but not all, the effects that native hGH can stimulate. We have shown that hGH and AOD9604 share a number of similar effects with respect to lipid metabolism when chronically administered to mice. For example, both compounds are capable of decreasing body weight gain in obese *ob/ob* mice following 2 weeks of chronic treatment using miniosmotic pumps. Both hGH and AOD9604 are also capable of increasing the levels of circulating glycerol (indicative of increased lipolysis) and increasing fat oxidation in obese mice. The effects of hGH and AOD9604 on carbohydrate metabolism, however, appear to be quite different, in that chronic administration of hGH in *ob/ob* mice markedly depresses glucose oxidation and increases circulating plasma glucose levels, apparently by inducing insulin insufficiency, whereas AOD9604 has no effect on glucose oxidation, plasma glucose, or plasma insulin levels.

We have also examined the ability of AOD9604 to act through the hGH receptor *in vitro*, and shown that there is no competition between AOD9604 and ¹²⁵I-hGH for this receptor. In addition, we have found that AOD9604 is incapable of inducing cell proliferation via the hGH receptor, a response that hGH induces with high potency. Together this data suggests that this new analogue of an hGH fragment, has similar actions on lipid metabolism to hGH, yet works independently to hGH on carbohydrate metabolism, receptor recognition, and cell proliferation. Hence, AOD9604 is a novel peptide, which works through its own effector-mediated pathway to induce some of the lipid mobilizing effects performed by the parent hormone, hGH. Our data provides strong support for the concept that hGH's diverse functions are mediated by specific domains of the hormone, and raises the possibility that some of these domains may have potential in the treatment of obesity.

Chronic administration of both hGH and AOD9604 blunts weight gain and reduces fat mass in obese (*ob/ob*) mice.^{14,20} Although we have previously shown an acute stimulatory effect of AOD9604 on resting energy expenditure,¹² we did not find a significant effect of chronic treatment of AOD9604 on resting energy expenditure in the present study even when we examined this data as a change from pre-treatment. From these observations, we may conclude that AOD9604 exerts acute stimulatory effects on fat and glucose oxidation and energy expenditure, whereas chronic treatment increases fat oxidation alone. The differences may be attributed to duration and mode of administration of AOD9604.

The effect of AOD9604 and hGH on adiposity also does not appear to be mediated by a reduction in caloric intake. The decrease in body fat is, however, associated with a marked increase in fat oxidation and, based on the circulating glycerol levels, a marked increase in lipolysis. The

increases in fat oxidation in lean mice were much smaller than observed in the obese mice, and had no significant impact on body weight, presumably because there is less adipose tissue for the compounds to target in the lean mice. It should also be noted that the leptin-deficient *ob/ob* mouse has an inherently low rate of fat oxidation (Table 1) which may make this animal more receptive to agents which promote fat oxidation.

The actions of hGH on adipocyte metabolism are varied, complex and not yet fully elucidated. However, it is known that chronic hGH treatment induces insulin-antagonistic effects, involving enhanced lipolysis with increased production of glycerol and free fatty acids,²¹ decreased lipid uptake and lipogenesis²² and importantly, a suppression of glucose metabolism in adipose tissue both basally and following insulin stimulation.²³ The decreased body weight and increased glycerol levels observed in our study after 2 weeks of hGH treatment support these findings. Mediators of the lipid mobilizing effect of hGH include an enhanced susceptibility to catecholamine-induced lipolysis suggesting an increment in the levels of β -adrenoceptors,²⁴ enhanced activity of hormone sensitive lipase,²⁵ inhibition of the guanine-nucleotide binding protein (Gi),²⁶ and effects on the activity of key enzymes involved in lipid synthesis such as acetyl-CoA carboxylase.²⁷

Chronic hGH treatment in humans⁶ and rodents²⁰ does, however, have serious drawbacks in that it has consistently been found to produce hyperglycaemia. In this study, we also have noted that hGH treatment is capable of inducing a marked hyperglycaemic change following 14 days of chronic administration in *ob/ob* mice. This hyperglycaemia may be a consequence of increased hepatic glucose production and/or peripheral tissue insulin resistance, as suggested by Cameron.²⁰ In agreement with this, Napoli *et al*²⁸ have shown that chronic treatment of rats with hGH results in a reduction in the levels of the glucose transporter GLUT 1 mRNA and protein. We also observed a fall in the rate of glucose oxidation with long-term hGH treatment. The hyperglycaemia and reduced glucose oxidation seen in the hGH-treated *ob/ob* mice may reflect the suppression of glucose metabolism in peripheral tissues which has been seen with chronic hGH treatment, as noted above. Alternatively, the hyperglycaemia and reduced glucose oxidation may be a consequence of the reduced circulating insulin levels induced by hGH treatment. This reduction in insulin was dramatic with circulating levels falling by 71% post-hGH treatment in the *ob/ob* mice ($P < 0.02$). This fall could be due to decreased insulin secretion by the pancreatic islets, or increased clearance of insulin from the circulation by the kidneys, liver or adipose tissue. There is no evidence in the literature that hGH induces either of these effects. In fact, shorter-term hGH treatment (3 days) in *ob/ob* mice has been shown to potentiate insulin secretion.²⁹ The fact that some studies show increased insulin secretion after hGH treatment while our study clearly shows decreased insulin secretion after hGH treatment may be due to the mode of treatment

(continual infusion by minipump vs i.p. injection) or, most likely, the length of treatment (14 vs 3 days). Indeed it is possible that hGH-mediated hypersecretion of insulin promotes β -cell 'burnout' and hyposecretion of insulin in the longer term.³⁰ Although the explanation is unclear, these results fit with the idea that hGH promotes glucose metabolism acutely but becomes diabetogenic when given chronically. The diabetogenic effects of hGH have been also demonstrated in the circulating fragment hGH44-191.³¹ Given that we have found no adverse effects on circulating glucose and insulin levels with hGH 177-191 it is likely that the deleterious effects on glucose metabolism reside in hGH44-177; however, this remains to be shown.

In contrast, we found that lean control animals showed no significant alteration in their circulating levels of glucose and insulin following hGH treatment. This suggests that the effect is predominantly a result of the obese, insulin resistant phenotype of the *ob/ob* mouse, and may be a consequence of a leptin deficiency or high circulating glucocorticoids.³² This is a possibility worth further exploration.

Assessment of the binding of hGH fragments to the hGH receptor, show that most do not bind, or in the case of hGH44-191, bind with very low affinity.³³ In agreement with this, we have shown that AOD9604 does not produce its effects through the hGH receptor, and therefore must activate an alternative receptor site. In addition, AOD9604 does not induce a proliferative effect in cells by acting on the hGH receptor. This contrast with the effect of the native hGH hormone and another circulating fragment, hGH1-43.¹⁷ This fragment is a naturally occurring amino-terminal fragment isolated from human plasma, and as well as being capable of cell proliferation, it has a marked insulin-potentiating action. However, it also acts independent of hGH, insulin or IGF-1 receptors, indicating novel mechanisms. hGH fragmentation may be likened to the actions of the ACTH-lipotropin precursor molecule, which acts to induce its multiple functions through smaller bioactive molecules.³⁴

In summary, this study further substantiates that hGH is composed of discrete bioactive domains which may account for the diverse array of hGH effects. The domain hGH177-191 appears to induce its actions through a site distinct from the hGH receptor, but acts like hGH in *ob/ob* mice, to increase levels of fat oxidation, promote lipolysis and decrease body weight, without the diabetogenic effects associated with hGH. The development of bioassays to detect whether this, or a similar fragment, circulates in human plasma may prove that this compound is a key regulator of lipid metabolism, which possibly has depleted function in obese subjects. There is a need to develop safe and more effective compounds for the treatment of human obesity. Work presented in this study highlights the possibility that large multi-functional proteins may exert their effects through post-translational fragmentation, and that the identification of these fragments allows for modification to target specific therapeutic disorders, such as obesity.

Acknowledgements

Thanks to Mr Anthony Civitarese for assistance with indirect calorimetry, and to Mr Ray Spark for plasma glucose analysis. This work was supported by Metabolic Pharmaceuticals.

References

- Davidson MB. Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev* 1987; **8**: 115–131.
- Dietz J, Schwartz J. Growth hormone alters lipolysis and hormone-sensitive lipase activity in 3T3-F442A adipocytes. *Metabolism* 1991; **40**: 800–806.
- Boss O, Muzzin P, Giacobino J-P. The uncoupling proteins; a review. *Eur J Endocrinol* 1998; **139**: 1–9.
- Watt PW, Finley E, Cork S, Clegg RA, Vernon RG. Chronic control of the beta- and alpha 2-adrenergic systems of sheep adipose tissue by growth hormone and insulin. *Biochem J* 1991; **273**(Pt 1): 39–42.
- Donkin SS, Chiu PY, Yin D, Louveau I, Swencki B, Vockroth J, Evock-Clover CM, Peters JL, Etherton TD. Porcine somatotrophin differentially down-regulates expression of the GLUT4 and fatty acid synthase genes in pig adipose tissue. *J Nutr* 1996; **126**: 2568–2577.
- Spyer G, Ellard S, Hattersley A. Growth-hormone treatment and risk of diabetes. *Lancet* 2000; **355**: 1913–1914.
- Carrel AL, Allen DB. Effects of growth hormone on body composition and bone metabolism. *Endocrine* 2000; **12**: 163–172.
- Baumann G. Growth hormone heterogeneity in human pituitary and plasma. *Horm Res* 1999; **51**: 2–6.
- Jeoung DI, Allen DL, Guller S, Yen V, Sonenberg M. Mitogenic and receptor activities of human growth hormone 108–129. *J Biol Chem* 1993; **268**: 22520–22524.
- Sinha YN, Jacobsen BP. Human growth hormone (hGH)-(44–191), a reportedly diabetogenic fragment of hGH, circulates in human blood: measurement by radioimmunoassay. *J Clin Endocrinol Metab* 1994; **78**: 1411–1418.
- Lim N, Ng FM, Wu ZM, Ede N, Hearn MT. Hypoglycemic action of a novel constrained analog of human growth hormone (6–13). *Endocrinology* 1992; **131**: 835–840.
- Heffernan MA, Jiang WJ, Thorburn AW, Ng FM. Effects of oral administration of a synthetic fragment of human growth hormone on lipid metabolism. *Am J Physiol* 2000; **279**: E501–E507.
- Wu Z, Ng FM. Antilipogenic action of synthetic C-terminal sequence 177–191 of human growth hormone. *Biochem Mol Biol Int* 1993; **30**: 187–196.
- Jiang WJ. Investigation into human Growth Hormone 177–191 peptides and analogues as anti-obesity agents. PhD thesis, Monash University, 1999.
- Rowlinson SW, Behncken SN, Rowland JE, Clarkson RW, Strasburger CJ, Wu Z, Baumbach W, Waters MJ. Activation of chimeric and full-length growth hormone receptors by growth hormone receptor monoclonal antibodies. A specific conformational change may be required for full-length receptor signaling. *J Biol Chem* 1998; **273**: 5307–5314.
- Gobius KS, Rowlinson SW, Barnard R, Mattick SC, Waters MJ. The first disulphide loop of rabbit growth hormone receptor is required for binding to the hormone. *J Mol Endocrinol* 1992; **9**: 213–220.
- Rowlinson SW, Waters MJ, Lewis UJ, Barnard R. Human growth hormone fragments 1–43 and 44–191: *in vitro* somatogenic activity and receptor binding characteristics in human and non-primate systems. *Endocrinology* 1996; **137**: 90–95.
- Casanueva FF, Dieguez C. Interaction between body composition, leptin and growth hormone status. *Baillieres Clin Endocrinol Metab* 1998; **12**: 297–314.

- 19 Natera SH, Jiang WJ, Ng FM. Reduction of cumulative body weight gain and adipose tissue mass in obese mice: response to chronic treatment with synthetic hGH 177–191 peptide. *Biochem Mol Biol Int* 1994; **33**: 1011–1021.
- 20 Cameron CM, Kostyo JL, Adamafo NA, Dunbar JC. Metabolic basis for the diabetogenic action of growth hormone in the obese (*ob/ob*) mouse. *Endocrinology* 1987; **120**: 1568–1575.
- 21 Marcus C, Margery V, Kamel A, Bronnegard M. Effects of growth hormone on lipolysis in humans. *Acta Paediatr* 1994; **406**(Suppl): 54–58.
- 22 Vernon RG. GH inhibition of lipogenesis and stimulation of lipolysis in sheep adipose tissue: involvement of protein serine phosphorylation and dephosphorylation and phospholipase C. *J Endocrinol* 1996; **150**: 129–140.
- 23 Salem MA, Wolff GL. Potentiation of response to insulin and anti-insulin action by two human pituitary peptides in lean agouti A/a, obese yellow Avy/A, and C57BL/6J-*ob/ob* mice. *Soc Exp Biol Med* 1989; **191**: 113–123.
- 24 Watt PW, Finley E, Cork S, Clegg RA, Vernon RG. Chronic control of the beta- and alpha 2-adrenergic systems of sheep adipose tissue by growth hormone and insulin. *Biochem J* 1991; **273**: 39–42.
- 25 Slavin BG, Ong JM, Kern PA. Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *J Lipid Res* 1994; **35**: 1535–1541.
- 26 Doris R, Vernon RG, Houslay MD, Kilgour E. Growth hormone decreases the response to anti-lipolytic agonists and decreases the levels of Gi2 in rat adipocytes. *Biochem J* 1994; **297**: 41–45.
- 27 Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, Noguchi T, Tanaka T. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur J Biochem* 1990; **190**: 435–441.
- 28 Napoli R, Cittadini A, Chow JC, Hirshman MF, Smith RJ, Douglas PS, Horton ES. Chronic growth hormone treatment in normal rats reduces post-prandial skeletal muscle plasma membrane GLUT1 content, but not glucose transport or GLUT4 expression and localization. *Biochem J* 1996; **315**: 959–963.
- 29 Adamafo NA, Kostyo JL, Cameron CM, Trimark JR, Dunbar JC. Acute effects of S-carboxymethylated human growth hormone on insulin resistance in the obese (*ob/ob*) mouse. *Metabolism* 1988; **37**: 900–905.
- 30 Ferrannini E. Insulin resistance versus insulin deficiency in NIDDM: problems & prospects. *Endocrine Rev* 1998; **19**: 477–490.
- 31 Lewis UJ, Lewis LJ, Salem MA, Staten NR, Galosy SS, Krivi GG. A recombinant-DNA-derived modification of human growth hormone (hGH44-191) with enhanced diabetogenic activity. *Cell Endocrinol* 1991; **78**(1–2): 45–54.
- 32 Evans BA, Papaioannou M, Anastasopoulos F, Summers RJ. Differential regulation of beta3-adrenoceptors in gut and adipose tissue of genetically obese (*ob/ob*) C57BL/6J-mice. *Br J Pharmacol* 1998; **124**: 763–771.
- 33 Hettiarachchi M, Watkinson A, Leung KC, Sinha YN, Ho KK, Kraegen EW. Human growth hormone fragment (hGH44-91) produces insulin resistance and hyperinsulinemia but is less potent than 22 kDa hGH in the rat. *Endocrine* 1997; **6**: 47–52.
- 34 Odagiri E, Sherrell BJ, Mount CD, Nicholson WE, Orth DN. Human placental immunoreactive corticotropin, lipotropin, and beta-endorphin: evidence for a common precursor. *Proc Natl Acad Sci, USA* 1979; **76**: 2027–2031.