Effects of oral administration of a synthetic fragment of human growth hormone on lipid metabolism

M. A. HEFFERNAN,1 W. J. JIANG,1 A. W. THORBURN,2 AND F. M. NG1

1Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3148; and 2Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

Received 15 December 1999; accepted in final form 11 April 2000

The lipolytic/antiligogenic property of hGH is well known (11, 19). For example, it is well documented that hGH is a potent inhibitor of lipoprotein lipase and can increase circulating free fatty acids, ultimately reducing fat cell mass (20). The association of circulating hGH with fat mass is well characterized in adult GH-deficient patients, where a strong correlation between excess abdominal adiposity and reduced circulating GH levels exists that can be normalized after GH replacement therapy (10). However, clinical applications of hGH for long-term obesity treatment have not been successful because of its diabetogenic and other unwanted side effects (3). Advances in peptide synthesis technology have made it possible to produce specific and discrete functional domains of hGH (9), and there is now considerable evidence supporting the concept of discrete structural domains within hGH responsible for the different metabolic functions of the intact hGH (4). For example, Ng et al. (13) have reported that the insulin-like actions of hGH may reside in the amino-terminal region of the molecule [hGH-(6–13)]. This was later confirmed to be within hGH-(1–43), another well-known hypoglycemic fragment that exists in the circulation of humans and results from posttranslational cleavage in vivo (20, 29). On the other hand, the carboxy terminus of the hGH molecule [hGH-(177–191), or AOD-9401] has been identified as the lipid mobilizing domain of the intact hormone. This fragment inhibits the activity of acetyl-CoA carboxylase in adipocytes and hepatocytes, and it acts to reduce glucose incorporation into lipid in both isolated cells and tissues (30). It has been suggested to be the lipolytic domain of the hGH molecule. Previous studies in our laboratory have shown that weight loss can be induced by chronic intraperitoneal treatment of AOD-9401 (12).

This study aims to extend these findings by examining whether oral administration of this fragment of hGH can also reduce body weight and affect lipid metabolism. This study had three parts. First, we investigated the effectiveness of oral administration of AOD-9401 on body weight reduction, energy balance, lipolysis, and lipogenesis in obese C57BL/6J (ob/ob) mice. Next, we examined the in vitro antiligogenic, lipolytic, and metabolic effects of AOD-9401 on isolated adipose tissue from obese and lean animals. Finally, we investigated the metabolic effects of AOD-9401 on isolated adipocytes and hepatocytes from obese and lean animals.

Obesity is a major public health concern in most developed countries. For example, in Australia almost one in five adults is obese, making them highly susceptible to diabetes, coronary heart disease, and high blood pressure, as well as reduced psychological health (1). Obesity is normally treated by diet and exercise, but attempts to sustain significant weight loss by dieting and exercise nearly always meet with failure (6). There is a great need to develop better pharmacotherapy for obesity (18). Here, we begin an assessment of the potential use of AOD-9401, a fragment of human growth hormone (hGH), in the treatment of obesity.

The lipolytic/antiligogenic property of hGH is well known (11, 19). For example, it is well documented that hGH is a potent inhibitor of lipoprotein lipase and can increase circulating free fatty acids, ultimately reducing fat cell mass (20). The association of circulating hGH with fat mass is well characterized in adult GH-deficient patients, where a strong correlation between excess abdominal adiposity and reduced circulating GH levels exists that can be normalized after GH replacement therapy (10). However, clinical applications of hGH for long-term obesity treatment have not been successful because of its diabetogenic and other unwanted side effects (3). Advances in peptide synthesis technology have made it possible to produce specific and discrete functional domains of hGH (9), and there is now considerable evidence supporting the concept of discrete structural domains within hGH responsible for the different metabolic functions of the intact hGH (4). For example, Ng et al. (13) have reported that the insulin-like actions of hGH may reside in the amino-terminal region of the molecule [hGH-(6–13)]. This was later confirmed to be within hGH-(1–43), another well-known hypoglycemic fragment that exists in the circulation of humans and results from posttranslational cleavage in vivo (20, 29). On the other hand, the carboxy terminus of the hGH molecule [hGH-(177–191), or AOD-9401] has been identified as the lipid mobilizing domain of the intact hormone. This fragment inhibits the activity of acetyl-CoA carboxylase in adipocytes and hepatocytes, and it acts to reduce glucose incorporation into lipid in both isolated cells and tissues (30). It has been suggested to be the lipolytic domain of the hGH molecule. Previous studies in our laboratory have shown that weight loss can be induced by chronic intraperitoneal treatment of AOD-9401 (12).

This study aims to extend these findings by examining whether oral administration of this fragment of hGH can also reduce body weight and affect lipid metabolism. This study had three parts. First, we investigated the effectiveness of oral administration of AOD-9401 on body weight reduction, energy balance, lipolysis, and lipogenesis in obese C57BL/6J (ob/ob) mice. Next, we examined the in vitro antiligogenic, lipolytic, and metabolic effects of AOD-9401 on isolated adipose tissue from obese and lean animals. Finally, we investigated the metabolic effects of AOD-9401 on isolated adipocytes and hepatocytes from obese and lean animals.

Address for reprint requests and other correspondence: M. Heffernan, Dept. of Biochemistry and Molecular Biology, Monash Univ., Clayton, Melbourne, Victoria 3168, Australia (E-mail: mark.heffernan@med.monash.edu.au).
and fat oxidation activity of AOD-9401 in peripheral adipose tissues from obese rodents. Finally, to assess the feasibility of human treatment, the in vitro action of AOD-9401 on lipolysis and lipogenesis in adipose tissue from obese human adipose tissue was examined.

MATERIALS AND METHODS

Chemical synthesis of hGH functional domain (AOD-9401). AOD-9401, a synthetic fragment of hGH consisting of the amino acid residues 177–191, was prepared with solid-phase synthesis procedure and purified with reverse-phase HPLC methodology in our laboratories at Monash University (9). The structure of the peptide analog was verified with mass spectrometry and amino acid analysis.

In vitro tests for nonenzymic and enzymic degradation of AOD-9401. AOD-9401 was tested for its in vitro stability against potential gastrointestinal degradation, according to the standard protocols of enzyme digestion for protein (26). The procedure of De Laureto et al. (4) was used to evaluate the rate of degradation by measuring the residual peptide with RP-HPLC techniques as well as amino acid analysis.

Experimental animals and oral treatment. Eighteen male C57BL/6J (ob/ob) mice aged 10–12 wk and weighing 46.4 ± 1.1 (SE) g were used in this study. The animals were divided into saline (n = 8) or AOD-9401 treatment groups (n = 10) and were matched for body weight and sex. Animals were housed in a normal 12:12-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, a standard laboratory nonpurified diet (Clark King, Melbourne, Australia) and allowed free access to water at all times. The mice were given daily oral doses of either AOD-9401 (500 μg/kg body wt) dissolved in 0.3 ml saline or only saline of equivalent volume for 30 days. Accurate dosing was facilitated by a stainless steel gavage needle (7.5 × 0.1 cm diameter). The dose was administered slowly to avoid reflux.

Measurements of body weight gain, food consumption, energy expenditure, and physical activity. The body weights of the mice were measured before treatment and then every 2 days until the end of the study. Food consumption was measured every 2 days after treatment started and averaged to give a daily measurement. Grams of nonpurified diet consumed were multiplied by 2.85 to give caloric intake in tissue per hour.

Acute effect of AOD-9401 on fat oxidation in vivo. The acute effect of AOD-9401 on the rates of fat and glucose oxidation was assessed at the conclusion of the 30-day oral treatment period in the obese ob/ob mice after food intake, physical activity, and resting energy expenditure studies had been completed. On the morning of the last day of the study, a group of three saline-treated mice and four AOD-9401-treated mice were food-deprived for 1 h; then basal fat oxidation, glucose oxidation, and energy expenditure were measured for 10 min with the indirect calorimetry procedure described previously. The mice were then given an intraperitoneal injection of saline (in the saline-treated group) or AOD-9401 (250 μg/kg in the AOD-treated group), and rates of fat oxidation, glucose oxidation, and energy expenditure were measured for a further 18 min.

Isolation of adipose tissues. Groups of mice were killed with a lethal dose of pentobarbitone (0.2 ml) 24 h after the last treatment with oral AOD-9401 on day 30. Energy expenditure measurements after the intraperitoneal AOD-9401 dosing were not conducted on these mice. Epididymal fat pads from male mice were isolated as in our previous studies (14). The tissues were washed in room-temperature saline before being weighed into ~200-kg pieces for ex vivo assays. For in vitro assays, male C57BL/6J (ob/ob) mice were used. Microsomal rates (200–300 mg 12 wk of age) were used to assess adipose tissue fat oxidation rates in vitro in response to AOD-9401. Human subcutaneous abdominal adipose tissue was obtained with consent from an overweight female patient (age: 42 yr; body mass index: 28.4) who had no other known medical complications and who had undergone fat-reduction surgery for cosmetic reasons.

Assay for lipogenic activity in adipose tissue. The rate of incorporation of exogenous [14C]glucose into total lipid in adipose tissue was used as an index of lipogenic activity. Tissues were placed in 2 ml of Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing 2% defatted BSA and 0.1 mg/ml glucose and then gassed with 95% O2-5% CO2 in a shaking water bath, with temperature controlled at 37°C. After 30 min of preincubation, the tissues were transferred to another 2 ml of fresh medium containing [14C]glucose (final specific activity of 0.05 μCi/μmol) for a further 90 min (same conditions as above). Tissues were then removed and rinsed thoroughly in saline, and lipid was extracted with a chloroform-methanol (2:1) mixture. 14C radioactivity was counted on a Wallace 1410 liquid scintillation counter (Turku, Finland).

The rates of total lipid synthesis were expressed as picomoles of glucose incorporated into fat per milligram of tissue per hour.

Assay for lipolytic activity in adipose tissue. The rate of lipolytic activity was measured by the release of glycerol into the incubation medium. Tissue pieces were placed in 2 ml KRB buffer with 2% BSA and 0.1 mg/ml glucose and incubated for 60 min (same conditions as above). The tissues were then removed and discarded, and the amount of glycerol present in the incubation medium was enzymatically assayed using glycerol phosphate oxidase reactions (Sigma Diagnostics, catalog no. GPO-337; St Louis, MO). Glycerol was determined with a spectrophotometer and converted to micromoles of glycerol released per gram of tissue per hour.

Plasma measurements. Blood samples were collected from the tail vein of anesthetized animals in capillary tubes after chronic treatment. Plasma was stored at −20°C until used. Plasma glucose was measured using a 2300 STAT glucose analyzer. Free fatty acids (FFAs) were determined by the method developed from Noma (15). Triglycerides (TGs) were measured with a kit according to the recommendations of the manufacturer (Sigma).

In vitro FAA oxidation assay. FAA oxidation was determined by measuring the converted [14C]O2 from [1,14C]palmitic acid (23). [14C]O2, a final product of β-oxidation of FFA, was trapped by hyamine hydroxide and measured by a liquid scintillation
counter. Adipose tissues removed from laboratory animals were sliced into segments of ~200 mg each. The tissues were placed in 25-ml vials containing 2 ml of Krebs-Ringer phosphate buffer and 4% defatted BSA and then were preincubated at 37°C for 30 min under an atmosphere of carbogen (95% O₂-5% CO₂). Tissues were then transferred to Konte flasks containing fresh incubation medium, with 0.15 mM sodium palmitate and 0.20 μCi/μmol of 14C specific activity and different concentrations of hGH-(177–191) peptide. A filter paper roll was placed in a well inside the flask and then was sealed with a rubber septum stopper. Flasks were incubated at 37°C for 1 h, and the reaction was terminated by injecting 250 μl of 4.5 M H₂SO₄ with a needle through the rubber septum into the medium of a flask. Hyamine hydroxide (250 μl) was then injected into the filter paper roll in the center well. Incubation proceeded for another 60 min to ensure the complete absorption of released [14C]O₂ by the paper roll. The filter paper rolls were then carefully removed and transferred to scintillation vials, and the 14C radioactivity was measured by a liquid scintillation counter. The rate of [14C]palmitic acid oxidation to [14C]O₂ was calculated and expressed as micromoles per gram of tissue per hour.

Statistical analysis. The Student’s t-test was used to analyze the results. All data are expressed as means ± SE. P values of <0.05 were accepted as statistically significant.

RESULTS

Molecular stability of AOD-9401. Synthetic AOD-9401 was stable in the aqueous KRB buffer at pH 7.4. Less than 5% of the analog was found degraded after 16-h incubation at 37°C (data not shown). The half-life (t₁/₂) of AOD-9401 was ~50 and 170 min in enzymic digestion with pepsin and trypsin, respectively (Fig. 1). These t₁/₂ values indicate that the structure of the peptide was reasonably stable in the presence of trypsin and pepsin, the two major gastrointestinal enzymes in the digestive tract of the body. These findings are supported by our observation that AOD-9401 is detected in rat plasma 30 min after oral administration to animals (unpublished data).

In vivo effect of AOD-9401 on energy balance in obese (ob/ob) mice. Figure 2 shows the change in body weight after mice were orally treated with AOD-9401 or saline for 30 days. The rate of weight gain was 58% lower in mice treated with AOD-9401 from day 16 of treatment onward (0.33 to 0.14 g/day, P < 0.05). This is the first time that oral administration of an hGH peptide fragment was shown to reduce body weight gain. Body weights for control animals started at 44.5 ± 2.3 g on day 0 and finished at 54.7 ± 1.8 g after 30 days of saline treatment (10.2 g). In contrast, AOD-9401-treated animals weighed 47.8 ± 0.9 g on day 0 and at the conclusion of treatment weighed 52.5 ± 0.6 g (4.7 g).

The reduction in body weight gain in the AOD-9401-treated obese mice was not due to a decrease in food intake. Figure 3 shows that the average daily caloric intake from day 2 to day 30 was the same in the saline- and AOD-9401-treated groups. Nor did the reduction in body weight gain correlate with increased resting energy expenditure measured 2 h after gavage treatment (0.00525 ± 0.00028 vs. 0.00518 ± 0.00042 kcal/min in AOD-9401- and saline-treated mice, respectively). Resting energy expenditure was the same in AOD-9401- and saline-treated mice even when normalized for body weight (0.103 ± 0.004 vs. 0.101 ± 0.009 kcal·min⁻¹·kg⁻¹ in AOD-9401- and saline-treated mice, respectively). AOD-9401 did not increase running wheel activity in obese ob/ob mice (data not shown). The mouse strain is very inactive compared with other strains of mice and remains so even after AOD-9401 treatment.
Ex vivo effect of AOD-9401 on lipogenesis and lipolysis. The data in Fig. 4A show that AOD-9401 increased the lipolytic rate from $0.63 \pm 0.20 \mu \text{mol g tissue}^{-1} \cdot \text{h}^{-1}$ in control animals to $1.02 \pm 0.25 \mu \text{mol g tissue}^{-1} \cdot \text{h}^{-1}$ ($P < 0.001$) as measured by the rate of glycerol release. The lipogenic activity of the tissue (Fig. 4B) was conversely inhibited by 22% ($4.23 \pm 0.08$ vs. $3.31 \pm 0.2$ pmol mg tissue$^{-1} \cdot \text{h}^{-1}$, $P < 0.0025$) after 30 days of oral administration of the compound as measured by the incorporation of $[14\text{C}]$glucose into lipid. These alterations in lipid metabolism are consistent with the observed decrease in cumulative body weight gain of the treated animals.

In addition to these observations, effects on plasma metabolites were also noted (Table 1). Results indicate that, after chronic administration of AOD-9401, a significant increase in plasma FFAs can be recorded. Levels of glucose and TGs are slightly lower in treated animals but are not significantly different from controls.

Acute effect of AOD-9401 on in vivo fat oxidation. An acute dose of AOD-9401 can markedly increase fat oxidation in vivo in obese ob/ob mice. Figure 5 shows the effect of a single intraperitoneal injection of AOD-9401 or saline on the rates of energy expenditure, fat, and glucose oxidation (Fig. 5, A, B, and C, respectively). After 18 min, AOD-9401 increased energy expenditure 45% above basal ($P < 0.02$) and increased fat oxidation 83% above basal values ($P < 0.02$). It also increased glucose oxidation 2.4-fold after 9 min ($P < 0.05$); however, this effect disappeared 15 min after the AOD-9401 injection. In contrast, an intraperitoneal dose of saline had no significant effect on either energy expenditure or fat or glucose oxidation in the obese ob/ob mice.

In vitro effect of AOD-9401 on lipogenesis, lipolysis, and fat oxidation. The effect of AOD-9401 on lipogenesis and lipolysis is also evident in vitro. Figure 6 shows a clear dose-dependent effect of AOD-9401 on lipogenesis (Fig. 6A) and lipolysis (Fig. 6B). Peripheral epididymal adipose tissue from male C57BL/6J (ob/ob) mice was incubated in the presence of AOD-9401 for 1 h.

The effect of AOD-9401 on fat oxidation was also measured, this time in obese male Zucker rats. AOD-9401 acted dose dependently to increase the rate of fat oxidation (Fig. 7), with a significant 25% increase in fat oxidation.

Table 1. Effects of AOD-9401 treatment on plasma glucose and lipids

<table>
<thead>
<tr>
<th></th>
<th>Glucose, mg/dl</th>
<th>FFA, mmol/l</th>
<th>TG, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>381.6 ± 3.2</td>
<td>1.0 ± 0.04</td>
<td>343.2 ± 5.5</td>
</tr>
<tr>
<td>AOD-9401, 500 µg kg$^{-1}$ day$^{-1}$</td>
<td>366.85 ± 5.96</td>
<td>1.38 ± 0.04*</td>
<td>326.85 ± 3.65</td>
</tr>
</tbody>
</table>

Values of plasma glucose and lipids are shown here as means ± SE of control ($n = 5$) and AOD-9401 treated ($n = 7$) ob/ob mice after chronic oral administration (*$P < 0.005$). FFA, free fatty acid; TG, triglyceride.
oxidation observed at concentrations of 1.0 μM AOD-9401 (P < 0.05). Maximal fat oxidation rates appeared to be achieved at 3.0 μM.

In vitro effect of AOD-9401 on human adipose tissue. AOD-9401 and synthetic analogs have the potential to be developed for therapeutic applications, including management of human obesity. Thus, we also evaluated the effect of AOD-9401 on human subcutaneous adipose tissue. Table 2 indicates that AOD-9401 enhanced lipolysis in human adipose tissue, resulting in a threefold increase in glycerol release, and decreased lipogenic activity by 50%, similar to our in vitro findings in ob/ob mouse tissue.

DISCUSSION

Administration of exogenous GH to GH-deficient human subjects and experimental animals affects body composition by stimulating lipid mobilization and energy expenditure (16, 22). However, hGH is not a potentially useful drug for treating obesity, because prolonged use results in several adverse side effects, namely glucose intolerance, insulin resistance, edema, and hypertension (2, 3). There is strong evidence implicating the effects of hGH to specific fragments of its sequence (22). There is therefore the potential of developing a peptide analog of hGH that modifies body composition without the adverse effects of the intact molecule. We have synthesized a carboxy-terminal fragment of the hGH molecule [hGH(177–191)] that appears to regulate lipid metabolism (12) but lacks the

Fig. 5. Measurements of basal energy expenditure (A), fat oxidation (B), and glucose oxidation (C) before and after ip injection of AOD-9401 (250 μg/kg, n = 4) or saline (n = 3) in obese (ob/ob) mice. Results are expressed as means ± SE (*P < 0.05).

Fig. 6. In vitro lipogenesis (A) and lipolysis (B) in epididymal adipose tissue from obese (ob/ob) mice in the presence of saline (control) or various concentrations of AOD-9401. Each bar represents means ± SE of 6 determinations (*P < 0.05).
E506  EFFECT OF hGH FRAGMENT ON LIPID METABOLISM

Fig. 7. In vitro rates of fat oxidation in male Zucker rat epididymal adipose tissue incubated in the presence of various concentrations of AOD-9401. Bars express means ± SE of 6 determinations. (*P < 0.05).

hGH fragment hGH-(1–43) that is associated with diabetes (13). Glucose clamp experiments from our laboratory have also indicated that AOD-9401 does not induce glucose intolerance, as shown by euglycemic clamp experiments (M. A. Heffernan, M. J. Waters and F. M. Ng, unpublished observations). Our previous studies have shown that intraperitoneal AOD-9401 treatment is capable of inducing weight loss in obese mice (30). The present study extends this work by demonstrating that the peptide is as effective when given orally, reducing the rate of weight gain in obese ob/ob mice by 58%. This has significant implications for its potential use as a therapeutic agent. Enzyme digestion data presented in this study suggest that AOD-9401 is relatively resistant to enzymatic degradation in the gastrointestinal (GI) tract and is available for absorption across the GI tract and into the bloodstream.

Interestingly, the lower weight gain after 30 days of oral treatment with AOD-9401 was not due to reduced energy intake. Nor did there appear to be an increase in resting energy expenditure or voluntary physical activity that was associated with this lower weight gain. There are several possible reasons for this. First, even a small alteration in the metabolic efficiency of an organism may result in weight loss or gain (27), and it is possible that the method we used could not detect these small changes. Studies in our laboratory, for example, indicate that, for a single mouse, the day-to-day coefficient of variation in food intake is substantial (23%). In addition, measuring energy expenditure in mice that have been deprived of food for 2 h after gavage with AOD-9401 may not have been the optimal time to look for changes in energy expenditure. AOD-9401 may increase diet-induced thermogenesis or nocturnal energy expenditure, which we did not measure in this study. We could, however, detect increases in energy expenditure, fat oxidation, and glucose oxidation in the ob/ob mice after acute intraperitoneal ad-

ministration of AOD-9401, which means that this peptide is capable of altering metabolic efficiency. The profound effect of AOD-9401 on fat oxidation was also illustrated in vitro in epididymal fat tissue isolated from obese Zucker rats.

The fact that AOD-9401 is able to increase fat oxidation in vivo and in vitro, and is able to increase lipolysis and reduce lipogenesis ex vivo as well as in vitro, makes it likely that AOD-9401 acts directly on adipose tissue. It must also be noted that an increase in circulating FFAs is difficult to explain; however, many acute factors may induce such transient metabolic changes. Plasma levels of glucose and TGs are slightly lower in treated animals compared with controls, but not significantly different. This may indicate that AOD-9401 can somehow improve glucose tolerance in hyperglycemic mice. A slight reduction in the TG level may be ascribed to the normalization of hyperphagia and reduction in activity of lipogenic enzymes involved in lipid reesterification.

Whether central administration of AOD-9401 is also capable of influencing peripheral tissue lipid metabolism has not yet been investigated. The mechanism of action of AOD-9401 is not known but is currently being examined in our laboratory. hGH is thought to increase lipolysis by increasing hormone-sensitive lipase expression and phosphorylation (4a), increasing β3-adrenergic receptor level (27), inhibiting Gi protein (5), and/or preventing insulin-induced antilipolysis (20). Previous work in our laboratory has shown that the increase in lipolysis induced by AOD-9401 is also associated with an increase in activity of the lipolytic enzyme hormone-sensitive lipase in adipose tissue (9). In addition, we have shown that the reduction in lipogenesis is associated with a reduction in activity of the lipogenic enzyme acetyl-CoA carboxylase (12). Identifying the receptor that AOD-9401 binds to would help to decipher the mode of action of AOD-9401. It is possible that the hGH receptor is not involved, because other peptide fragments isolated from hGH [hGH-(95–133) and hGH-(108–129)] appear to work in a receptor-mediated fashion distinct from the hGH receptor to induce mitogenic activity in 3T3-F442A fibroblasts (8, 25). Whatever the site and mode of action, the fact that AOD-9401 influences lipid metabolism when orally ad-

Table 2. In vitro lipolysis and antilipogenesis in human subcutaneous adipose tissue by AOD-9401

<table>
<thead>
<tr>
<th>Concentration of AOD-9401 (μM)</th>
<th>Lipolysis, μmol glycerol/g tissue -1h -1</th>
<th>Lipogenesis, pmol mg tissue -1h -1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.52 ± 0.03</td>
<td>4.98 ± 0.34</td>
</tr>
<tr>
<td>AOD-9401 (0.1 μM)</td>
<td>1.39 ± 0.34 (+167%)*</td>
<td>2.74 ± 0.32 (-45%)*</td>
</tr>
<tr>
<td>AOD-9401 (1.0 μM)</td>
<td>1.22 ± 0.19 (+134%)*</td>
<td>3.01 ± 0.17 (-40%)*</td>
</tr>
</tbody>
</table>

In vitro lipolysis and antilipogenesis induced with two concentrations of AOD-9401 in obese human subcutaneous adipose tissue. Tissue was obtained from abdominoplasty surgery and was incubated in the presence of AOD-9401 at various concentrations for 1 h. Lipolysis and lipogenesis were determined. Results represent means ± SE of 4 samples in each group (with % change), isolated from a single obese human (*P < 0.05).
ministered and is capable of increasing lipolysis and decreasing lipogenesis in human adipose tissue means that it is worthwhile pursuing this peptide as a possible treatment for human obesity.

It is possible that AOD-9401 is a naturally occurring peptide that exists in the circulation. Wroblewski et al. (29) have shown that proteolytically cleaved two-chain forms of hGH do exist in vivo, which together may account for much of the activity of hGH. Such protein fragments can persist longer in the circulation than hGH and have led to the hypothesis that hGH may behave as a prohormone, which requires proteolytic cleavage to induce the diversity of effects observed. The existence of such peptides, which may or may not share the biological outcomes of hGH, helps to explain the diversity of action of hGH.

Because conventional dietary modification and physical exercise programs are usually unable to maintain weight loss in the long term, there is an urgent need to develop safe, effective pharmacotherapy to treat human obesity. Although further studies are required, this study suggests that AOD-9401 given orally may be useful in the treatment of obesity because of its ability to increase fat oxidation and lipolysis and decrease lipogenesis in adipose tissue.

This study was supported by Special Research Grants to F. M. Ng from Monash University, and by Metabolic Pharmaceuticals Limited, Australia. M. A. Heffernan is a recipient of an Australian Postgraduate Research Scholarship.

REFERENCES


