(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 January 2003 (23.01.2003)

PCT

(10) International Publication Number
WO 03/006051 A1

(51) International Patent Classification[7]: A61K 38/18

(21) International Application Number: PCT/US02/22747

(22) International Filing Date: 15 July 2002 (15.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/305,284 13 July 2001 (13.07.2001) US

(71) Applicant: ZYMOGENETICS, INC. [US/US]; 1201 Eastlake Avenue East, Seattle, Wa 98102 (US).

(72) Inventors: KELLY, James, D.; 3330 West Mercer Way, Mercer Island, WA 98040 (US). WEBSTER, Philippa, J.; 5823 17th Avenue NE, Seattle, WA 98105 (US).

(74) Agent: LUNN, Paul, G.; ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seatle, WA 98102 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/006051 A1

(54) Title: USE OF CORTICOTROPH-DERIVED GLYCOPROTEIN HORMONE TO INDUCE LIPOLYSIS

(57) Abstract: The use of corticotroph-derived glycoprotein hormone (CGH) to induce lipolysis, treat obesity, insulin resistance, and type II diabetes is described.

USE OF CORTICOTROPH-DERIVED GLYCOPROTEIN HORMONE TO INDUCE LIPOLYSIS

5

FIELD OF THE INVENTION

The present invention relates to the treatment of obesity. More particularly, the invention relates to the use of corticotroph-derived glycoprotein hormone (CGH) to
10 stimulate lipolysis for the treatment of obesity and diabetes.

BACKGROUND OF THE INVENTION

The teachings of all of the references cited herein are incorporated in their
15 entirety herein by reference.

Obesity is a public health problem, which is both serious and widespread. One third of the population in industrialized countries has an excess weight of at least 20% relative to the ideal weight. This phenomenon has spread to the developing world, particularly to the regions of the globe where economies are modernizing. As of the
20 year 2000, there were an estimated 300 million obese people worldwide.

Obesity considerably increases the risk of developing cardiovascular or metabolic diseases. For an excess weight greater than 30%, the incidence of coronary diseases is doubled in subjects under 50 years of age. Studies carried out for other diseases are equally revealing. For an excess weight of 20%, the risk of high blood
25 pressure is doubled. For an excess weight of 30%, the risk of developing non-insulin dependent diabetes is tripled, and the incidence of dyslipidemia increased six fold. The list of additional diseases promoted by obesity is long; abnormalities in hepatic function, digestive pathologies, certain cancers, and psychological disorders are prominent among them.

30 Treatments for obesity include restriction of caloric intake, and increased caloric expenditure through physical exercise. However, the treatment of obesity by dieting, although effective in the short-term, suffers from an extremely high rate of recidivism. Treatment with exercise has been shown to be relatively ineffective when applied in the absence of dieting. Other treatments include gastrointestinal surgery or agents that limit
35 the absorption of dietary lipids. These strategies have been largely unsuccessful due to side-effects of their use.

Clearly there remains a need for novel treatments that are useful for reducing body weight in humans. Therapies that can be administered to promote lipolysis and weight loss would help to control obesity and thereby alleviate many of the negative consequences associated with this condition.

5

DESCRIPTION OF THE INVENTION

Introduction

The present invention fills the need for a novel therapy to promote weight loss.

10 The present invention is comprised of administering corticotroph-derived glycoprotein hormone (CGH) to an individual to promote weight loss and in particular to promote lipolysis. The present invention is further comprised of a method for treating type-2 diabetes in an individual comprising administering a pharmaceutically effective amount of CGH to said individual. In another embodiment the present invention is

15 comprised of a method for improving insulin sensitivity in an individual comprising administering a pharmaceutically effective amount of CGH to said individual.

Herein we disclose methods that are useful for the treatment of obesity. As described below, the ability to stimulate lipolysis in adipose tissue provides a means of intervening in a wide number of pathologies associated with obesity. In particular, we

20 have discovered that CGH, when administered *in vitro* or *in vivo*, stimulates lipolysis. As a consequence, metabolic rate is increased, leading to decreased weight and increased insulin sensitivity.

When used to promote lipolysis, CGH can promote weight loss. The invented composition and methods are useful for treating conditions that include: obesity,

25 atherosclerosis associated with obesity, diabetes, hypertension associated with obesity or diabetes, or more generally the various pathologies associated with obesity.

In another aspect of the invention, this agent can be used for the maintenance of weight loss in individuals treated with other medicaments that induce weight loss.

A preferred embodiment of the invention is the treatment of non-insulin

30 dependent diabetes, especially that associated with obesity. In one embodiment, the use of CGH to treat non-insulin dependent diabetes is envisioned in non-obese individuals.

Yet another aspect of the invention relates to the use of CGH to increase resting metabolic rate in individuals. In one embodiment of this aspect, individuals with low resting metabolic rate are administered CGH to promote lipolysis and increase energy

35 utilization.

Definitions and Terms

One aspect of the invention is the use of the novel glycoprotein hormone CGH to stimulate lipolysis. CGH is disclosed in International Patent Application No. PCT/US01/09999, publication no. WO 01/73034. It is comprised of an alpha subunit, glycoprotein hormone alpha2 (GPHA2), and a beta subunit, glycoprotein hormone beta 5 (GPHB5). GPHA2 was previously called Zsig51 (International Patent Application No. PCT/US99/03104, publication no. WO 99/41377 published August 19, 1999). SEQ ID NO: 1 is the human cDNA sequence that encodes the full-length polypeptide GPHA2, and SEQ ID NO:2 is the full-length polypeptide sequence of human GPHA2. SEQ ID NO:3 is the mature GPHA2 polypeptide sequence without the signal sequence. SEQ ID NO: 4 is the human cDNA sequence that encodes the full-length GPHB5 polypeptide. SEQ ID NO: 5 is the full-length GPHB5 polypeptide. SEQ ID NO: 6 is the mature GPHB5 polypeptide without the signal sequence. SEQ ID NO: 7 is the human genomic DNA sequence that encodes the full-length GPHB5 polypeptide.

The present invention relates generally to methods that are useful for stimulating lipolysis in adipose tissue. Those having ordinary skill in the art will understand that lipolysis is the biochemical process by which stored fats in the form of triglycerides are released from fat cells as individual free fatty acids into the circulation. Stimulation of lipolysis has been clearly linked to increased energy expenditure in humans, and several strategies to promote lipolysis and increase oxidation of lipids have been investigated to promote weight loss and treat the diabetic state associated with obesity. These therapeutic efforts primarily focus on creating compounds that stimulate the sympathetic nervous system (SNS) through its peripheral β-adrenoreceptors. The discovery of CGH-promoted lipolysis in adipose tissue presents a novel and specific method of treating obesity, and the insulin-resistant diabetic state associated with obesity.

As used herein, the terms "obesity" and "obesity-related" are used to refer to individuals having a body mass which is measurably greater than ideal for their height and frame. Preferably these terms refer to individuals with body mass index values of greater than 20, more preferably with body mass index values of greater than 30, and most preferably with body mass index greater than 40.

Overview

Energy expenditure represents one side of the energy balance equation. In order to maintain stable weight, energy expenditure should be in equilibrium with energy intake. Considerable efforts have been made to manipulate energy intake (*i.e.*, diet and appetite) as a means of maintaining or losing weight; however, despite enormous sums of money devoted to these approaches, they have been largely unsuccessful. There have

also been efforts to increase energy expenditure pharmacologically as a means of managing weight control and treating obesity. Increasing energy metabolism is an attractive therapeutic approach because it has the potential of allowing affected individuals to maintain food intake at normal levels. Further, there is evidence to
5 support the view that increases in energy expenditure due to pharmacological means are not fully counteracted by corresponding increases in energy intake and appetite. See Bray, G. A. (1991) *Annu Rev Med* **42**, 205-216.

Energy expenditure can be stimulated pharmacologically by manipulation of the central nervous system, by activation of the peripheral efferents of the SNS, or by
10 increasing thyroid hormone levels. Much of the energy expended on a daily basis derives from resting metabolic rate (RMR), which comprises 50-80% of the total daily energy expenditure. For a review, see Astrup, A. (2000) *Endocrine* **13**, 207-212. Noradrenaline turnover studies have shown that most of the variability in RMR unexplained by body size and composition is related to differences in SNS activity,
15 suggesting that SNS activity does modulate RMR. See Snitker, S., et al. (2001) *Obes. Rev.* **1**, 5-15. Meal ingestion is accompanied by increased SNS activity, and studies have demonstrated that increased SNS activity in response to a meal accounts for at least part of meal-induced thermogenesis.

The peripheral targets of the SNS involved in the regulation of energy
20 utilization are the β-adrenoreceptors (β-AR's). These receptors are coupled to the second messenger cyclic adenosine monophosphate (cAMP). Elevation of cAMP levels leads to activation of protein kinase A (PKA), a multi-potent protein kinase and transcription factor eliciting diverse cellular effects. See Bourne, H. R., et al. (1991) *Nature* **349**, 117-127. Adipose tissue is highly enervated by the SNS, and possesses
25 three known subtypes of β-adrenoreceptors, β_1-, β_2-, and β_3-AR. Activation of the SNS stimulates energy expenditure via coupling of these receptors to lipolysis and fat oxidation. Increased serum free fatty acids (FFAs) produced by adipose tissue and released into the bloodstream stimulate energy expenditure and increase thermogenesis. For a review, see Astrup, A. (2000) *Endocrine* **13**, 207-212. In addition, elevated PKA
30 levels increase energy utilization in fat by up-regulating uncoupling protein-1 (UCP-1), which creates a futile cycle in mitochondria, generating waste heat.

Over the past two decades, investigation of the physiological benefits of SNS activation for the treatment of obesity and diabetes related to obesity has centered on pharmacological activation of the β_3-AR. Expression of the β_3-AR is restricted to a
35 narrower range of tissues than the β_1 or β_2 isoforms, and is highly expressed in rodent adipose tissue compared to the other isoforms. Experimental work in rodents treated with β_3-AR agonists has demonstrated that stimulation of lipolysis and fat oxidation

produces increased energy expenditure, weight loss, and increased insulin sensitivity. See de Souza, C. J. and Burkey, B. F. (2001) *Curr Pharm Des* **7**, 1433-1449. The potential benefits of these compounds have not been not realized, however, due to their lack of efficacy at the human β_3-AR. Further, it was only subsequently realized that the
5 levels of β_3-AR in rodent adipose tissue are much higher than in human adipose tissue. In human adipose tissue, the β_1 and β_2 isoforms represent the predominant adrenoreceptor isoforms. See Arch, J. R. (2002) *Eur J Pharmacol* **440**, 99-107. Thus, although the biochemical premise of stimulation of lipolysis for treatment of obesity has been clearly demonstrated, the mechanism for therapeutically producing the
10 corresponding effects in humans is unrealized.

Strategies to promote lipid oxidation through lipolysis have demonstrated improved insulin sensitivity at doses that do not promote weight loss, and over time periods that do not affect body weight. It is not suprising that an insulin-sensitizing effect is more readily detectable than an anti-obesity effect. Stimulation of fat oxidation
15 may rapidly lower the intracellular concentration of metabolites that modulate insulin signaling. The anti-obesity effect, by contrast, must develop gradually as large stores of fat are oxidized.

BRIEF DESCRIPTION OF THE DRAWINGS

20

Figure 1. Dose response of CGH and isoproterenol-induced lipolysis in 3T3 L1 adipocytes. Glycerol (panel A) and FFA (panel B) accumulations were determined following a 4-hour treatment with CGH (solid squares) or isoproterenol (solid triangles) at the indicated concentrations.

25

Figure 2. Stimulation of lipolysis *in vivo* by CGH. Mice (n=4, each group) were injected IP with vehicle saline, CGH (300 μg/kg), or CL 316,243 (1mg/kg). Changes in serum glycerol (upper panel, A) and FFA (lower panel, B) over a 2-hour period as described in Example 3 are shown for each group.

30

<u>CGH Promotes Elevation of cAMP in Adipose Tissue</u>

CGH exerts its effects through interaction with the thyrotropin-stimulating hormone (TSH) receptor. See Nakabayashi, K., et al. (2002) *J Clin Invest* **109**, 1445-1452. The TSH receptor (TSHR) is a member of the G-protein coupled, seven
35 transmembrane receptor superfamily. Activation of the TSH receptor leads to coupling

with heterotrimeric G proteins, which evoke downstream cellular effects. The TSH receptor has been shown to interact with G proteins of subtypes G_s, G_q, G_{12}, and G_i. In particular, interaction with G_s leads to activation of adenyl cyclase and increased levels of cAMP. See Laugwitz, K. L., et al. (1996) *Proc Natl Acad Sci U S A* **93**, 116-120.

5 Although the presence of TSH receptors in adipose tissue has been the subject of controversy for some time, recent reports have documented the presence of TSHR in adipose tissue of humans and rodents. Se, Bell, A., et al. (2000) *Am J Physiol Cell Physiol* **279**, C335-340, and Endo, T., et al. (1995) *J Biol Chem* **270**, 10833-10837.

Example 1 demonstrates the production of elevated cAMP by CGH in cultured
10 murine 3T3-L1 adipocytes and in primary human adipocytes. We have discovered that CGH produces activation of a luciferase reporter gene construct under the control of cAMP response element (CRE) enhancer sequences. We typically observe a 15-40 fold induction of the luciferase reporter gene in response to CGH treatment, indicating significant production of cAMP in adipocytes following activation of the TSHR. These
15 data suggest that CGH could be an important physiological regulator of adipose tissue lipolysis, which is primarily controlled by intracellular cAMP levels. For a review, see Astrup, A. (2000) *Endocrine* **13**, 207-212.

CGH Promotes Lipolysis in Adipocytes and Whole Animals

20 CGH was examined for its ability to activate lipolysis in cultured 3T3-L1 murine adipocytes. Following treatment of adipocytes for 4 hours, lipolysis was assessed by the accumulation of glycerol and FFA in the adipocyte culture medium. Treatment of adipocytes with 10 nM human recombinant CGH produced significantly elevated levels of extracellular glycerol and FFA. Example 2 compares the lipolytic
25 activity of CGH to isoproterenol, a non-specific β-adrenergic agonist. Maximal lipolysis achieved with CGH is at least 50% of that produced by isoproterenol. Lipolysis was significantly stimulated by CGH at concentrations of 0.1 nM, indicating that CGH is a potent regulator of lipolysis in adipocytes.

CGH also produced elevations in serum glycerol and FFA following IP injection
30 into mice. As described in example 3, mice were fasted overnight before IP injection of either CGH (300 μg/kg), β3-AR agonist CL 316,243 (1 mg/kg), or vehicle saline. Serum was withdrawn before injection, or 2 hours post-injection. Although the vehicle controls showed decreases in serum glycerol and FFA levels, the animals treated with CGH showed significant elevations in both, indicating that CGH is a potent stimulator
35 of lipolysis in vivo.

Advantages of CGH as a Lipolysis Stimulating Agent

CGH presents a novel method of producing lipolysis and increasing metabolic rate. Other strategies employed thus far have suffered from lack of specificity, such as β-AR agonists in general, or lack of efficacy, as for the most specific of the β_3-AR agonists developed thus far. Most of the agents investigated for human use have not exhibited sufficient selectivity and as a result, have produced increased blood pressure and heart rate due to activation of sympathetic pathways in tissues other than adipose. See Arch, J. R. (2002) *Eur J Pharmacol* **440**, 99-107.

In spite of the emphasis on development of β_3-AR specific agonists, recent human studies have implicated the β_1- and β_2-adrenoreceptors as the primary mediators of sympathetically induced thermogenesis and energy expenditure. Further, studies in human obese populations suggest that decreases in resting metabolic rate observed in these individuals are the result of impaired function of β_2-adrenoreceptors in adipose tissue. See Schiffelers, S. L., et al. (2001) *J Clin Endocrinol Metab* **86**, 2191-2199, and Blaak, E. E., et al. (1993) *Am J Physiol* **264**, E11-17. Thus, a novel mechanism of increasing lipolysis without invoking sympathetic enervation presents a unique opportunity for the treatment of obesity.

Other studies in human lean and obese subjects have found that increases in plasma FFA levels lead to similar increases in lipid oxidation and energy expenditure. These studies conclude that the accumulation of fat in obese subjects may be due to a defect in adipose tissue lipolysis rather than to defects in lipid utilization. See Schiffelers, S. L., et al. (2001) *Int J Obes Relat Metab Disord* **25**, 33-38.

Increased adipose lipolysis and the resulting decrease in adipocyte size are negatively correlated with insulin resistance in human cross-sectional studies. See Weyer, C., et al. (2000) *Diabetologia* **43**, 1498-1506. Thus a method for stimulating lipolysis and reducing adipocyte size is predicted to decrease the insulin-resistant diabetic state associated with obesity. The presence of significant numbers of CGH receptors in adipose tissue represents a novel method for the control of lipolysis and RMR in human obese populations.

Use of CGH to Treat Type-2 Diabetes

CGH can also be administered to treat type-2 diabetes mellitus (Type II DM). Type II DM is usually the type of diabetes that is diagnosed in patients older than 30 years of age, but it also occurs in children and adolescents. It is characterized clinically

by hyperglycemia and insulin resistance. Type II DM is commonly associated with obesity, especially of the upper body (visceral/abdominal), and often occurs after weight gain.

Type II DM is a heterogeneous group of disorders in which hyperglycemia results from both an impaired insulin secretory response to glucose and a decreased insulin effectiveness in stimulating glucose uptake by skeletal muscle and in restraining hepatic glucose production (insulin resistance). The resulting hyperglycemia may lead to other common conditions, such as obesity, hypertension, hyperlipidemia, and coronary artery disease.

CGH can be administered to an individual at dosages described below. CGH can also be administered in conjunction with insulin, and other diabetic drugs such as tolbutamide, chlorpropamide, acetohexamide, tolazamide, glyburide, glipizide, glimepiride, metformin, acarbose, troglitazone and repaglinide.

Formulations and Administration of CGH

CGH can be administered to a human patient, alone or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at therapeutically effective doeses to treat or ameliorate diseases associated with obesity and diabetes. Treatment dosages of CGH should be titrated to optimize safety and efficacy. Methods for administration include intravenous, intraperitoneal, rectal, intranasal, subcutaneous, and intramuscular. Pharmaceutically acceptable carriers will include water, saline, and buffers, to name just a few. Dosage ranges would ordinarily be expected from 0.1μg to 0.1mg per kilogram of body weight per day. A useful dose to try initially would be 25 μg/kg per day. However, the doses may be higher or lower as can be determined by a medical doctor with ordinary skill in the art. For a complete discussion of drug formulations and dosage ranges see *Remington's Pharmaceutical Sciences*, 17th Ed., (Mack Publishing Co., Easton, Penn., 1990), and *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 9th Ed. (Pergamon Press 1996).

Example 1

CGH Activation of 3T3 L1 Adipocytes and Human Adipocytes Results in cAMP Production

Summary

5 Differentiated murine 3T3 L1 adipocytes and primary human adipocytes were used to study signal transduction of CGH. 3T3 L1 fibroblasts were differentiated into adipocytes and the cells were transduced with recombinant adenovirus containing a reporter construct, a firefly luciferase gene under the control of cAMP response element (CRE) enhancer sequences. This assay system detects cAMP-mediated gene induction 10 downstream of activation of G_s-coupled G-protein coupled receptors (GPCR's). Treatment of the differentiated 3T3 L1 cells with isoproterenol, a β-adrenoreceptor agonist, resulted in elevation of cAMP levels and an 80-fold induction of luciferase expression. Treatment of differentiated 3T3 L1 cells with CGH also resulted in elevated cAMP levels and a 27-fold induction of luciferase expression. In a separate 15 experiment, undifferentiated 3T3 L1 fibroblasts were transduced with the recombinant adenovirus. Treatment of the fibroblasts with CGH did not result in an increase in reporter gene induction. In another experiment, human primary adipocytes were also transduced with the recombinant adenovirus containing a reporter construct. Treatment of the human adipocytes with isoproterenol produced a 17-fold induction of luciferase 20 expression. Treatment of the human adipocytes with CGH resulted in a 14-fold induction of the reporter gene. These results demonstrate CGH signaling through a GPCR in murine adipocytes and human adipocytes, and the production of cAMP levels similar to those achieved through β-adrenoreceptor stimulation.

25 ***Experimental Procedure***

3T3 L1 cells were obtained from the ATCC (CL-173) and cultured in growth medium as follows: the cells were propagated in DMEM high glucose (Life Technologies, cat. # 11965-092) containing 10% bovine calf serum (JRH Biosciences, cat. # 12133-78P). Cells were cultured at 37°C in an 8% CO_2 humidified incubator. Cells were seeded to 30 collagen-coated 96-well plates (Becton Dickinson, cat. # 356407) at a density of 5,000 cells per well. Two days later, differentiation medium was added as follows: DMEM high glucose containing 10% fetal bovine serum (Hyclone, cat. # SH30071), 1 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-methyl xanthine (ICN, cat. #195262). The cells were incubated at 37°C in 8% CO_2 for 4 days and the medium 35 replaced with DMEM high glucose containing 10% fetal bovine serum and 1 μg/ml insulin. The cells were incubated at 37°C in 8% CO_2 for 3 days, then the medium was

replaced with DMEM high glucose containing 10% fetal bovine serum. The cells were incubated at 37°C in 8% CO_2 for 3 days, and the medium was replaced with DMEM low glucose (Life Technologies, cat. # 12387-015) containing 10% fetal bovine serum. The day before the assay, the cells were rinsed with F12 Ham (Life Technologies, cat. # 12396-016) containing 2 mM L-glutamine (Life Technologies, cat. # 25030-149), 0.5% bovine albumin fraction V (Life Technologies, cat. # 15260-037), 1 mM MEM sodium pyruvate (Life Technologies, cat. # 11360-070), and 20 mM HEPES. Cells were transduced with AV KZ55, an adenovirus vector containing KZ55, a CRE-driven luciferase reporter cassette, at 5,000 particles per cell. Following overnight incubation, the cells were rinsed once with assay medium (F12 HAM containing 0.5% bovine albumin fraction V, 2 mM L-glutamine, 1 mM sodium pyruvate, and 20 mM HEPES). 50 μl of assay medium were added to each well followed by 50 μl of 2X concentrated test protein. The plate was incubated at 37°C at 5% CO_2 for 4 hours. Medium was removed from the plate and the cells were lysed with 25 μl per well of 1X cell culture lysis reagent supplied in a luciferase assay kit (Promega, cat. # E4530). The cells were incubated at room temperature for 15 minutes. Luciferase activity was measured on a microplate luminometer (PerkinElmer Life Sciences, Inc., model LB 96V2R) following automated injection of 40 μl of luciferase assay substrate into each well. The method described above, with modifications, was also used to test CGH and isoproterenol on human adipocytes obtained from Stratagene (cat. # 937236) seeded in 96-well plates. Human adipocytes were rinsed once with basal medium (Stratagene, cat. # 220002) containing 0.5% bovine albumin fraction V, then transduced with AV KZ55 at 5,000 particles per cell. Following overnight incubation, the cells were rinsed once with assay medium comprised of basal medium containing 0.5% bovine albumin fraction V and assayed as described above.

Example 2
CGH-Induced Lipolysis in 3T3 L1 Adipocytes

Summary

3T3 L1 Adipocytes were treated with CGH and the non-specific β-adrenoreceptor agonist isoproterenol for 4 hours. Lipolysis was assessed by the accumulation of glycerol and FFAs in the conditioned medium. Figure 1 displays dose-response curves of CGH and isoproterenol for glycerol (panel A) and FFA (panel B). CGH potently stimulated lipolysis in the murine adipocytes, as shown in Figure 1.

Measurement of free fatty acids in conditioned media from differentiated 3T3 L1 Cells

Free fatty acids were measured using the Wako NEFA C kit for quantitative determination of non-esterified (or free) fatty acids with a modified protocol. Isoproterenol (ICN), a lipolysis-inducing positive control, was diluted to a starting concentration of 2 μM in assay medium (Life Technologies low glucose DMEM, 1mM sodium pyruvate, 2 mM L-glutamine, 20 mM HEPES, and 0.5% BSA). The isoproterenol was further diluted in half log serial dilutions. CGH was serially diluted down to 0.06 nM. Medium was removed from 3T3 L1 adipocytes in 96-well plates. 50 μl of assay medium were added to each well, followed by 50 μl of CGH or isoproterenol to each well. The plates were incubated for 4 hours at 37 degrees. 40 μl of conditioned medium were collected for glycerol assay analysis, and 40 μl of conditioned medium were collected for free fatty acid analysis. Oleic acid (Sigma) was dissolved in methanol and used as a reference for determining the amount of free fatty acids in the conditioned media. Wako reagents A and B were reconstituted to 4X the recommended concentration. Conditioned media samples were assayed in 96-well plates. 50 μl of Wako reagent A were added to 5 μl of oleic acid standard plus 40 μl of assay medium. 50 μl of Wako reagent A were added to 40 μl of conditioned medium from differentiated 3T3 L1 cells and 5 μl of methanol. The 96-well plates were incubated at 37° C for 10 minutes. 100 μl of Wako reagent B were added to each well. The 96-well plates were incubated at 37 degrees for 10 minutes. The 96-well plates were then allowed to sit at room temperature for 5 minutes. The 96-well plates were centrifuged in a Beckman Coulter Allegra 6R centrifuge at 3250Xg for 5 minutes to remove air bubbles. The absorbance at 530 nm was measured on the Wallac Victor2 Multilabel counter.

Measurement of glycerol in conditioned media from differentiated 3T3 L1 cells

Glycerol was measured in conditioned media using the Sigma Triglyceride (GPO-Trinder) kit with a modified protocol. Isoproterenol was diluted to a starting concentration of 2 μM. The isoproterenol was further diluted in half log serial dilutions. CGH was diluted to starting concentrations of 300 nM in assay medium. CGH was then serially diluted down to 0.06 nM. Medium was removed from 3T3 L1 adipocytes in 96-well plates. 50 μl of assay medium were added to each well, followed by 50 μl of CGH or isoproterenol to each well. The plates were incubated for 4 hours at 37 degrees. 40 μl of conditioned medium were collected for glycerol assay analysis, and 40 μl of conditioned medium were collected for free fatty acid analysis. The

glycerol standard was diluted in water to a range from 200 nmols/10 μl to 0.25 nmols/10 μl. Glycerol was used as a reference for determining the amount of glycerol in the conditioned media. Sigma reagent A was reconstituted to the recommended concentration. Conditioned media samples were assayed in 96-well plates. 150 μl of Sigma reagent A were added to 10 μl of glycerol standard plus 40 μl of assay medium. 150 μl of Sigma reagent A were added to 40 μl of conditioned medium from differentiated 3T3 L1 cells plus 10 μl of water. The 96-well plates were incubated for 15 minutes at room temperature. The 96-well plates were centrifuged in a Beckman Coulter Allegra 6R centrifuge at 3250Xg for 5 minutes to remove air bubbles. The absorbance at 530 nm was measured on the Wallac Victor2 Multilabel counter.

Example 3
Stimulation of Lipolysis by CGH in Vivo

Summary

CGH, the β_3-adrenoreceptor agonist CL 316,243 (CL), and saline vehicle were examined for stimulation of lipolysis in mice following an overnight fast. Mice (n=4) were bled immediately before IP injection of CGH (300 μg/kg), CL (1 mg/kg), or vehicle, and then sacrificed 2 hours later. Lipolysis was assessed as the percent change in serum glycerol or FFA over the 2 hour period. Figure 2 shows the changes in glycerol (upper panel) and FFA (lower panel) for the treatment groups. The serum glycerol and FFA for the vehicle groups decreased by 7% +/- 9% and 24% +/-15%, respectively. The serum glycerol for the CGH group increased by 57% +/-20%; p=0.0254, and the FFA levels increased 25% +/-5%; p=0.0188. The serum glycerol for the CL group increased 168% +/-23%; p=0.0004, and the FFA increased 82% +/-16%; p=0.0029.

Treatment Protocol

C57 BL/6 male mice, age 19 weeks, were grouped to normalize weight (n=4 for each treatment; average group weight = 37.8 g +/- 0.4 g). Mice were housed individually for 18 hours prior to treatment, at which time food was withdrawn, with free access to water given. At approximately 8 a.m., the subjects were anesthetized with halothane and blood samples taken by retro-orbital eye bleed. The blood was allowed to clot, and the serum was separated by centrifugation and frozen for later analysis. Test substances were administered by IP injection in a volume of 0.1 ml, and the animals replaced in

their cages for 2 hours with free access to water. At 2 hours, the mice were sacrificed and blood drawn by cardiac puncture.

Measurement of glycerol and FFA in murine serum

5 For measuring free fatty acids in serum, the method previously described for measuring free fatty acids in conditioned media was followed, with the following modifications. Wako reagents A and B were reconstituted to 2X the recommended concentration. 75 μl of Wako reagent A were added to 5 μl of oleic acid standard plus 5 μl of water. 75 μl of Wako reagent A were added to 5 μl of serum plus 5 μl of methanol (to mirror the 10 oleic acid standard conditions). The 96-well plates were incubated at 37degrees for 10 minutes. 150 μl of Wako reagent B were added to each well. The 96-well plates were incubated at 37° C for 10 minutes. The 96-well plates were allowed to sit at room temperature for 5 minutes. The 96-well plates were centrifuged in a Beckman Coulter Allegra 6R centrifuge at 3250Xg for 5 minutes to remove air bubbles. The absorbance 15 at 530 nm was measured on the Wallac Victor2 Multilabel counter.

For measuring glycerol in serum, the method previously described for measuring glycerol in conditioned media was followed, with the modifications described below. Sigma reagent A was reconstituted to 0.5X the recommended concentration. 200 μl of Sigma reagent A were added to 10 μl of glycerol standard. 200 μl of Sigma reagent A 20 were added to 5 μl of serum plus 5 μl of water. The 96-well plates were incubated for 15 minutes at room temperature. The 96-well plates were centrifuged in a Beckman Coulter Allegra 6R centrifuge at 3250Xg for 5 minutes to remove air bubbles. The absorbance at 530 nm was measured on the Wallac Victor2 Multilabel counter.

25

Example 4

Expression and purification of recombinant CGH

Summary

A Chinese Hamster Ovary (CHO) cell line overexpressing both GPHA2 and GPHB5, 30 the subunits of CGH, was generated and named CHO 180. CHO 180 was found to secrete active, heterodimeric CGH. CGH was purified from the supernatant of CHO 180 using standard biochemical techniques.

Generation of CHO 180

35 The CGH-producing cell line CHO 180 was generated in two stages. A construct expressing GPHA2, GPHB5 and drug resistance (dihydrofolate reductase) from the CMV promoter was transfected to protein-free CHO DG44 cells (PF CHO) by

electroporation. The resulting pool was selected and amplified using methotrexate. Early analysis indicated a high level of GPHA2 expression, but a low level of GPHB5 expression. Therefore, a second construct expressing GPHB5 from the CMV promoter and zeocin resistance from the SV-40 promoter was transfected into the selected, amplified pool by electroporation. After zeocin selection, the final pool (CHO 180) expressed significant levels of both GPHA2 and GPHB5; the proteins were secreted as the non-covalent heterodimer, CGH.

Purification of CGH from CHO culture supernatant

CGH was purified from CHO culture supernatant by established chromatographic procedures: first the CGH was captured on a strong cation exchanger, POROS HS50; next it was affinity purified using ConA Sepharose; and finally was polished and buffer-exchanged into PBS by Superdex 75 size exclusion chromatography.

Cation Exchange Chromatography

The CHO culture supernatant was 0.2 μm filtered and adjusted to pH 6 and 20 mM 2-Morpholinoethanesulfonic Acid (MES). The CGH in the adjusted supernatant was captured at 55 cm/hr using a 1:2 online dilution with 20 mM MES pH 6 onto a POROS HS 50 column that was previously equilibrated in 20 mM MES pH 6. After loading was complete, the column was washed with 20 column volumes (CV) of equilibration buffer. This was followed by a 3 CV wash with 250 mM NaCl in 20 mM MES pH 6 at 90 cm/hr. Next the CGH was eluted from the column with 3 CV of 500 mM NaCl in 20 mM MES pH 6 at the same flow rate. Finally the column was stripped with steps of 1M and 2M NaCl and then re-equilibrated with 20 mM MES pH 6. The 500 mM NaCl-eluted pool containing the CGH was adjusted with NaOH to pH 7.4 for the next step.

ConA Sepharose Chromatography

ConA Sepharose is Concanavalin A coupled to Sepharose. Concanavalin A is a lectin, which binds reversibly to molecules, which contain D-mannopyranosyl, D-glucopyranosyl and related residues. The adjusted pool of CGH from the cation exchange chromatography was applied directly at 2 cm/hr to the ConA column equilibrated in 20 mM Tris pH 7.4 containing 0.5 M NaCl. After loading, the column was washed with 20 CV of equilibration buffer. The CGH was then competed off the column at 1-2 cm/hr with 3 CV of 0.5M Methyl-D-Manno-Pyranoside in 20 mM Tris

pH 7.4. This CGH pool was concentrated via ultrafiltration using an Amicon stirred cell with a 5kDa-cutoff membrane.

Size-Exclusion Chromatography

5 The concentrated CGH ConA pool was then applied to an appropriately sized bed of Superdex 75 resin (i.e. ≤5% of bed volume) for removal of remaining HMW contaminants and for buffer exchange into PBS. The CGH eluted from the Superdex 75 column at about 0.65 to 0.7 CV and was concentrated for storage at –80 °C using the Amicon stirred cell with a 5kDa-cutoff ultrafiltration membrane. The heterodimeric 10 protein was pure by Coomassie-stained SDS PAGE, had the correct NH2 termini, the correct amino acid composition, and the correct mass by SEC MALS. The overall process recovery estimated by RP HPLC assay was 50-60%.

CLAIMS

We claim:

1. A method for inducing lipolysis in an individual comprising administering a pharmaceutically effective amount of corticotroph-derived glycoprotein hormone (CGH) to said individual, wherein CGH is a heterodimeric protein comprised of the polypeptides of SEQ ID NO:3 and SEQ ID NO:6.

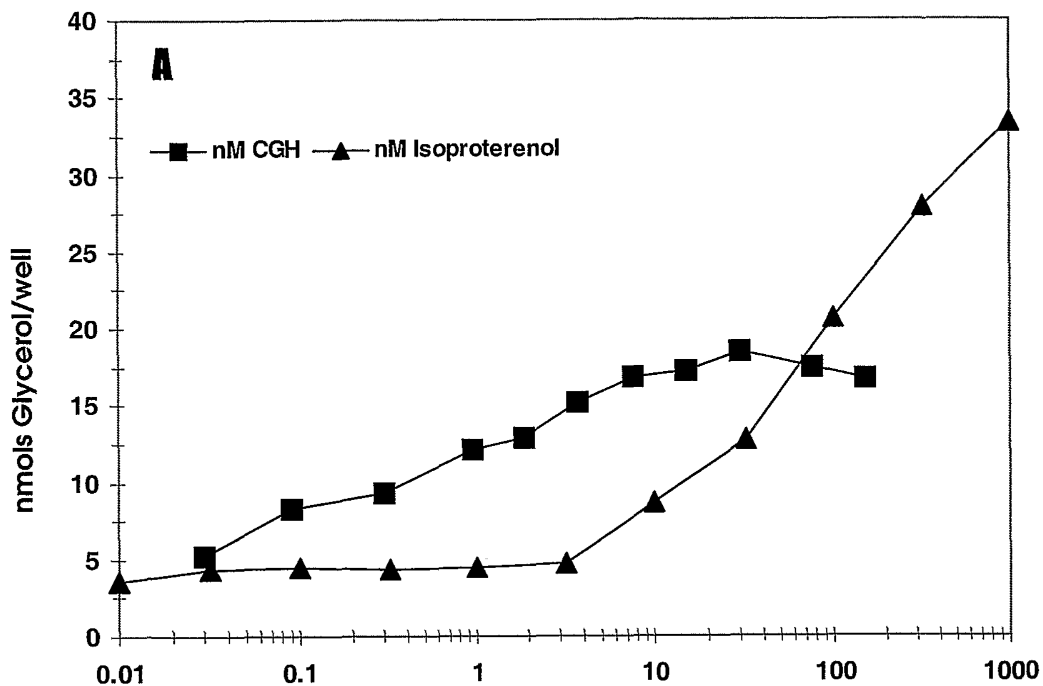
2. A method for inducing weight loss in an individual comprising administering a pharmaceutically effective amount of CGH to said individual.

3. A method for treating type-2 diabetes in an individual comprising administering a pharmaceutically effective amount of CGH to said individual.

4. A method for improving insulin sensitivity in an individual comprising administering a pharmaceutically effective amount of CGH to said individual.

5. The method of claim 4 wherein said individual is obese.

Figure 1



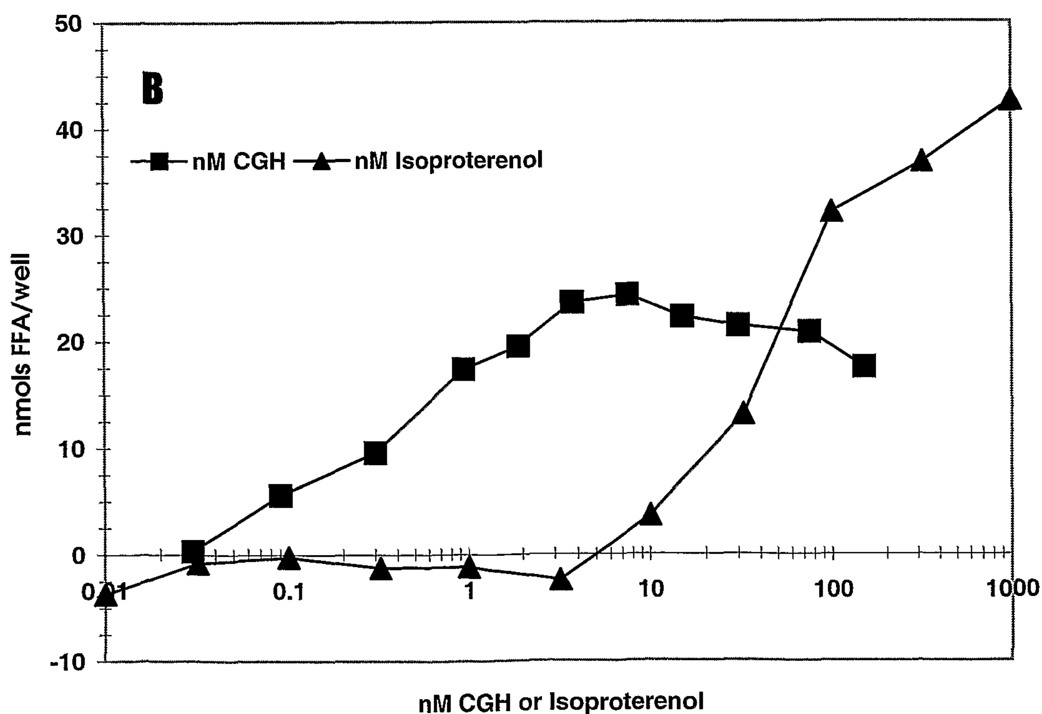
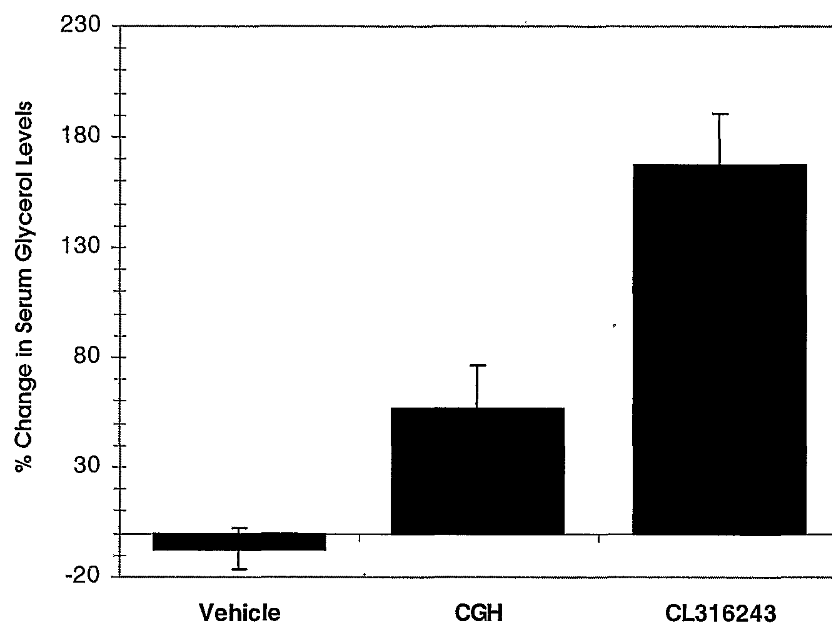
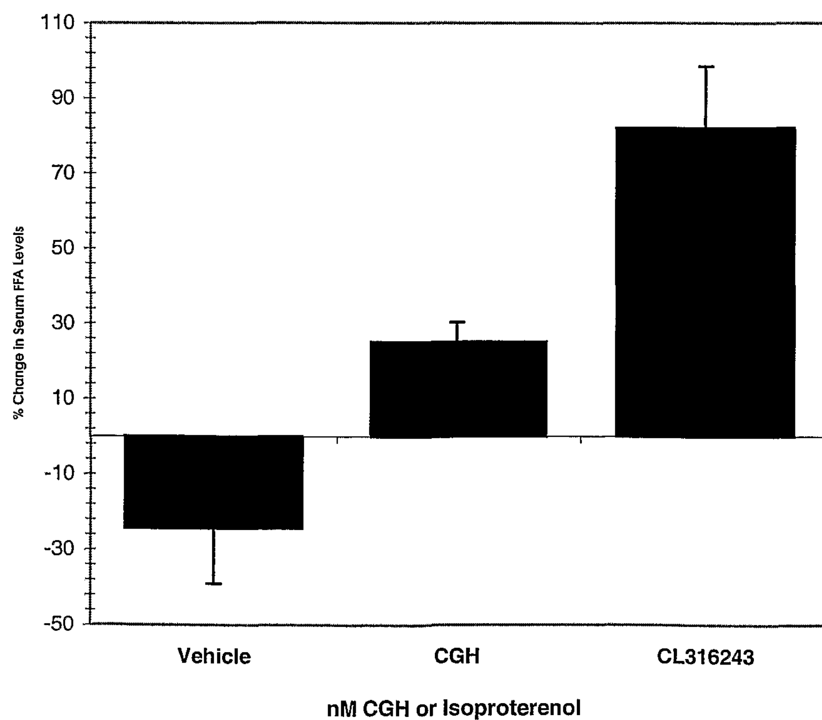


Figure 2





SEQUENCE LISTING

<110> ZymoGenetics, Inc.

<120> USE OF CORTICOTROPH-DERIVED GLYCOPROTEIN
HORMONE TO INDUCE LYPOLYSIS

<130> 01-16PC

<150> 60/305,284
<151> 2001-07-13

<160> 11

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 746
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (56)...(442)

<400> 1

```
ccagcaggag gcacaggaaa actgcaagcc gctctgttcc tgggcctcgg aagtg atg      58
                                                             Met
                                                              1

cct atg gcg tcc cct caa acc ctg gtc ctc tat ctg ctg gtc ctg gca      106
Pro Met Ala Ser Pro Gln Thr Leu Val Leu Tyr Leu Leu Val Leu Ala
            5                  10                  15

gtc act gaa gcc tgg ggc cag gag gca gtc atc cca ggc tgc cac ttg      154
Val Thr Glu Ala Trp Gly Gln Glu Ala Val Ile Pro Gly Cys His Leu
        20                  25                  30

cac ccc ttc aat gtg aca gtg cga agt gac cgc caa ggc acc tgc cag      202
```

His Pro Phe Asn Val Thr Val Arg Ser Asp Arg Gln Gly Thr Cys Gln
35 40 45

ggc tcc cac gtg gca cag gcc tgt gtg ggc cac tgt gag tcc agc gcc 250
Gly Ser His Val Ala Gln Ala Cys Val Gly His Cys Glu Ser Ser Ala
50 55 60 65

ttc cct tct cgg tac tct gtg ctg gtg gcc agt ggt tac cga cac aac 298
Phe Pro Ser Arg Tyr Ser Val Leu Val Ala Ser Gly Tyr Arg His Asn
70 75 80

atc acc tcc gtc tct cag tgc tgc acc atc agt ggc ctg aag aag gtc 346
Ile Thr Ser Val Ser Gln Cys Cys Thr Ile Ser Gly Leu Lys Lys Val
85 90 95

aaa gta cag ctg cag tgt gtg ggg agc cgg agg gag gag ctc gag atc 394
Lys Val Gln Leu Gln Cys Val Gly Ser Arg Arg Glu Glu Leu Glu Ile
100 105 110

ttc acg gcc agg gcc tgc cag tgt gac atg tgt cgc ctc tct cgc tac 442
Phe Thr Ala Arg Ala Cys Gln Cys Asp Met Cys Arg Leu Ser Arg Tyr
115 120 125

tagcccatcc tctcccctcc ttcctcccct gggtcacagg gcttgacatt ctggtggggg 502
aaacctgtgt tcaagattca aaaactggaa ggagctccag ccctgatggt tacttgctat 562
ggaatttttt taaataaggg gagggttgtt ccagctttga tcctttgtaa gattttgtga 622
ctgtcacctg agaagagggg agtttctgct tcttccctgc ctctgcctgg cccttctaaa 682
ccaatctttc atcattttac ttccctcttt gcccttaccc ctaaataaag caagcagttc 742
ttga 746

<210> 2
<211> 129
<212> PRT
<213> Homo sapiens

<400> 2
Met Pro Met Ala Ser Pro Gln Thr Leu Val Leu Tyr Leu Leu Val Leu
1 5 10 15
Ala Val Thr Glu Ala Trp Gly Gln Glu Ala Val Ile Pro Gly Cys His
20 25 30
Leu His Pro Phe Asn Val Thr Val Arg Ser Asp Arg Gln Gly Thr Cys
35 40 45

```
Gln Gly Ser His Val Ala Gln Ala Cys Val Gly His Cys Glu Ser Ser
    50                  55                  60
Ala Phe Pro Ser Arg Tyr Ser Val Leu Val Ala Ser Gly Tyr Arg His
65                  70                  75                  80
Asn Ile Thr Ser Val Ser Gln Cys Cys Thr Ile Ser Gly Leu Lys Lys
                85                  90                  95
Val Lys Val Gln Leu Gln Cys Val Gly Ser Arg Arg Glu Glu Leu Glu
            100                 105                 110
Ile Phe Thr Ala Arg Ala Cys Gln Cys Asp Met Cys Arg Leu Ser Arg
        115                 120                 125
Tyr


      <210> 3
      <211> 106
      <212> PRT
      <213> Homo sapiens

      <400> 3
Gln Glu Ala Val Ile Pro Gly Cys His Leu His Pro Phe Asn Val Thr
 1               5                  10                  15
Val Arg Ser Asp Arg Gln Gly Thr Cys Gln Gly Ser His Val Ala Gln
            20                  25                  30
Ala Cys Val Gly His Cys Glu Ser Ser Ala Phe Pro Ser Arg Tyr Ser
        35                  40                  45
Val Leu Val Ala Ser Gly Tyr Arg His Asn Ile Thr Ser Val Ser Gln
    50                  55                  60
Cys Cys Thr Ile Ser Gly Leu Lys Lys Val Lys Val Gln Leu Gln Cys
65                  70                  75                  80
Val Gly Ser Arg Arg Glu Glu Leu Glu Ile Phe Thr Ala Arg Ala Cys
                85                  90                  95
Gln Cys Asp Met Cys Arg Leu Ser Arg Tyr
            100                 105

      <210> 4
      <211> 390
      <212> DNA
      <213> Homo sapiens

      <220>
      <221> CDS
      <222> (1)...(390)
```

```
<400> 4
atg aag ctg gca ttc ctc ttc ctt ggc ccc atg gcc ctc ctc ctt ctg      48
Met Lys Leu Ala Phe Leu Phe Leu Gly Pro Met Ala Leu Leu Leu Leu
 1               5                  10                  15

gct ggc tat ggc tgt gtc ctc ggt gcc tcc agt ggg aac ctg cgc acc      96
Ala Gly Tyr Gly Cys Val Leu Gly Ala Ser Ser Gly Asn Leu Arg Thr
            20                  25                  30

ttt gtg ggc tgt gcc gtg agg gag ttt act ttc ctg gcc aag aag cca     144
Phe Val Gly Cys Ala Val Arg Glu Phe Thr Phe Leu Ala Lys Lys Pro
        35                  40                  45

ggc tgc agg ggc ctt cgg atc acc acg gat gcc tgc tgg ggt cgc tgt     192
Gly Cys Arg Gly Leu Arg Ile Thr Thr Asp Ala Cys Trp Gly Arg Cys
    50                  55                  60

gag acc tgg gag aaa ccc att ctg gaa ccc ccc tat att gaa gcc cat     240
Glu Thr Trp Glu Lys Pro Ile Leu Glu Pro Pro Tyr Ile Glu Ala His
65                  70                  75                  80

cat cga gtc tgt acc tac aac gag acc aaa cag gtg act gtc aag ctg     288
His Arg Val Cys Thr Tyr Asn Glu Thr Lys Gln Val Thr Val Lys Leu
                85                  90                  95

ccc aac tgt gcc ccg gga gtc gac ccc ttc tac acc tat ccc gtg gcc     336
Pro Asn Cys Ala Pro Gly Val Asp Pro Phe Tyr Thr Tyr Pro Val Ala
            100                 105                 110

atc cgc tgt gac tgc gga gcc tgc tcc act gcc acc acg gag tgt gag     384
Ile Arg Cys Asp Cys Gly Ala Cys Ser Thr Ala Thr Thr Glu Cys Glu
        115                 120                 125

acc atc                                                             390
Thr Ile
    130

<210> 5
<211> 130
<212> PRT
<213> Homo sapiens
```

<400> 5

```
Met Lys Leu Ala Phe Leu Phe Leu Gly Pro Met Ala Leu Leu Leu Leu
 1               5                  10                  15
Ala Gly Tyr Gly Cys Val Leu Gly Ala Ser Ser Gly Asn Leu Arg Thr
            20                  25                  30
Phe Val Gly Cys Ala Val Arg Glu Phe Thr Phe Leu Ala Lys Lys Pro
        35                  40                  45
Gly Cys Arg Gly Leu Arg Ile Thr Thr Asp Ala Cys Trp Gly Arg Cys
    50                  55                  60
Glu Thr Trp Glu Lys Pro Ile Leu Glu Pro Pro Tyr Ile Glu Ala His
65                  70                  75                  80
His Arg Val Cys Thr Tyr Asn Glu Thr Lys Gln Val Thr Val Lys Leu
                85                  90                  95
Pro Asn Cys Ala Pro Gly Val Asp Pro Phe Tyr Thr Tyr Pro Val Ala
            100                 105                 110
Ile Arg Cys Asp Cys Gly Ala Cys Ser Thr Ala Thr Thr Glu Cys Glu
        115                 120                 125
Thr Ile
    130
```

<210> 6
<211> 106
<212> PRT
<213> Homo sapiens

<400> 6

```
Ala Ser Ser Gly Asn Leu Arg Thr Phe Val Gly Cys Ala Val Arg Glu
 1               5                  10                  15
Phe Thr Phe Leu Ala Lys Lys Pro Gly Cys Arg Gly Leu Arg Ile Thr
            20                  25                  30
Thr Asp Ala Cys Trp Gly Arg Cys Glu Thr Trp Glu Lys Pro Ile Leu
        35                  40                  45
Glu Pro Pro Tyr Ile Glu Ala His His Arg Val Cys Thr Tyr Asn Glu
    50                  55                  60
Thr Lys Gln Val Thr Val Lys Leu Pro Asn Cys Ala Pro Gly Val Asp
65                  70                  75                  80
Pro Phe Tyr Thr Tyr Pro Val Ala Ile Arg Cys Asp Cys Gly Ala Cys
                85                  90                  95
Ser Thr Ala Thr Thr Glu Cys Glu Thr Ile
            100                 105
```

<210> 7
<211> 5605

<212> DNA
<213> Homo sapiens

<400> 7

```
atgaagctgg cattcctctt ccttggcccc atggccctcc tccttctggc tggctatggc      60
tgtgtcctcg gtgcctccag tgggaacctg cgcacctttg tgggctgtgc cgtgagggag     120
tttactttcc tggccaagaa gccaggctgc aggggccttc ggatcaccac ggatgcctgc     180
tggggtcgct gtgagacctg ggaggtgagt tgctaagttg tgcagatgac agtgtcttct     240
aggccagcag cttgggtctg attcttaaga gttcactttt taaatgatat gaggtagagc     300
tgggacatct gccctttcct gtggacttaa aaaaccaaaa caaaactatg attggcatct     360
tccaaaagtg atttgaaaaa catgatgttg cccctctaac aaagcattga taaggttaag     420
aatttggttt acattgtgtc tatgtatctg ggaatcatct ctgggaggtc aagatgtact     480
gttctacccg ttttacagat gacatggagg gattcaaggg agagtggctg caaagtcacg     540
tagagcgtca gtgtaaagct gggaatcaat ttgtggttca agcttgtgac ccaaactcct     600
ccctatgttt cctcattttg gataaattag ccagtttcca agaaagaggc cctgagctga     660
agggtgagcg ttggtcccag tgaagggtga gaccccttca ctgcctcttc tgcagccctt     720
ttcctcctca agtctctggg agccctctgg ggttatcact gacggatcca ttaagttcct     780
tcatattcaa ttatacctgg cctttttaga gacatttaat ttaaagtgga gataacactc     840
tcaaacaaag ttaaaatcct attgggctaa gaggagctgt ttgagtgatg aagaggaaga     900
gagctattca gcaccccagc agatcacatt acgtagtgac tgtgggctct tccccctgag     960
gcctgcccac ttggtaacca atgaagtgct gtctctgatc ttgtcactcc ctggcccaaa    1020
aaccttgaat gtccacacac tactacagat tcaataacta actttcaagg tgctcagcaa    1080
tatggcgtct gcctgctttc ctggagacag cacattttct tactctggcc ttggtaagtg    1140
actttcaaag gttttatcaa atagccctta tggatctcat tttgttcctt ccctcatatc    1200
ccttctcctt cccatctgtc attatcatat ttattcctga tgcctatctg cagtgccagc    1260
tccctttctg ggcctttttt gacttgcagg taagcccttg actatgctct acttttcgtc    1320
ttacttcctc ccccaccaca cgcgtgattt aaattttttc aggacagagg ttcattctta    1380
taaccttcac agcttttgtc aagatgtcgt gtatgaacaa ggcattcaat acacatttgt    1440
tggttgactg ggatggacct ccccctggag ctgtagatcc tccagcctaa tggaaggcca    1500
tttagaatca cacttgcact gtgagtggac actgccattg ggaaaaatag ccttctcttt    1560
ggggacccag agggtaacct gctcttgctt aggtacaatt acggccctgt gaatggaatt    1620
gggtcatagt gatgaaatct ccaaattgga tgaaactact ctatcaaagt agttttcttt    1680
tgcctcattc aggggcttga gccctactag cccaatgaaa atcgggtttt gctaagtaga    1740
ctttgcctgt caattggcag caaattcacc tggggcactt ggcacctcct cctgttcagg    1800
gactggcctg gcagggcctc tccctgttcg catctagtgt ctgggctatt tgaagccctc    1860
tctgtgccaa atcctcaaac tcctgcttcc gttcgattca gcccatcttc tcttcttttt    1920
aaaaactgat gaatgtcttt aattggatca tggtcaccca taggaggtca ggaactgtgc    1980
tctcactgga aagatggaaa caccaaaacc gttaaagaac aagattctcc ctgatgttag    2040
ccagctttca ttcatgtctt gactgtgtta tgaaaaggga ggttacctat agaaaataaa    2100
taaaagaatg agattcattt tcccagcaat ctgaaagttt ctgcgctata aagcacttga    2160
ttttttggtg ggggggatct taactgaaag catgtctgaa aataaggatg ttcatgatga    2220
caggctggct ggatttacat ttgaaggttg ttgaaaatag ctattcctca taatctgggt    2280
atagagttgc cagatttagc aaacaaacaa acagacaaac aaaataaaac aaaaccaatc    2340
```

```
ccctccccac agaaacccaa actgaaataa aaccagaaaa ccaggaagcc caggtaaatt    2400
tgaatttaag ataaataata aataaatttt tagcataagt ctgtctgtct catacagtat    2460
ttgggatgac ttatactaaa aaattatgta tctgaaaatg aaattttatg gggcgtttgg    2520
tctgcctagg ttcccagagt actaatggta agaggactta aagcaaatac gggaaggtag    2580
gagaaaacag ttgaggacaa attcagctct tctggtcttt gtcaaaggca aggctggccg    2640
ggcgtggtgg ctaacacctg taatctcagc actttgggag gctgtggtgg gtggataatg    2700
aggtcaggag ttcgagacca gcctggccag tttttagtaa agaggtgagt aaaaccctgt    2760
ctctactaaa aatacaaaaa ttagccgggc atggtggtat gcacctgtag tcccagctac    2820
ttgggaggct gaggcagaag acttgcttga acccaggagg tggaggttac agtgagccaa    2880
gatcatgcca ctatactcca gcctggcgac agagtgagac tccatctcaa aaaaaaaaaa    2940
aaaaagaaaa aagaaaaaaa aaaggtaagg ctgctatttt catgacattc atgcaagaac    3000
atcttgagtt acatatgtat atatattctt ttttgcctag aacaaagaag aaccaaaaag    3060
caaaggtact gtcatttgaa agcttgttat tatttacatt actttcttat aataattgca    3120
ctaataagaa caatggattg gctgggcgtg gtggctcacg cctgtaatcc cagcactttg    3180
ggaggccgag gcaggcagat cacgaggtca ggaaatcgag accatcctgg ctaacatggt    3240
gaaaccctgt ctctactaaa aatacaaaaa atgagccagg cgtggtggtg ggtgcctgta    3300
gtcccgggag gctgaggcag gagaatggcg tgaacccggg aggcggagat tgcaatgagc    3360
tgagattgcg ccactgaact ccagcctggg agacagcaag actccgtctc aaaaaaaaaa    3420
aaaaatggat tgcatttttt gaacatttac tttgttctag acattgtgca ttgcgtatat    3480
catcttacct tatctctcaa acaatggtgg gaggtagcta ttttgtttta cagaggagga    3540
aacttgagtc ttcaggaagt taagtggatt ttccaaggtc tccagcaagt ggcagaacag    3600
ggactcaagc tccttagttc tgactgcagg gctcgagatt ttaactccag ctaggtgctg    3660
atattttttc tgatctgtgt gttctgttta tcaaaattgt ctttgaactt aagatttata    3720
aaaggtgaag gaaggaaatg aatctttttg atgatcagaa cagtgcacag agtattcggg    3780
aacctgtctt gtaatgtttt ctttcattga ttcaatgaca aatagttatt gaaactctcc    3840
cagggtctgt tttgggtact tgaggcacag tgggcaaaaa tctctgtcct aaaagagctt    3900
actttctaga gtgggaggaa tatcacacga atgaaaggta gactacgtcg tgtggtattg    3960
atcagtgctg tggtggaaaa taaagcaaga tgggggatgg gaagtttctg ggcatggaga    4020
tggaatgttg caattttaaa taggatggtc aggaaatgct tccctgagag ggtgacattc    4080
taacaaaaac ccaaggttgg tgaaagagtg aatcatacgg gagaagaatg ttccaggcag    4140
aaggaacagt aagtgcaaag gccctgagct ggggctgttc ctggtgggtc agaggagcaa    4200
taaggagacc gccgtgagcc tagtgaggaa gtcagtgagg tgggaatggt tgcaggcatt    4260
tcagaaggta gagttgcaga gaaggtgatg taggtcttga aggtgatcat aaggtctttg    4320
atgtttgttc tgagtgagat gggaaatcac tggggctttg ggcagaggag taacatgatc    4380
tgacttaggt ttaaacagga tcactcaggg ccgctgtgtt gcaaatagat tgtagggagt    4440
aaaaatggaa gaggggagac cagttagaag gtatttgcaa tgactaagat gattcatttg    4500
ctgactatgc atggagcact tgctgtgtgc tatggtctct cctgggagct tagaatatgg    4560
tcttgagtga aatcagcttc ttgctttcag gagtttgttt tctactggga gacgacagag    4620
caacaagtaa atcaacgaat aacaagttaa tttctgatag tgataaatga tactaaaaaa    4680
ctgaaacaag atcatatgtt ctaatgaatt ctctgtttct atctatgggg acagaaaccc    4740
attctggaac ccccctatat tgaagcccat catcgagtct gtacctacaa cgagaccaaa    4800
caggtgactg tcaagctgcc caactgtgcc ccgggagtcg accccttcta cacctatccc    4860
gtggccatcc gctgtgactg cggagcctgc tccactgcca ccacggagtg tgagaccatc    4920
```

```
tgaggccgct agctgctctc tgcagaccca cctgtgtgag cagcacatgc agttatactt    4980
cctggatgca agactgttta atttcgacca cacccatgga ggaggttacc tgtcgcccct    5040
taggtccagc tcaggcaaaa ggcccaaatg cagcctactt atgctaaaag ttcaaaacaa    5100
tattcgtgcc ttcaccaaaa taatttctcc agctcacata cctgcaaatt aatttttctt    5160
tgccttgagt cttggaacat aatttgtgta tcacaatcct cccccaattt ggacttataa    5220
tatgctaatg atttaaacac atgggatgta attaggatat ggggctggaa agtctttaaa    5280
ttctcatgtt ctatttaacc tctgatctcc aaccggattt atgattaaag ggctagaaat    5340
gaacaaaacc catgtactag tcttccttac cccagaggaa ttccagctgc aagcttcttt    5400
agggaaaatg ctcccttccc cttttaactg agcaattatc tacacaagaa ataagactgc    5460
tcagatatac aaagagagta gcttcaatga aaagatgttt ggatttggat aattcttttc    5520
cctagcaaaa ttcgctagct cccttaagag tcttaataaa gaggctacgt tgggattaaa    5580
agaaaaaaaa acagaaataa aatat                                          5605
```

<210> 8
<211> 22
<212> DNA
<213> Homo sapiens

<400> 8
tcagaagaaa atcagaggaa tc 22

<210> 9
<211> 23
<212> DNA
<213> Homo sapiens

<400> 9
gggacgttca gtagcggttg tag 23

<210> 10
<211> 20
<212> DNA
<213> Homo sapiens

<400> 10
ctgcccatgg acaccgagac 20

<210> 11
<211> 23
<212> DNA
<213> Homo sapiens

<400> 11
ccgtttgcat atactcttct gag 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/22747

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/18
US CL :514/2, 12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/40291 A2 (CURAGEN CORPORATION) 07 JUNE 2001 (07-06-2001), pages 2, 4, 49-51 and 74.	1-5
Y	WO 00/78964 A1 (AMGEN INC.) 28 DECEMBER 2000 (28-12-2000), pages 2, 4, 9-10 and 65-68.	1-5
Y	WO 99/41377 A1 (ZYMOGENETICS, INC.) 19 AUGUST 1999 (19-08-99), pages 11-12, 26 and 35.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

24 SEPTEMBER 2002

Date of mailing of the international search report

12 DEC 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer
ROBERT C. HAYES, PH.D.

Telephone No. (703) 308-0196

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

MEDLINE, BIOSIS, USPATFULL, PCTFULL, GENCORE
search terms: corticotroph-derived glycoprotein hormone, CGH, heterodimer##, lipolysis, weight loss, diabet##, insulin, obes###