Lipolytic and Antilipolytic Effects of Human Growth Hormone, Its 20-Kilodalton Variant, A Reduced and Carboxymethylated Derivative, and Human Placental Lactogen on Chicken Adipose Tissue *In Vitro* (43034)

ROBERT M. CAMPBELL,[1] JACK L. KOSTYO, AND COLIN G. SCANES

Department of Animal Sciences, Rutgers-The State University, New Brunswick, New Jersey 08903 and Department of Physiology, The University of Michigan Medical School, Ann Arbor, Michigan 48109

Abstract. **The lipolytic and antilipolytic effects of human growth hormone (22K-hGH), its 20-kilodalton variant (20K-hGH), a reduced and *S*-carboxymethylated derivative (RCM-hGH), and human placental lactogen were examined using chicken adipose tissue explants *in vitro*. Lipolysis, as determined by glycerol release, was stimulated by 22K-hGH (biosynthetic and pituitary derived), 20K-hGH (pituitary derived), and RCM-hGH (modified biosynthetic). These growth hormone preparations also exhibited similar antilipolytic activity (i.e., transient inhibition of glucagon-induced lipolysis). However, unlike human growth hormone, human placental lactogen neither stimulated lipolysis nor inhibited glucagon-stimulated lipolysis. Some augmentation of glucagon-stimulated lipolysis was observed in the presence of human placental lactogen. These results indicate that the disulfide bridges ($Cys^{53} \rightarrow Cys^{165}$; $Cys^{182} \rightarrow Cys^{189}$) and amino acid residues 32–46 of hGH are not required for lipolytic or antilipolytic activities of human growth hormone on chicken adipose tissue.** [P.S.E.B.M. 1990, Vol 193]

Considerable attention has been focused on the relationship between the structure of growth hormone (GH) and its growth-promoting activity (reviewed in ref. 1). However, the structural requirements for the various metabolic actions of GH have been less intensively investigated. These metabolic effects of GH in mammals include (i) stimulation of lipolysis (2, 3); (ii) transient inhibition of epinephrine-induced lipolysis (4, 5); (iii) simulation of "insulin-like" responses, such as glucose oxidation (6) and lipogenesis (7–9); and (iv) impaired glucose tolerance or "diabetogenic" effects (10). In the chicken, GH (chicken or bovine) stimulates basal lipolysis and also inhibits glucagon-induced lipolysis by adipose tissue explants (11, 12). Thus, chicken adipose tissue may be a useful model to ascertain the structural determinants of GH action, both as a stimulator of lipolysis and as an inhibitor of glucagon-induced lipolysis.

The present studies examine the ability of human GH (22K-hGH), its 20-kilodalton variant (20K-hGH), a reduced *S*-carboxymethylated derivative (RCM-hGH), and human placental lactogen (hPL) to evoke lipolytic and/or antilipolytic responses in chicken adipose tissue. The high degree of amino acid sequence homology between these polypeptides presents distinct advantages in probing the relative importance of structural modifications on GH activity. Native 22K-hGH is only 56% homologous to chicken GH (13), but is 85% homologous with hPL (14, 15). Surprisingly, hPL has very low growth-promoting activity in rat tibia assays (16, 17). The naturally occurring 20K-hGH variant of human GH (20K-hGH) differs from 22K-hGH, by only a 15-amino acid residue deletion (residues 32–46) (18–20). 20K-hGH and 22K-hGH have similar somatomedin-generating (21), growth-promoting (rat tibial growth or weight gain assay) (18, 19), and lactogenic (pigeon crop sac) (18–22) activities. However,

[1] Present address: Department of Animal Sciences, Research, Hoffmann-La Roche, Inc., Nutley, NJ 07110.

Received May 9, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted December 11, 1989.

0037-9727/90/1934-0269$2.00/0

when compared with 22K-hGH, 20K-hGH displays lower potency (≅10–30%) in stimulating glucose oxidation (22) and increasing lipogenesis (potency ≅2–6%) *in vitro* (9), and in producing insulin-like effects *in vivo* (transiently decreasing plasma glucose and free fatty acid levels) (23). 20K-hGH and 22K-hGH appear to be equipotent in eliciting diabetogenic effects, as indicated by glucose intolerance (22) *in vivo*. The 20K-hGH variant has reduced affinity for GH receptors in liver (24), mammary gland (24), and adipocyte (25) plasma membranes.

When 22K-hGH is reduced and *S*-carbamidomethylated (RCAM-hGH), it retains growth-promoting and lactogenic activities (26), while the *in vitro* insulin-like activity (stimulation of glucose oxidation) is greatly decreased (27). Following reduction and *S*-carboxymethylation (RCM-hGH), hGH has little growth-promoting or *in vitro* insulin-like (stimulation of glucose oxidation) activities and attenuated lactogenic activity (26–28). In addition, RCM-hGH possesses approximately 50% of the diabetogenic activity of native hGH (28).

Materials and Methods

In all studies, abdominal adipose tissue from adult (24- to 30-week old) male chickens (strain: Single Comb White Leghorn) was employed. Birds were housed in brooder pens with free access to water and feed (Chick Grower diet; Agway). Explants were prepared and incubated as described previously (11, 12). Briefly, this entailed dicing adipose tissue (from three chickens per trial) and distribution into incubation vials (7–10 explants/vial, 50–100 mg total weight). Adipose tissue explants were incubated in 1 ml of Krebs-Ringer-Hepes medium (pH 7.4) supplemented with 15 mM glucose, 1% bovine serum albumin (Armour, Fraction V), and 2.54 m*M* calcium chloride under 95% O_2-5% CO_2 in a shaking water bath (70 oscillations/min, 37.5°C). Following a 1-hr preincubation, the medium was removed and fresh medium, containing test hormones, added. Tissues were incubated for 1 hr (Tables II–IV) or 4 hr further (Table I), removed from the vials (for weight determination), and the media rapidly frozen. Media glycerol content was determined by enzymatic/fluorometric assay (11) and used as the index of lipolysis. In all studies, bovine GH (bGH), whose lipolytic and antilipolytic activities have been well characterized in this *in vitro* model (11, 12), was included for comparison.

Pituitary-derived 22K-hGH and 20K-hGH variant were kindly donated by Dr. U. J. Lewis (La Jolla, CA). Biosynthetic methionyl 22K-hGH (Somatonorm) and bGH were provided by Kabi-Vitrum (Stockholm, Sweden) and Eli Lilly Research Laboratories (Indianapolis, IN), respectively. The biosynthetic human GH preparation was chemically modified, reduced, and *S*-carboxymethylated according to the method of Cameron *et al.* (28). Porcine glucagon and hPL were obtained, respectively, from Sigma Chemical (St. Louis, MO) and Dr. S. Raiti (NIADDK).

Statistical differences between means were determined (Table I: independently for the first and fourth hr of incubation) by analysis of variance (ANOVA), followed by least significant differences (LSD). Biologic potencies were estimated by a Parallel Line Bioassay Computer Program (Hoffmann-La Roche) using data from all doses and with 22K-hGH as the standard for comparison. Potency comparisons for RCM-hGH could not be performed due to the lack of dose-response curve parallelism with 22K-hGH.

Results

Lipolytic Effects. Table I summarizes the lipolytic effects of pituitary-derived 22K-hGH, 20K-hGH variant, and hPL. At a concentration of 1 μg/ml, 22K-hGH, 20 K-hGH, and bGH were similarly effective in stimulating lipolysis after 1 hr of incubation. At the 0.1-μg/ml dose, 22K-hGH caused a small, but significant ($P < 0.05$), increase in lipolysis while 20K-hGH was ineffective. hPL did not affect lipolysis at either 0.1 or 1.0 μg/ml. When the tissue incubation was continued for three additional periods of 1 hr (4-hr total treatment), the magnitude of lipolytic response in the fourth hour of the incubation increased (30–98%) for all of the GH preparations. In the fourth hr of incubation (noncumulative glycerol release), 20K-hGH and 22K-hGH were each more effective than bGH (1 μg/ml). Unlike the first hour of incubation, both 20K-hGH and 22K-hGH stimulated lipolysis at 0.1 μg/ml. hPL inhibited basal lipolysis in the fourth hr of incubation. The potency of 20K-hGH (0.10 [95% confidence limits, 0.07–0.51]) was lower that of 22K-hGH in the first hour of incubation. However, in the fourth hr of incubation, no difference in potency was observed, 20K-

Table I. Effect of Pituitary-derived Human GH (22K-hGH), Its 20K-Variant (20K-hGH), hPL, and bGH on Chicken Adipose Tissue Lipolysis *In Vitro*

	Glycerol release during incubation (nmol/g tissue ± SEM, $n = 3$)[a]	
	First hour	Fourth hour
Control	284.0 ± 19.5a, b	287.5 ± 2.3b
bGH (1 μg/ml)	459.4 ± 29.0c	595.0 ± 23.2c, d
22K-hGH (0.1 μg/ml)	373.0 ± 21.5b, c	573.8 ± 19.1c
22K-hGH (1 μg/ml)	426.2 ± 20.7c	819.5 ± 51.3e
20K-hGH (0.1 μg/ml)	322.0 ± 16.2a, b	501.0 ± 16.5c
20K-hGH (1 μg/ml)	371.5 ± 19.8b, c	737.0 ± 70.9d, e
hPL (0.1 μg/ml)	240.0 ± 17.9a	148.1 ± 15.6a, b
hPL (1 μg/ml)	236.6 ± 21.5a	96.9 ± 21.5a

[a] Values represent mean ± SE of three independent trials, with six replicates/treatment group/trial. Means with similar letters are not statistically different ($P < 0.05$) by ANOVA, followed by LSD.

hGH having 0.61 (95% confidence limits, 0.31–1.18) the potency of 22K-hGH.

In another study (Table II), increasing concentrations (0.01, 0.10, and 1.00 μg/ml) of biosynthetic methionyl 22K-hGH and reduced, carboxymethylated methionyl hGH (RCM-hGH) progressively enhanced lipolysis. In the first hr of incubation, the maximal effects observed with 22K-hGH or RCM-hGH were virtually identical to those of bGH (1 μg/ml).

Antilipolytic Effects. As observed previously (12), glucagon (1 ng/ml) elevated glycerol release, and this effect was partially suppressed (29–37%) by bGH (Tables III and IV). Pituitary-derived 22K-hGH, 20K-hGH, and bGH inhibited glucagon-induced lipolysis, with no significant difference observed between preparations or doses (0.1 or 1.0 μg/ml) (Table III). In contrast to the antilipolytic effects of hGH, hPL (at 1 μg/ml, but not 0.1 μg/ml) augmented the lipolytic effect of glucagon.

Like its native counterpart, biosynthetic methionyl 22K-hGH (0.01, 0.10, and 1.00 μg/ml) reduced glucagon-stimulated lipolysis in a dose-responsive manner (Table IV). Similarly, glucagon-induced lipolysis was inhibited by RCM-hGH (Table IV).

Table II. Effect of Biosynthetic Methionyl Human GH (22K-hGH), a Reduced Carboxymethylated Derivative (RCM-hGH), and bGH on Chicken Adipose Tissue Lipolysis *In Vitro*

	Glycerol release during incubation (nmol/g tissue ± SEM, $n = 3$)[a]
	First hour
Control	272.9 ± 7.0a
bGH (1 μg/ml)	436.8 ± 10.1c–e
22K-hGH (0.01 μg/ml)	383.0 ± 10.5b, c
22K-hGH (0.1 μg/ml)	402.7 ± 9.0c–e
22K-hGH (1 μg/ml)	446.9 ± 20.0d, e
RCM-hGH (0.01 μg/ml)	358.4 ± 4.5b
RCM-hGH (0.1 μg/ml)	400.8 ± 15.5b–d
RCM-hGH (1 μg/ml)	473.9 ± 23.0e

[a] Values represent mean ± SE of three independent trials, with six replicates/treatment group/trial. Means with similar letters are not statistically different ($P < 0.05$) by ANOVA, followed by LSD.

Discussion

In previous studies, we have shown that chicken and bovine GH (biosynthetic or pituitary derived) stimulate lipolysis in chicken adipose tissue *in vitro* (11). We have now determined that 22K-hGH (biosynthetic or pituitary derived) is lipolytic using chicken adipose tissue explants. There is good evidence that 22K-hGH is lipolytic with rat adipose tissue *in vitro*. Biosynthetic and pituitary-derived 22K-hGH have been observed to stimulate lipolysis in adipose tissue from hypophysectomized or intact rats (29). Another laboratory (30, 31) has, however, been unable to demonstrate dose-dependent lipolytic effects of 22K-hGH using adipose tissue from intact rats.

The 20K-variant (pituitary derived) of hGH is lipolytic in chicken adipose tissues; the potency of the 20K-hGH being lower than the 22K-hGH in the first hour of incubation but equipotent in the fourth hour of incubation. Lipolytic responses to 20K-hGH have been observed with rat adipose tissue incubated for 4 hr (H. Goodman, personal communication). The extremely low specific binding of ^{125}I-20K-hGH to rat adipocytes does not support the concept of an independent 20K-hGH receptor in rat adipocytes (25). Moreover, Scatchard analyses of 22K-hGH binding to rat adipocytes are linear, indicating a single class of GH receptors (32, 33). To date, receptor binding studies of 22K- or 20K-hGH have not been performed using chicken adipocytes.

The lipolytic and antilipolytic activities of 20K-hGH were similar to those of 22K-hGH. It is not possible to discriminate between these lipolytic and antilipolytic potencies. Therefore, the possibility that different receptors or receptor subunits might be re-

Table III. Effect of Human GH (22K-hGH), Its 20K-variant (20K-hGH), hPL, and bGH on Glucagon-Induced Lipolysis by Chicken Adipose Tissue *In Vitro*

	Glycerol release during incubation (nmol/g tissue ± SEM, $n = 3$)[a]
Control	246.8 ± 28.2a
Glucagon (1 ng/ml)	757.0 ± 40.2c
Glucagon (1 ng/ml) + bGH (0.1 μg/ml)	536.2 ± 42.3b
Glucagon (1 ng/ml) + bGH (1 μg/ml)	472.7 ± 42.7b
Glucagon (1 ng/ml) + 22K-hGH (0.1 μg/ml)	600.5 ± 56.6b
Glucagon (1 ng/ml) + 22K-hGH (1 μg/ml)	575.1 ± 30.1b
Glucagon (1 ng/ml) + 20K-hGH (0.1 μg/ml)	584.7 ± 47.2b
Glucagon (1 ng/ml) + 20K-hGH (1 μg/ml)	521.8 ± 49.3b
Glucagon (1 ng/ml) + hPL (0.1 μg/ml)	904.3 ± 52.0c, d
Glucagon (1 ng/ml) + hPL (1 μg/ml)	950.1 ± 40.5d

[a] Values represent mean ± SE of three independent trials, with six replicates/treatment group/trial. Means with similar letters are not statistically different ($P < 0.05$) by ANOVA, followed by LSD.

Table IV. Effect of Biosynthetic Methionyl Human GH (22K-hGH), a Reduced Carboxymethylated Derivative and bGH on Glucagon-Induced Lipolysis by Chicken Adipose Tissue *In Vitro*

	Glycerol release during incubation (nmol/g tissue ± SEM, $n = 3$)[a]
Control	271.9 ± 11.2a
Glucagon (1 ng/ml)	903.8 ± 34.1e
Glucagon (1 ng/ml) + bGH (1 μg/ml)	638.6 ± 12.6c
Glucagon (1 ng/ml) + 22K-hGH (0.01 μg/ml)	753.7 ± 14.2d
Glucagon (1 ng/ml) + 22K-hGH (0.1 μg/ml)	648.6 ± 11.1c
Glucagon (1 ng/ml) + 22K-hGH (1 μg/ml)	592.5 ± 10.0b, c
Glucagon (1 ng/ml) + RCM-hGH (0.01 μg/ml)	635.2 ± 19.3c
Glucagon (1 ng/ml) + RCM-hGH (0.1 μg/ml)	597.1 ± 13.3b, c
Glucagon (1 ng/ml) + RCM-hGH (1 μg/ml)	572.5 ± 19.0b

[a] Values represent mean ± SE of three independent trials, with six replicates/treatment group/trial. Means with similar letters are not statistically different ($P < 0.05$) by ANOVA, followed by LSD.

sponsible for antilipolytic and lipolytic activity of GH cannot be precluded.

The reduced *S*-carboxymethylated 22K-hGH (modified biosynthetic; RCM-hGH) was found to be effective in stimulating lipolysis by chicken adipose tissue explants (Table II). The lipolytic response to RCM-hGH has not been examined in any other species for comparison. With rat adipocytes, two large fragment complexes of reduced *S*-carbamidomethylated hGH (residues 1–134:141–191; and (42–134:141–191) were determined to be lipolytic (34). Therefore reduction, in combination with carboxymethylation or carbamidomethylation, does not greatly alter the lipolytic activity of hGH. As the lipolytic and antilipolytic effects of GH appear to be mechanistically distinct in rat (2, 4, 5) and chicken (35) adipose tissue, it is conceivable that the receptors or receptor subunits (and hence structural determinants) for these activities also differ.

In contrast to hGH, hPL (1 μg/ml) was without either lipolytic or antilipolytic activities on chicken adipose tissue. The lack of lipolytic response to hPL (1 μg/ml) is consistent with that observed by investigators employing rat or mouse adipose tissue/adipocytes (36, 37). Furthermore, hPL (3–5 μg/ml) does not compete with radiolabeled 22K-hGH binding to rat (38) or human (39) fat cells. It should be noted, however, that very high concentrations (≥20 μg/ml) of hPL have been reported to stimulate lipolysis (36). Based on the present data, hPL does not appear to be inherently lipolytic or antilipolytic on chicken adipose tissue. Although, in view of the augmentation we observed with glucagon-induced lipolysis (in the presence of hPL) (Table III), hPL may modify the postreceptor response to glucagon, possibly by way of the cyclic AMP-second messenger system.

The basis for the absence of lipolytic and antilipolytic activities of hPL is not readily apparent. hPL possesses only 26 amino acid residues which are not homologous with hGH and 21 amino acid residues which differ from hGH, bGH, or chicken GH (1, 13–15, 40–42). Of these residues, 13 are highly conserved in the human, bovine, and chicken GH sequences (Phe^{1}, Pro^{2}, Asn^{12}, Arg^{16}, Leu^{20}, Asn^{47}, Glu^{56}, Gln^{84}, Gln^{91}, Val^{96}, Gly^{104}, Val^{110} and Pro^{133} of the hGH sequence). These are, therefore, likely to be in loci associated with the biologic determinants of GH. The relative contribution of residue 109 (Asp^{109} of hPL) is unclear as it differs from 22K-hGH (Asn^{109}), while a deletion is seen in the chicken and bovine sequences. The His^{112} residue of hPL may also be considered variant as Glu^{112} (chicken GH and bGH) and Asp^{112} (hGH) are nonconservative substitutions. Residues, 12, 16 and 20 all lie within helix-1; 84 lies within helix-2; and 110 and 112 lie within helix-3. It may be speculated that these substitutions are responsible for the lack of lipolytic activity of hPL.

Chicken adipose tissue exhibits lipolytic and antilipolytic responses to chicken, bovine, or human GH (22K- , 20K- , or RCM-hGH). Therefore, these GH preparations all contain the structural determinants necessary for lipolytic and antilipolytic action. It appears that amino acid residues 32–45 and the disulfide bonds ($Cys^{53} \rightarrow Cys^{165}$, $Cys^{182} \rightarrow Cys^{189}$) of human Gh are not required for either of these activities.

This is a paper of the Journal Series of the New Jersey State Agricultural Experiment Station (Project 18140) supported by State and Hatch Act funds and grants from the National Science Foundation (DCB-8600447) and the United States Department of Agriculture (623-2341-37265).

1. Nicoll CS, Mayer GL, Russell SM. Structural features of prolactins and growth hormones that can be related to their biological properties. Endocr Rev **7**:169–203, 1986.
2. Fain JN, Kovacev VP, Scow RO. Effect of growth hormone on lipolysis and metabolism in isolated fat cells of the rat. J Biol Chem **240**:3522–3529, 1965.
3. Goodman HM. Multiple effects of growth hormone on lipolysis. Endocrinology **83**:300–308, 1968.
4. Goodman HM. Antilipolytic effects of growth hormone. Metabolism **19**:849–855, 1970.

5. Birnbaum RS, Goodman HM. Studies on the mechanism of the antilipolytic effects of growth hormone. Endocrinology **99**:1336–1345, 1976.
6. Goodman HM. *In vitro* actions of growth hormone on glucose metabolism in adipose tissue. Endocrinology **76**:216–225, 1965.
7. Goodman HM. Effects of chronic growth hormone treatment on lipogenesis by rat adipose tissue. Endocrinology **72**:95–99, 1968.
8. Pandian MR, Gupta SL, Talwar GP. Studies on the early interactions of growth hormone: Effect *in vitro* on lipogenesis in adipose tissue. Endocrinology **88**:928–936, 1971.
9. Smal J, Closset J, Hennen, DeMeyts P. Receptor binding properties and insulin-like effects of human growth hormone and its 20 kDa-variant in rat adipocytes. J Biol Chem **262**:11071–11079, 1987.
10. Kostyo JL, Gennick SE, Sauder SE. Diabetogenic activity of native and biosynthetic human growth hormone in the obese (ob/ob) mouse. Am J Physiol **246**:E356–E360, 1984.
11. Campbell RM, Scanes CG. Lipolytic activity of purified pituitary and bacterially-derived growth hormone on chicken adipose tissue *in vitro*. Proc Soc Exp Biol Med **180**:513–517, 1985.
12. Campbell RM, Scanes CG. Growth hormone inhibition of glucagon and cAMP-induced lipolysis by chicken adipose tissue *in vitro*. Proc Soc Exp Biol Med **184**:456–460, 1987.
13. Souza LM, Boone TC, Murdock D, Langley K, Wypych J, Fenton D, Johnson S, Lai PH, Everett R, Hsu R-Y, Bosselman R. Application of recombinant DNA technologies to studies on chicken growth hormone. J Exp Zool **232**:465–473, 1984.
14. Niall HD, Hogan ML, Sayer R, Rosenblum IY, Greenwood FC. Sequences of pituitary and placental lactogenic and growth hormones: Evolution from a primordial peptide by gene duplication. Proc Natl Acad Sci USA **68**:866–869, 1971.
15. Bewley TA, Dixon JS, Li CH. Sequence comparisons of human pituitary growth hormone, human chorionic somatomammotropin, and ovine pituitary growth and lactogenic hormones. Int J Pept Protein Res **4**:281–287, 1972.
16. Friesen H. Purification of a placental factor with immunological and chemical similarity to human growth hormone. Endocrinology **76**:369–381, 1965.
17. Florini JR, Tonelli G, Brener CB, Coppola J, Ringler I, Bell PH. Characterization and biological effects of purified placental protein (human). Endocrinology **79**:692–708, 1966.
18. Lewis UJ, Dunn JT, Bonewald LF, Seavey BK, Vanderlaan WP. A naturally occurring structural variant of human growth hormone. J Biol Chem **253**:2679–2687, 1978.
19. Lewis UJ, Bonewald LF, Lewis LJ. The 20,000-dalton variant of human growth hormone: Location of the amino acid deletions. Biochem Biophys Res Commun **92**:511–516, 1980.
20. Chapman GE, Rogers KM, Brittain T, Bradshaw RA, Bates OJ, Turner C, Cary PD, Crane-Robinson C. The 20,000 molecular weight variant of human growth hormone. Preparation and some physical and chemical properties. J Biol Chem **256**:2395–2401, 1981.
21. Spencer EM, Lewis LJ, Lewis UJ. Somatomedin generating activity of the 20,000-dalton variant of human growth hormone. Endocrinology **109**:1301–1302, 1981.
22. Kostyo JL, Cameron CM, Olson KC, Jones AJS, Pai R-C. Biosynthetic 20-kilodalton methionyl-human growth hormone has diabetogenic and insulin-like activities. Proc Natl Acad Sci USA **82**:4250–4253, 1985.
23. Tinsley FC, Grinnan EL, Baker SH, Powell JG, Bemis KG, Shaar CJ. The 20,000 dalton structural variant of recombinant DNA-derived methionyl human growth hormone has early insulin-like effects of hypophysectomized rats. Biochem Biophys Res Commun **138**:342–348, 1986.
24. Sigel MB, Thorpe NA, Kobrin MS, Lewis UJ and Vanderlaan WP. Binding characteristic of a biologically active variant of human growth hormone (20K) to growth hormone and lactogen receptors. Endocrinology **108**:1600–1603, 1981.
25. Smal J, Closset J, Hennen G, DeMeyts P. The receptor binding properties of the 20K variant of human growth hormone explain its discrepant insulin-like and growth promoting activities. Biochem Biophys Res Commun **134**:159–165, 1986.
26. Bewley TA, Brovetto-Cruz J, Li CH. Human pituitary growth hormone. Physicochemical investigations of the native and reduced-alkylated protein. Biochemistry **8**:4701–4708, 1969.
27. Mills JB, Kostyo JL, Reagan CR, Wagner SA, Moseley MH, Wilhelmi AE. Fragments of human growth hormone produced by digestion with thrombin: Chemistry and biological properties. Endocrinology **107**:391–399, 1980.
28. Cameron CM, Kostyo JL, Rillema JA, Gennick SE. Reduced and *S*-carboxymethylated human growth hormone: a probe for diabetogenic action. Am J Physiol **247**:E639–E644, 1984.
29. Goodman HM, Grichting G. Growth hormone and lipolysis: A re-evaluation. Endocrinology **113**:1697–1702, 1983.
30. Frigieri LG. Absence of *in vitro* dexamethasone-dependent lipolytic activity from highly purified growth hormone. Endocrinology **107**:738–743, 1980.
31. Frigieri LG, Robel G, Stebbing N. Bacteria-derived human growth hormone lacks lipolytic activity in rat adipose tissue. Biochem Biophys Res Commun **104**:1041–1046, 1982.
32. Fagin KD, Lackey SL, Reagan CR, DiGirolamo M. Specific binding of growth hormone by rat adipocytes. Endocrinology **107**:608–615, 1980.
33. Grichting G, Levy LK, Goodman HM. Relationship between binding and biological effects of human growth hormone in rat adipocytes. Endocrinology **113**:1111–1120, 1983.
34. Goodman HM, Kostyo JL. Altered profiles of biological activity of growth hormone fragments on adipocyte metabolism. Endocrinology **108**:553–558, 1981.
35. Campbell RM, Scanes CG. Pharmacological investigations on the lipolytic and antilipolytic effects of growth hormone (GH) in chicken adipose tissue *in vitro*: Evidence for involvement of calcium and polyamines. Proc Soc Exp Biol Med **188**:177–184, 1988.
36. Turtle JR, Kipnis DM. The lipolytic action of human placental lactogen on isolated fat cells. Biochim Biophys Acta **144**:583–593, 1967.
37. Fielder PJ, Talamantes F. The lipolytic effects of mouse placental lactogen II, mouse prolactin, and mouse growth hormone on adipose tissue from virgin and pregnant mice. Endocrinology **121**:493–497, 1987.
38. Gavin JR, Saltman RJ, Tollefsen SE. Growth hormone receptors in isolated rat adipocytes. Endocrinology **110**:637–643, 1982.
39. DiGirolamo M, Eden S, Enberg G, Isaksson O, Lonnroth P, Hall K, Smith U. Specific binding of human growth hormone but not insulin-like growth factors by human adipocytes. FEBS Lett **205**:15–19, 1986.
40. Bewley TA, Li CH. Sequence comparison of human pituitary growth hormone, human chorionic somatomammotropin and ovine pituitary lactogenic hormone. Experentia **27**:1368–1371, 1971.
41. Wallis M. The primary structure of bovine growth hormone. FEBS Lett **5**:11–14, 1973.
42. Niall HD. Revised primary structure of human growth hormone. Nature **230**:90–91, 1971.