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Lipolytic Activity of Purified Pituitary and Bacterially Derived Growth Hormone on Chicken Adipose Tissue *in Vitro* (42210)

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Abstract. The ability of growth hormone (GH) to stimulate lipolysis was examined using chicken abdominal adipose tissue explants incubated *in vitro* and purified pituitary and bacterially derived chicken and bovine GH. Consistently in the fourth hour of incubation, lipolysis (as determined by glycerol release) was increased by the presence of GH (1 μg/ml), irrespective of pituitary or bacterial derivation or of chicken or bovine origins. This effect of GH was observed with adipose tissue originating from young (6–8 weeks old) intact and hypophysectomized chicks and adult (6–9 months old) male chickens. Glycerol release was also enhanced by lower doses of GH (10 ng/ml with tissue from young and 100 ng/ml with tissue from adult chickens).

The effects of growth hormone (GH) on lipid metabolism have been studied extensively in mammals and also to some extent in birds. In the presence of glucocorticoid, a GH preparation was shown to increase fatty acid and glycerol release from rat adipocytes and adipose tissue segments (1, 2). However, there is still controversy as to whether GH itself stimulates lipolysis in adipose tissue. It has been suggested that the lipolytic effect observed by GH preparations are due to acidic peptide contaminants which can be removed by further purification (3), although another report does not support this proposition (4). The availability of recombinant bacterially derived GH (which would not be expected to be contaminated with other pituitary factors) would allow for the determintion of whether GH per se stimulates lipolysis. Bacterially derived human GH has been found to stimulate lipolysis in the presence of dexamethasone in adipose tissue from fed rats (4, 5), but not fasted rats (5). In addition, incubating adipose tissue from hypophysectomized rats with recombinant human GH, followed by theophylline, led to increased glycerol release (6).

The present study examines the ability of native (pituitary) and biosynthetic GH, of either bovine or chicken origin, to stimulate lipolysis in chicken adipose tissue *in vitro*. It has been previously observed that chicken GH of pituitary origin stimulated *in vitro* glycerol release by adipose explants from chickens, turkeys, and pigeons (7). Thus, the domestic fowl provides a suitable system with which to investigate the possible lipolytic role of GH. Furthermore, the existence of purified pituitary (8) and bacterially-derived (9) chicken GH for studies in a homologous species provide an additional advantage.

Materials and Methods. Young (6–8 weeks) and adult (6–9 months) male chickens (strain, White Leghorn) were employed in these studies. Food (commercial "grower" diet) and water were available *ad libitum*. Adipose tissue explants were prepared by a modification of the method of (7). In one study, tissue was obtained from young chickens (6 weeks) which had been surgically hypophysectomized (for 24 days) by the method of King (10). Following sacrifice, abdominal adipose was rapidly excised and placed into incubation media (Krebs–Ringer–Hepes medium containing 20 m*M* glucose, 1 mg/ml bovine serum albumin (Armour, Fraction V), and 2.54 m*M* $CaCl_2$) at 37°C. The adipose tissue from three animals was diced into explants (approximately 1–2 mm^3) and randomly pooled. Each incubation flask contained 7–10 explants with a total weight of 50–100 mg. The explants were incubated in 1 ml Krebs–Ringer–Hepes medium (at 37°C, pH 7.4) under an atmosphere of 95% O_2/5% CO_2 in a shaking water bath. Following a 1-hr preincubation period, the medium was discarded and replaced with fresh medium and treatment in 50 μl. The explants were then incubated for four successive 1-hr periods, with media and treatment being replaced at the end

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of each. Previous studies have indicated glycerol release using a single glycerol determination at the end of a 4-hr incubation period. Therefore, data for glycerol release were expressed per hour (nanomoles per gram tissue per hour) and also as a cumulative total (nanomoles per gram tissue per 4 hr). Glycerol release into the media (a relative index of lipolysis) was determined by a fluorometric modification (11) of the enzymatic method of Wieland (12).

The following hormonal preparations and chemicals were employed: native bovine GH (Miles Laboratories), native chicken GH (purified by the method of Harvey and Scanes (8)), biosynthetic bovine somatotropin (GH, donated by Eli Lilly Research Laboratories), biosynthetic chicken GH (9, donated by Hoffman-La Roche). Statistical differences between means were determined by analysis of variance (ANOVA), followed by least significant differences (LSD).

Results. The effects of native and biosynthetic GH, of either bovine or chicken origin, on lipolysis are summarized in Table I. The basal release of glycerol (control values) was observed to be somewhat variable between studies. This is consistent with data from previous investigations using avian adipose tissue or adipocytes (7, 13, 14, 15). In all studies, glycerol release from adipose tissue was increased in the fourth hour of incubation, irrespective of the GH preparation employed and whether tissue from young (intact or hypophysectomized) or adult (intact) chickens was used. Dealing first with the studies from young chickens, glycerol release was not affected in the first hour of incubation by native or biosynthetic chicken GH or by native bovine GH. Biosynthetic bovine GH stimulated lipolysis, however, in all four incubation periods. There was a marked increase in GH-induced lipolysis in adipose tissue from intact chickens with time of incubation. For instance, bacterially derived chicken GH stimulated glycerol release by adipose tissue from young chicks; the effect being progressively greater in the second (19% above control), third (40% above control), and fourth (50% above control) incubation periods. Not only did biosynthetic GH evoke an earlier lipolytic response than native GH in young chicken adipose tissue, but also the cumulative total for glycerol released was greater than for pituitary-derived GH. Tissue from hypophysectomized young chicks responded to GH at all incubation times examined.

In adipose tissue from adult chickens, native chicken GH increased glycerol release throughout the incubation; the increases in lipolysis being 18, 51, 79, and 84% of the control, in the first to fourth hours of incubation, respectively. The time course of the effect of biosynthetic chicken GH was similar with glycerol release significantly increased only in the second, third, and fourth hours of incubation (by 11, 28, and 59%, respectively). Native bovine GH stimulated glycerol release in the first (17% above control), second (30% above control), third (47% above control), and fourth (57% above control) hours of incubation. The time course of the effect of biosynthetic bovine GH was similar with glycerol release increased in all four hourly incubation periods (by 22, 34, 51, and 67% that of the control, respectively).

The ability of lower doses of biosynthetic bovine GH to affect lipolysis was investigated using adipose explants from both young and adult chickens (Table II). In view of the greater responses to GH observed in the fourth hour of incubation, the dose–response studies were performed only at this time point. Lipolysis in adipose tissue from young chickens was increased by GH at all doses tested (10 ng/ml by 26%; 100 ng/ml by 38%; 1000 ng/ml by 43%). However, responses were only observed with the higher doses of GH (100 ng/ml by 52% and 1000 ng/ml by 58%) in tissue from adult domestic fowl.

Discussion. The present study demonstrates that GH per se stimulates lipolysis in the domestic fowl. This lipolytic response was observed with either bovine or chicken GH and irrespective of whether the hormone was of pituitary or recombinant bacterial origins. Thus, the lipolytic activity of the GH preparations on chicken adipose tissue would appear to be due to their GH contents and not some lipolytic contaminant as suggested by other studies [e.g. (3)]. This is the first report where biosynthetic GH has been shown to be lipolytic in its homologous species, the domestic fowl. Furthermore, it is also the first report of the lipolytic actions of biosynthetic bovine GH; previous studies (4, 5, 6) having been limited

TABLE I. EFFECT OF PITUITARY AND RECOMBINANT-DERIVED GROWTH HORMONE[a] (1 μg/ml) ON GLYCEROL RELEASE FROM CHICKEN ADIPOSE TISSUE EXPLANTS *IN VIVO*

Treatment	Source of tissue	Glycerol release during incubation (nmole/g ± SEM, $N = 6$)[b] 1st hr	2nd hr	3rd hr	4th hr	Total
Study 1						
Control	Young	288 ± 7	287 ± 12	287 ± 9	293 ± 13	1154 ± 27
Native cGH	Young	272 ± 11	282 ± 10	303 ± 12	419 ± 18***	1276 ± 31**
Biosyn. cGH	Young	294 ± 9	341 ± 12	403 ± 14***	440 ± 19***	1477 ± 33***
Study 2						
Control	Young	366 ± 13	359 ± 23	370 ± 17	362 ± 18	1456 ± 23
Native bGH	Young	380 ± 15	380 ± 18	396 ± 12	480 ± 19***	1636 ± 39**
Biosyn. bGH	Young	461 ± 22***	460 ± 22**	476 ± 15***	507 ± 16***	1904 ± 47***
Study 3						
Control	Young Hx[c]	198 ± 10	204 ± 11	207 ± 9	203 ± 12	811 ± 28
Native bGH	Young Hx	231 ± 8***	267 ± 10***	287 ± 8***	263 ± 9***	1047 ± 29***
Biosyn. bGH	Young Hx	274 ± 9***	293 ± 9***	278 ± 8***	282 ± 6***	1127 ± 27***
Study 4						
Control	Adult	379 ± 12	377 ± 6	380 ± 11	371 ± 10	1507 ± 31
Native cGH	Adult	447 ± 18**	569 ± 15***	681 ± 24***	682 ± 16***	2382 ± 62***
Biosyn. cGH	Adult	404 ± 6	417 ± 11*	487 ± 8***	590 ± 15***	1900 ± 36***
Study 5						
Control	Adult	236 ± 6	235 ± 11	227 ± 11	238 ± 7	936 ± 25
Native bGH	Adult	276 ± 16*	305 ± 16**	333 ± 21***	373 ± 15***	1287 ± 41***
Biosyn. bGH	Adult	289 ± 17***	314 ± 19***	343 ± 14***	397 ± 11***	1344 ± 33***

Note. Different from respective control $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by ANOVA and LSD.

[a] Tissue was incubated in the presence or absence of GH for four 1-hr periods. At the end of each period, media was removed and replaced with fresh media and treatment.

[b] Six replicates per treatment group.

[c] Hx denotes tissue from hypophysectomized chickens.

TABLE II. EFFECTS OF BIOSYNTHETIC BOVINE GH ON *IN VITRO* LIPOLYSIS IN CHICKEN ADIPOSE TISSUE

Dose of GH (ng/ml)	Glycerol Release[a] (nmole/g tissue ± SEM, $N = 6$)[b] Source of tissue: Young chick	Adult chicken
0	348 ± 19	316 ± 13
10	438 ± 18*	335 ± 15
100	479 ± 29***	479 ± 16***
1000	500 ± 24***	498 ± 20***

Note. Differs from control $*P < 0.05$, $***P < 0.001$ by ANOVA and LSD.

[a] Tissue was incubated in the presence or absence of GH for four 1-hr periods. At the end of each period, media was removed and replaced with fresh media and treatment. Data shown is for the fourth hour of the incubation.

[b] Six replicates per treatment group.

to bacterially derived human GH. In tissue from young chickens (intact or hypophysectomized), biosynthetic GH tended to evoke greater increases in glycerol release than did the native hormone. It is probable that this simply reflects the greater purity of the biosynthetic GH preparations.

In view of the low concentrations of GH required to stimulate lipolysis (Table II), it is tempting to suggest that GH has a physiological role, controlling lipolysis in the domestic fowl. Not only are similar concentrations of GH observed in the circulation of chickens [e.g. (22)], but also plasma concentrations of GH are affected by nutritional deprivation in chickens. For instance, in young chicks, plasma concentrations of GH are elevated by fasting (23) and chronic protein deprivation (24).

It should be noted that earlier investigations have provided evidence that GH is lipolytic in birds. In ducks, circulating concentrations of free fatty acids have been found to be depressed following hypophysectomy, while replacement therapy with bovine GH elevated the circulating concentrations of free fatty acids (16). Similarly, ovine GH increases plasma concentrations of fatty acids in pigeons (17). *In vitro,* purified pituitary chicken GH has been demonstrated to increase glycerol release by adipose explants from chickens, turkeys, and pigeons (7). In the present study, native chicken GH (1 μg/ml) increased glycerol release by adipose tissue from adult chickens by 59%, while previously native chicken GH (20 μg/ml) increased glycerol release by 93% (adult chicken adipose tissue). It should be noted that in earlier work (7), bovine GH did not significantly increase lipolysis by chicken adipose tissue, while bovine GH was effective in the present studies (Table I). This may reflect an increase in the precision of the methods employed with the mean coefficient of variance for studies with adult adipose tissue in (7) being 44.1 and 5.4% in the present study (Table I). In addition, greater lipolytic responses in adult adipose tissue were observed with native chicken GH than with biosynthetic chicken GH or with bovine GH (Table I). It is possible that part of the response to purified pituitary chicken GH may reflect contamination with other lipolytic factors (perhaps adrenocorticotropic hormone) (18). No evidence for species specificity of GH affecting lipolysis was found in the present study. This is similar to the observations that both chicken and bovine GH inhibit insulin-induced lipogenesis in chicken hepatocytes (7) and stimulate glucose uptake by chicken adipose tissue (19).

Although GH has been found to stimulate glycerol release from both chicken and rat adipose tissue *in vitro,* there are both similarities and differences in the responses. In tissue from both species, the greatest lipolytic effects of GH are observed in the fourth hour of incubation [e.g. (1, 6, 20)]. In intact rats, the effect of GH on lipolysis requires the presence of dexamethasone in the incubation medium (1). However, with chicken adipose tissue, the lipolytic effect of GH was demonstrated in the absence of glucocorticoids [see Table I and (7)]. Chicken adipose tissue responds to GH in a manner similar to that of hypophysectomized (6) or weanling rats (21).

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