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Inhibition of Growth Hormone-Stimulated Lipolysis by Somatostatin, Insulin, and Insulin-like Growth Factors (Somatomedins) *in Vitro* (42819)

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Abstract. The effects of somatostatin, insulin, insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II)/MSA on growth hormone (GH) (1 μg/ml)-induced lipolysis were examined employing chicken adipose tissue *in vitro*. Basal and GH-stimulated glycerol release were inhibited by somatostatin (1 ng/ml) and by IGF-II/MSA (10 and 100 ng/ml). Insulin and IGF-I (10 and 100 ng/ml) completely inhibited the lipolytic response to GH without affecting basal glycerol release. Insulin and IGF-I were equipotent in inhibiting GH-induced lipolysis while IGF-II is only 16% as potent as insulin.

Pituitary- and recombinant-derived growth hormone (GH) have been reported to stimulate lipolysis *in vitro* by adipose tissue/adipocytes from a number of species (rat (1,2), mouse (3), cattle (4), chicken/turkey/pigeon (5, 6)). In chicken adipose tissue, the lipolytic response to GH can be consistently observed within 1 hr of incubation, increasing in magnitude for up to 4 hr (6).

Hormonal influences on the lipolytic response to GH have received little attention, in any species, although several hormones have been reported to be antilipolytic including: somatostatin (SRIF), insulin, and somatomedins/insulin-like growth factors (IGF-I and IGF-II). Somatostatin has been demonstrated to inhibit basal and glucagon-stimulated lipolysis by chicken adipocytes (7). Insulin inhibits epinephrine- (8, 9) and glucagon (10, 11)-stimulated glycerol release by rat adipocytes *in vitro*. Insulin has also been reported to depress basal lipolysis by rat adipocytes *in vitro* (1, 11, 12), although this effect has not been consistently observed (10, 13). In contrast, no inhibitory effects of insulin on basal or glucagon-stimulated lipolysis are seen with chicken adipose tissue or adipocytes *in vitro* (14–16). Similar to the effects of insulin in rat adipocytes, partially purified somatomedins/insulin-like growth factors reduce epinephrine-stimulated, but not basal, glycerol release by adipose tissue from fed rats *in vitro* (17, 18). However, purified IGF-I and IGF-II do depress basal lipolysis by adipocytes from fasted–refed rats (19). The present studies examine the effects of SRIF, insulin, IGF-I, and IGF-II/MSA on basal and GH-induced lipolysis by chicken adipose tissue explants.

Materials and Methods. Abdominal adipose tissue from adult (6–9 months) White Leghorn male chickens was employed in all studies. Adult chicken body weights were relatively constant and hence, adipose cell size was expected to be stabilized. Chickens were provided a commercial diet (Chick Grower, Agway) and water *ad libitum* prior to experimentation. Abdominal adipose tissue explants were prepared as previously described (6). Adipose tissue was rapidly excised from two to four chickens per trial and randomly distributed into vials (7–10 explants per vial; 50–100 mg total weight, six replicate vials per treatment repeated in three studies) containing 1 ml Krebs–Ringer–Hepes medium (pH 7.4) supplemented with 15 m*M* glucose, 1% bovine serum albumin (Armour, Fraction V), and 2.54 m*M* $CaCl_2$. All incubations were conducted in a shaking water bath (37.5°C, 70 oscillations/min) under a 95% O_2/5% CO_2 atmosphere. Following a 1-hr preincubation period, the "preincubation" media was discarded and replaced with fresh Krebs–Ringer–Hepes media. The adipose tissue explants were then incubated for 1 hr in the presence or absence of GH and/or putative antilipolytic hormones. Glycerol release into the media, determined by an enzymatic/fluorometric method (20), was utilized as a relative index of lipolysis.

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TABLE I. EFFECT OF SOMATOSTATIN ON THE LIPOLYTIC RESPONSE TO GH BY CHICKEN ADIPOSE TISSUE EXPLANTS

	Glycerol release during incubation (nmole/g tissue ± SEM (N = 3)*)
Control	262 ± 6[b]
GH (1 μg/ml)	431 ± 19[c]
Somatostatin (1 ng/ml)	185 ± 5[a]
GH (1 μg/ml) + somatostatin (1 ng/ml)	284 ± 17[b]

[a-c] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

Biosynthetic bovine somatotropin (GH) and biosynthetic human insulin-like growth factor I were generously provided by Eli Lilly Research Laboratories (Indianapolis, IN) and Kabi Laboratories (Stockholm, Sweden), respectively. Buffalo rat hepatocyte (line BRL-3A) CR multiplication stimulating activity (MSA or IGF-II), the rat homolog of human IGF-II (21), was obtained from Collaborative Research, Inc. (Bedford, MA). Somatostatin (synthetic 1–14) and bovine insulin were obtained from Sigma Chemical Co. (St. Louis, MO). Mammalian insulin was employed to provide ready comparison to mammalian somatomedin preparations. Statistical differences comparing treatment means of three independent experiments (N = 3) were determined by analysis of variance followed by least significant differences. Within each experiment the coefficient of variation was less than or equal to 6.0%. However this variation was not considered in the analysis.

Results. The effect of somatostatin (1 ng/ml) on basal and GH-induced lipolysis in chicken adipose tissue explants is shown in Table I. Biosynthetic bovine GH stimulated lipolysis (by 65%, that of the control) within 1 hr of incubation. Somatostatin completely inhibited this lipolytic effect of GH by chicken adipose tissue *in vitro*. In addition, somatostatin depressed basal glycerol release (by 29%).

The actions of insulin, IGF-I, and IGF-II/MSA (Table II) on basal and GH-induced lipolysis have been investigated using chicken adipose tissue explants. Bovine insulin did not affect basal glycerol release at any of the concentrations examined (1, 10, or 100 ng/ml) (Table II). Insulin, at doses of 10 and 100 ng/ml, blocked GH-induced lipolysis. However, the 1 ng/ml dose of insulin did not affect GH-induced lipolysis. Recombinant-derived human IGF-I (1, 10, or 100 ng/ml) did not affect basal lipolysis by chicken adipose tissue explants (Table II). As observed with similar doses of insulin, GH-stimulated lipolysis was inhibited by 10 and 100 ng/ml, but not 1 ng/ml, of IGF-I.

Addition of rat IGF-II/MSA to chicken adipose tissue (Table II) yielded somewhat

TABLE II. EFFECT OF INSULIN, IGF-I, OR IGF-II/MSA ON GH-INDUCED LIPOLYSIS BY CHICKEN ADIPOSE TISSUE *IN VITRO*

	Glycerol release during incubation (nmole/g tissue ± SEM (N = 3)*)	
	No addition	GH (1 μg/ml)
Study 1		
Control	238 ± 5[a]	385 ± 28[b]
Insulin (1 ng/ml)	233 ± 5[a]	346 ± 12[b]
Insulin (10 ng/ml)	251 ± 9[a]	254 ± 4[a]
Insulin (100 ng/ml)	242 ± 19[a]	226 ± 13[a]
Study 2		
Control	241 ± 11[a]	395 ± 24[b]
IGF-I (1 ng/ml)	252 ± 14[a]	355 ± 33[b]
IGF-I (10 ng/ml)	264 ± 15[a]	243 ± 11[a]
IGF-I (100 ng/ml)	257 ± 10[a]	245 ± 7[a]
Study 3		
Control	247 ± 7[b]	397 ± 16[d]
IGF-II (10 ng/ml)	197 ± 10[a]	322 ± 15[c]
IGF-II (100 ng/ml)	165 ± 7[a]	243 ± 15[b]

[a-d] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

different results than that obtained with either insulin or IGF-I. Unlike insulin or IGF-I, IGF-II/MSA depressed basal glycerol release (10 ng/ml, by 19%; 100 ng/ml, by 32%). In addition, 10 ng/ml IGF-II/MSA partially inhibited the lipolytic response to GH, with 100 ng/ml IGF-II/MSA effecting a complete blockade.

Very similar rates of lipolysis (both basal and GH induced) were observed in the studies presented in Table II. By combining data from these studies, it was possible to compare the abilities of insulin, IGF-I, and IGF-II to inhibit GH-induced lipolysis. Insulin and IGF-I were equipotent in inhibiting GH-induced lipolysis (potency estimate IGF-I $\equiv$ 1.0 μg insulin/μg). However, IGF-II was less potent (IGF-II $\equiv$ 0.16 μg insulin/μg).

Discussion. The lipolytic effect of biosynthetic GH was inhibited by somatostatin, insulin, IGF-I, and IGF-II/MSA (Tables I and II). The inhibitory effect of insulin on GH-induced lipolysis is in contrast to its lack of effect on glucagon-stimulated lipolysis by chicken adipose tissue or adipocytes *in vitro* (15, 16). This is unlike the situation in rat adipose tissue, where insulin reduces glucagon-, epinephrine-, isoproterenol-, or ACTH-stimulated lipolysis *in vitro* (7–10). However, insulin does similarly block GH-stimulated lipolysis (in the presence of dexamethasone) with rat adipocytes (1). Hence, this is the first report describing an antilipolytic role for insulin in chickens (22). Antilipolytic effects on chicken adipose tissue have been previously observed with pancreatic polypeptide (23), prostaglandin E_1 (16), and somatostatin (7).

There are differences between the situation with chicken adipose tissue and that of the rat. Insulin and insulin-like growth factor (IGF-I and IGF-II) binding to rat adipocytes have been demonstrated *in vitro* (19). The antilipolytic, "insulin-like," effects of insulin-like growth factors (on epinephrine-stimulated lipolysis) in rat adipocytes are presumably exerted via the insulin receptor, since antilipolytic/insulin-like activity correlates strongly with IGF-I/IGF-II ability to compete for insulin receptor binding (24, 25). Rat adipocyte membranes have been suggested by some workers to contain only insulin and "type II" IGF receptors, with no detectable "type I" IGF receptors (26). With chicken adipose tissue, similar concentrations of insulin and IGF-I were observed to inhibit GH-stimulated lipolysis (Table II), but IGF-II was less effective. On the basis of this evidence, it is possible that these agents may bind to a similar receptor and activate common effector pathways. The biological potencies of insulin and the insulin-like growth factors (insulin = IGF-I > IGF-II) are in contrast to the presumed properties of receptor binding (27). For instance, binding of radiolabeled insulin to chick embryo brain (insulin) receptors can be displaced by insulin, IGF-I, and IGF-II, with IGF-I being approximately 20% as active as insulin and IGF-II being about 5% as active as insulin (28). Conversely, binding of either radiolabeled IGF-I or IGF-II was competitively inhibited by insulin, IGF-I, and IGF-II, with IGF-I being most potent, IGF-II less (with approximately 10% activity of IGF-I), and insulin being least potent (<10% activity of IGF-I). Indeed, there is little evidence for a distinct type II IGF receptor (with a greater affinity for IGF-II) in avian tissues. The lower activity of IGF-II in the present study may be explained by reduced affinity binding to this (insulin or IGF-I) single receptor site. However, the equivalence of insulin and IGF-I in the present studies is not consistent with the mutually exclusive involvement of either an insulin receptor or an IGF-II receptor. There may be a receptor, as yet undefined, with similar affinities for insulin and IGF-I on chicken adipose tissue. It is also possible that, following ligand binding, insulin and IGF-I receptors act cooperatively to exert an inhibitory effect on GH-stimulated lipolysis.

The growth-promoting effects of GH are presumed to be mediated by somatomedin C/IGF-I (28). However, Schwartz and Goodman (18) have suggested that somatomedins/IGF do not mediate GH-induced lipolysis, since semipurified rat somatomedins/IGF (in the presence or absence of dexamethasone) failed to reproduce the delayed lipolytic effects of GH with rat adipose tissue segments. With chicken adipose tissue explants, biosynthetic IGF-I and purified rat liver IGF-II/MSA do not mimic the effects of GH but, in fact, inhibit the lipolytic effect of GH (Table II). Therefore, as with rat studies (18), neither IGF-I nor IGF-II/MSA appears

to mediate the lipolytic action of GH in chicken adipose tissue.

The physiological relevance of both GH-induced lipolysis and the inhibition of GH-induced lipolysis by somatostatin, insulin, IGF-I, and IGF-II is open to question. The concentrations of insulin, IGF-I, and IGF-II which inhibited GH-induced lipolysis are broadly similar to the highest reported physiological circulating concentrations of these hormones in the chicken (insulin (29–31), IGF-I (32), IGF-II (32)). Thus, under these circumstances, GH is unlikely to be exerting a lipolytic effect *in vivo*. However, if a chicken was nutritionally deprived, then GH could ensure a steady release of free fatty acids to peripheral tissues via its inherent lipolytic activity and the absence of confounding inhibitory hormonal influences. There is substantial support for this notion. Fasting (34) and protein deprivation (35, 36) have been reported to elevate circulating GH levels in chickens. Somatomedin C/IGF-I is correspondingly depressed by fasting ((37–38); trend (39)) or protein deprivation (36) in chickens. Moreover, plasma insulin concentrations are observed to be either depressed (30) or not elevated in fasted chickens (22). In view of the ability of insulin to stimulate somatostatin release from pancreatic cells (40), it is reasonable to suggest that nutritional deprivation and consequent low insulin secretion reduce circulating concentrations of somatostatin. Indeed, a decrease in circulating concentrations of somatostatin have been observed in both sham-operated and partially pancreatectomized chickens (41).

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