Growth Hormone Alters Metabolic Effects and Proteolysis of Insulin in Adipose Tissue during Lactation (43394)

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Abstract. **To study the regulation of lipogenesis in adipose tissue by insulin and growth hormone during lactation, tissue was biopsied from primiparous bovines at 30 days antepartum and 60 days postpartum. Tissue was cultured for 24 hr or 48 hr in M199 with acetate and glucose, with a change of medium at 24 hr. The three *in vitro* treatments were: insulin and hydrocortisone at 10 and 50 ng/ml, respectively (IH); IH + 10 ng/ml of growth hormone (G10); and IH + 100 ng/ml of growth hormone (G100). IH allowed lipogenesis rates from 50% to 85% of those in fresh tissue. Addition of 10 ng/ml of growth hormone reduced ($P < 0.05$) lipogenesis; at 100 ng/ml, the effect was only slightly greater. The hypothesis that insulin and growth hormone could be degraded by bovine adipose tissue was tested. Adipose tissue cell-free extracts degraded ^{125}I-labeled insulin, but did not degrade labeled growth hormone. The insulin protease activity was further characterized and had a pH optimum of 7.1, a maximum hydrolysis of approximately 70%, and a hydrated molecular mass of approximately 23,000 daltons. Insulin proteolysis was inhibited by specific insulin protease inhibitors and stimulated by disulfide reducing agents. Bovine growth hormone, prolactin, and histone inhibited ($P < 0.05$) the proteolysis of insulin, while bovine serum albumin, egg albumin, trypsin inhibitor, and lysozyme did not. Adipose tissue from pregnant and lactating bovines was sensitive to insulin and growth hormone, and growth hormone may modulate activity of an insulin-specific protease.** [P.S.E.B.M. 1992, Vol 200]

In order to support the reproductive states of pregnancy and lactation, lipogenesis in adipose tissue is controlled by a system of regulatory hormones, two of the most important being insulin and growth hormone (1–4). Insulin usually increases the flux of carbon to fatty acids by increasing glucose entry and oxidation and by modulating the activity of a large number of enzymes in the conversion of carbohydrate to fat (1–7). Growth hormone can inhibit the increase in lipogenesis caused by insulin in adipose tissue of humans (8), rats (9), pigs (10–12), sheep, and cattle (13–16). However, the physiological and biochemical mechanisms of action of growth hormone and insulin on adipose tissue in pregnant and lactating animals are still unknown.

During lactation, adipose tissue responsiveness to insulin, as well as the rates of lipogenesis and esterification, decreases, while lipolysis and the responsiveness to catabolic hormones increase (1, 2, 4). Serum concentration of growth hormone increases during early lactation (1). The homeorhetic regulation of adipocyte metabolism by growth hormone appears to be quite complex, with effects being dependent upon species, physiological state, and nutritional status. *In vivo* treatment of lactating dairy cattle with bovine somatotropin may increase the sensitivity of adipose tissue to catabolic hormones (17); co-culture of adipose tissue with liver and mammary tissue from lactating cows in the presence of GH seems to decrease adipose tissue lipogenesis (18). One study reported that GH may increase or decrease insulin-stimulated lipogenesis in bovine adipose tissue *in vitro*, depending upon the lactational state (19). The ability of GH to inhibit the stimulatory effect of insulin appears to be well documented in

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growing domestic and laboratory animals (5, 9, 12, 13, 15); however, a direct, long-term *in vitro* antilipogenic effect of growth hormone on insulin action in adipose tissue from pregnant and lactating bovines has not been determined.

Adipocytes take up and hydrolyze insulin via a nonlysosomal pathway (20–24). Many of the longer term actions of insulin may rely on insulin-receptor complex internalization and metabolism (20, 21); however, the physiological significance of this pathway is unproven. Growth hormone can alter the ability of insulin to modulate the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase (25). Many tissues have insulin-specific proteases that are involved in the breakdown and subsequent action of insulin (20–22). It is possible that growth hormone may act via altering insulin degradation.

Therefore, we tested the following hypotheses: (i) growth hormone inhibits insulin-induced lipogenesis in *in vitro* tissue explant culture of adipose tissue from pregnant and lactating bovines; (ii) growth hormone is more effective at inhibiting lipogenesis in tissue from lactating than from pregnant animals; (iii) insulin and growth hormone can be degraded by bovine adipose tissue via specific proteases; and (iv) growth hormone can alter the proteolysis of insulin in bovine adipose tissue.

Materials and Methods

Animal Handling and Sampling. Animals were primiparous bovines of the Holstein breed, fed and housed at ambient temperature, as described previously (26, 27), and fed a grain and alfalfa hay ration according to National Research Council requirements via a computer-controlled feeding apparatus (26). After parturition, milk production and feed intake records were recorded daily and summarized weekly. Adipose tissue was biopsied under spinal block anesthesia, as described previously (28), from the perianal region at 30 days antepartum and 60 days postpartum. Milk production and fat and protein percentages for the month preceding the biopsy at 60 days of lactation were (mean ± SD) 30.5 ± 1.3 kg/day, 3.18 ± 1.3%, and 3.11 ± 0.05%. The net energy intake was 33.2 ± 7.3 Mcal/day.

Tissue Handling and Culture. *Sampling and preparation.* Seven animals provided tissue for culture studies. Adipose tissue was removed under sterile conditions, placed into vials containing sterile M199 (Gibco, Grand Island, NY) at 39°C. Tissue was cultured using techniques validated previously (12–15). Briefly, tissue was handled under sterile conditions in a closed-containment recycling filter chamber (CCI, Inc., Lansdale, PA) and sliced into 5- to 15-mg pieces, rinsed three times in sterile M199, and placed in 15-cm plastic petri dishes containing treatment medium. Tissue explants were cultured for 24 hr, then old medium was replaced with fresh medium of the same composition and explants were cultured for another 24 hr. Less than 90 min passed from biopsy to beginning of culture.

Treatments applied. M199 was prepared from dry powder with doubly distilled sterile water, and penicillin, streptomycin, and fungizone (ABAM; Flow Laboratories, McLean, VA) were added at 100 IU/ml, 100 μg/ml, and 250 ng/ml, respectively (final concentration). All media contained, in final concentration, 10 m*M* acetate, 5 m*M* glucose, 250 μ*M* sodium palmitate, and 25 m*M* HEPES. Treatment media were prepared by dissolving insulin in 0.005 *M* HCl, hydrocortisone in 100% ethanol, and growth hormone (GH) in 0.25 *M* Na_2CO_3 plus 0.25 *M* $NaHCO_3$ (pH 9.4) buffer. Initial dilutions of hormones from these stocks were made in sterile M199 and then diluted to final concentration in sterile medium containing nutrients, then the final medium was resterilized by filtration through a 0.22-μm filter (Corning, Corning, NY). Treatments were: insulin, 10 ng/ml, plus hydrocortisone, 50 ng/ml (IH); IH plus 10 ng/ml of growth hormone (G10); and IH plus 100 ng/ml of growth hormone (G100). These concentrations have been shown to promote biologically and statistically significant responses in similarly conducted experiments (12–15).

Determination of Lipogenesis, Cell Number, and Cell Size. Lipogenesis was determined in 2-hr incubations as described previously (15, 26). Cell number and size were determined as described previously (28). Metabolic data are expressed as nanomoles of acetate incorporated into lipid per gram of tissue or per cell.

Determination of Proteolytic Ability. *Preparation of labeled hormones.* Bovine insulin (I5500; Sigma Chemical Co., St. Louis, MO) and pituitary-derived growth hormone (lot AIP-S50; donated by the Upjohn Co.) were iodinated by the chloramine T method of Greenwood *et al.* (29), using $Na^{125}I$ (NEN-Dupont, Boston, MA). Hormones were purified by chromatography on a 0.7 × 30-cm Sephadex G-150 column, equilibrated, and eluted with 0.02 *M* Na-phosphate and 0.15 *M* NaCl buffer (pH 7.5). One milliliter fractions were collected in tubes containing 0.15 mg/ml of bovine serum albumin, final concentration. The monomeric form of the labeled hormone was used in subsequent assays. Specific activity of labeled, purified monomeric hormones was approximately 50 mCi/mg. Iodinated hormones were diluted to a concentration of 0.75 μg/ml in 0.02 *M* Na-phosphate buffer with 0.5 *M* NaCl at pH 7.4. Aliquots of 1 ml were stored at −20°C for less than 1 week and thawed once immediately prior to use.

Preparation of Cell-Free Extracts. Adipose tissue cell-free extracts were made in 0.25 *M* sucrose (pH 7.4) at 2 ml of buffer/gram tissue by homogenization three times at setting 7 of a Polytron homogenizer (Kinematika; Brinkman Instruments, Westbury, CT) and

centrifugation at 4°C at 1000*g* for 15 min. Homogenates were kept on ice during the procedure. Extract protein was determined by the method of Lowry *et al.* (30).

Assay of proteolysis and characterization. Proteolysis was determined on adipose cell-free extracts prepared as described above. Ten lactating animals supplied tissue for these studies; however, all characteristics were not determined on each homogenate from all animals. Each protease characteristic was determined on extracts pooled from two to six animals, as noted in the Figure Legends. The among-animal variation was determined in some cases and is reported in the Tables or Figure Legends.

The assay procedure was as follows: incubations were at 37°C, in a shaking water bath, in triplicate and contained 0.1–0.15 ml of homogenate, 0.9–0.85 ml of 0.2 *M* Na-phosphate, 0.2 *M* citrate buffer, 300 μg/ml of defatted bovine serum albumin (Bovuminar reagent; Armour Pharmaceuticals, Kankakee, IL), and 30 μl of ^{125}I-insulin or ^{125}I-growth hormone (15 ng/assay). At timed intervals, three 0.1-ml aliquots of incubate were added to 0.2 ml of ice-cold 10% trichloroacetic acid (TCA). Then, another 1 ml of ice-cold 10% TCA was added and tubes were held on ice for 30 min, then centrifuged at 1200*g* for 15 min. Supernatant was removed and the precipitate was drained and total radioactivity was counted. The percentage of hydrolysis was figured as ((total dpm ^{125}I) − (dpm in ppt))/total dpm. After initial characterization of activity, assays were conducted for 30 min, within the linear range of activity versus protein and time (Table I and Fig. 3). Control tubes without adipose extracts were run with every assay and activity was corrected for nonspecific hydrolysis. Assays were done in duplicate, and three aliquots were counted. Variation among duplicates was routinely less than 5%.

Approximate determination of hydrated molecular size was done by passing a 1.5-ml sample of cell-free extract through a Sephacryl S300 column (buffered with 0.02 *M* phosphate and 10 m*M* dithiothreitol and 0.5 *M* NaCl [pH 7.0]) (1.2 × 55 cm) calibrated against molecular size markers (carbonic anhydrase, bovine serum albumin, (β-amylase, apoferritin, and thyroglobulin). One-milliliter fractions were collected and protein content was determined by A_{280} in a calibrated uv flow-through detector. An assay of proteolysis was then done on the fractions as described above.

Qualitative studies designed to initially characterize the type of proteolytic activity included the effects of time, protein, and pH dependence, and of proteinase inhibitors (leupeptin, antipain, pepstatin, phenylmethylsulfonyl fluoride, *N*-ethylmaleimide, *P*-hydroxymercuribenzoic acid, and 1,10-phenanthroline) and activators (dithiothreitol and glutathione, reagent grade; Sigma). The latter studies were conducted by diluting the agents in methanol and adding 10 μl of agent to the assay. Control tubes received 10 μl of methanol. Inhibitors were used within the allotted time frame for optimum activity. Assays were run on homogenates from three animals, in duplicate, and triplicate samples from each duplicate were counted as described above.

Studies to initially determine the effects of growth hormone and other proteins on the proteolysis of insulin were conducted on pooled homogenates from two to four cows. Effects of bovine growth hormone, bovine prolactin (USDA-bPrl-B-1; donated by the USDA), histone protein (Sigma H-9250, Type II-A from calf thymus), bovine serum albumin, egg albumin, lysozyme, trypsin inhibitor, and unlabeled insulin were determined by adding graded doses from 5 to 300 μg/ml of test protein to the assay described above. The among-animal variation from the three animals providing tissue homogenates pooled for use in these assays is given in the Legend to Figure 6.

Experimental Design and Analysis. The tissue culture experiment was designed as a randomized complete block with a split-plot, with animals as blocks and physiological state as the main plot effect and treatment of hormone and time of treatment as the subplot effects (31). Effect of treatment over time in culture was determined over both physiological states (biopsy time) and within each state. Significance of effects was tested against the residual error mean square within each physiological state and overall, or, if a significant interaction among main effects occurred, against that error mean square. Calculations were performed with the SAS software package (32).

Table I. Degradation of ^{125}I-Insulin by Bovine Adipose Tissue Homogenates *In Vitro*[a]

	Time of incubation		
	20 min	30 min	60 min
Percent degraded	23.4 ± 1.2	34.1 ± 3.8	47.3 ± 4.0

[a] Tissue was biopsied from pregnant primiparous Holstein cows. Tissue was homogenized and used at 1 mg of protein per incubation. Incubations were at 37°C in triplicate, as described in Materials and Methods. Percentage of hydrolysis was figured as (total dpm ^{125}I − dpm in ppt)/total dpm. Results are means ± among-animal SE from homogenates from three cows (six replicates per homogenate). Intra-assay variation was less than 5%.

Results

Lipogenesis from acetate in fresh tissue was 648 ± 400 and 941 ± 352 nmol/2 hr/g tissue in pregnant and lactating primiparous animals, respectively; these were not different ($P > 0.05$). Activity of lipogenesis was lower after 24 and 48 hr of culture in IH medium (Fig. 1). Activity in IH treatments was 336 ± 74 and 785 ± 347 nmol/2 hr/g tissue after 24 hr in tissue from pregnant and lactating animals, respectively. This requirement of insulin and hydrocortisone for mainte-

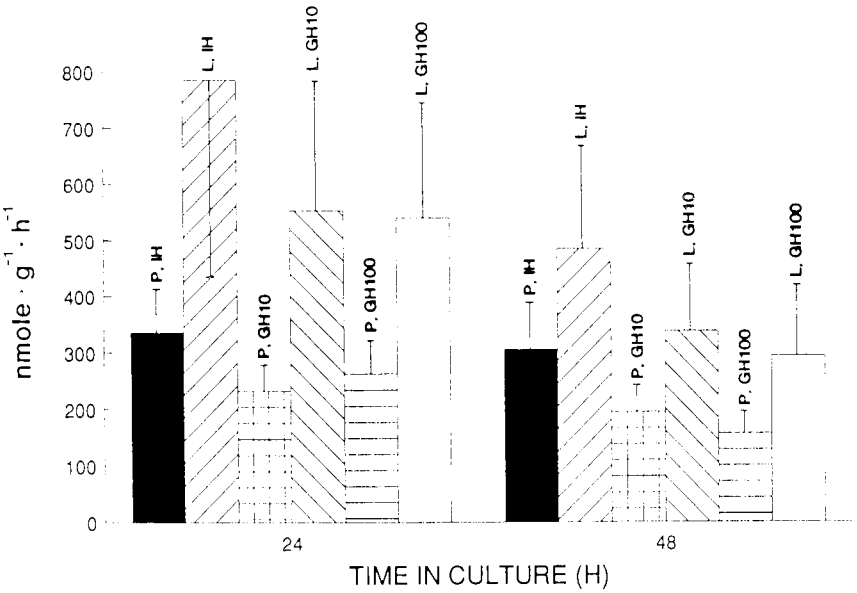

Figure 1. Lipogenesis from acetate per gram adipose tissue from pregnant or lactating bovines in *in vitro* tissue explant culture. Tissue was obtained via subcutaneous biopsy at −30 days (P; $n = 7$) and +60 days (L; $n = 6$) about parturition. Tissue was cultured under sterile conditions 24 or 48 hr in M199 containing 10 ng/ml of insulin and 50 ng/ml of hydrocortisone (IH) and either 10 (GH10) or 100 ng/ml of bovine GH (GH100). Tissue was removed and placed in 2 ml of Krebs-HEPES, 3% bovine serum albumin medium to measure lipogenesis from 2-[^{14}C] acetate for 2 hr. Bars are SE. The effect of treatment dose with GH was significant ($P < 0.05$ overall) and at each day. Rates of lipogenesis in fresh tissue were 648 ± 400 and 941 ± 352 for P and L, respectively.

nance of some anabolic activity has been demonstrated previously in adipose tissue of various species (12–16).

Growth hormone inhibited ($P < 0.05$) lipogenesis at either 10 or 100 ng/ml in tissue from both pregnant and lactating animals (Fig. 1). The effect was time dependent, with the inhibitory effect of GH being greater ($P < 0.05$) at 48 hr than at 24 hr. However, the effect was not significantly greater at 100 ng/ml than at 10 ng/ml. There was no interaction between GH effect and time of biopsy; that is, the effect of GH was not different in tissue from lactating versus pregnant animals.

Adipocyte size and number change during lactation in bovines (28). Therefore, we measured the adipocyte size and number per gram of tissue. At 30 days antepartum, mean cell diameter was 125.6 ± 4.3 μm; at 60 days postpartum, it was 118.7 ± 5.0 μm. This corresponded to volumes of 1053 ± 107 and 888 ± 64 pl in pregnant and lactating animals, respectively. Cells per gram were 533 (± 109) × 10^5 and 847 (± 110) × 10^5 in pregnant and lactating animals. These differences were not significant ($P > 0.05$). When lipogenesis was expressed per million cells, the results were similar to those for activity per gram (Fig. 2). The effect of GH on insulin-stimulated lipogenesis was similar when the data were calculated as activity per cell.

Because certain actions of insulin and growth hormone may require uptake and further processing in adipocytes (20, 21), we tested whether bovine adipose tissue possesses the ability to degrade insulin and growth hormone. Degradation of insulin was rapid in tissue extracts at each pH tested with a pH optimum of approximately 7 (Figs. 3–5). The maximum activity was approximately 70% of the radiolabeled insulin hydrolyzed to products unprecipitable in TCA, primarily amino acids and small peptides (Figs. 3 and 4). Addition of sulfhydryl protecting agents increased hydrolysis to 100% (Table II).

No significant degradation of ^{125}I-bovine growth hormone was noted at any pH under conditions identical to those for insulin degradation (less than 2% degradation, repeated on homogenates from three animals, data not shown). In order to determine whether some partial degradation of growth hormone was occurring that would not be measured by TCA precipitation, two additional protease assays with ^{125}I-GH were conducted as described, and then the entire assay incubates were eluted through 0.7 × 30-cm Sephadex G-100 columns (see Materials and Methods). The major peak (>95% total radioactivity) co-eluted with unlabeled, undegraded GH (data not shown). Although these methods cannot eliminate the possibility of internal cleavage of the protein, it is clear that bovine adipose tissue cannot degrade growth hormone to amino acids and small peptides in the same fashion that it can degrade insulin.

The activity of the putative insulin protease was sensitive to inhibitors (*n*-ethylmaleimide and *p*-hydroxymercuribenzoic acid) and activators (glutathione and dithiothreitol) of insulin-specific proteases (Table II). The inhibitory agents, known to inhibit insulin-specific proteases in other cell types (20–22), almost completely

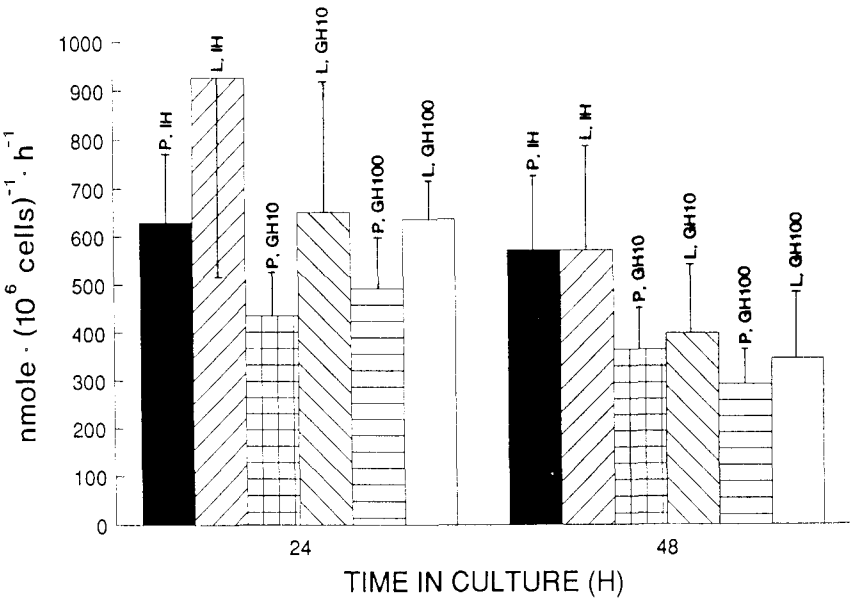

Figure 2. Lipogenesis from acetate per million adipocytes from pregnant or lactating bovines in *in vitro* tissue explant culture. Tissue was obtained and cultured as described in the Legend to Figure 1. Means represent seven animals in P and six in L, and bars are SE. The effect of a dose of GH was significant ($P < 0.05$) overall and at each day. Rates of lipogenesis in fresh tissue were 1215 ± 634 and 1111 ± 416 per million adipocytes for P and L, respectively.

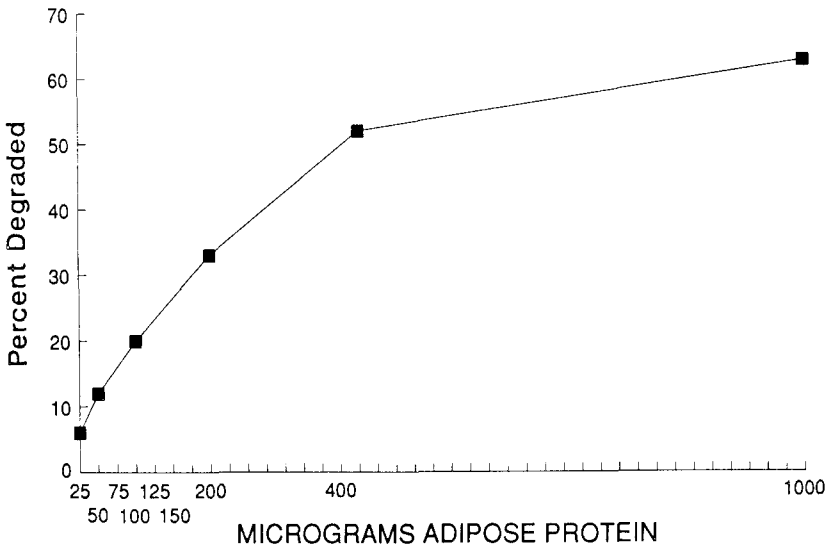

Figure 3. Degradation of ^{125}I-insulin by bovine adipose cell-free extracts and effects of extract protein level. Adipose tissue cell-free extracts were prepared by centrifugation and homogenization from adipose tissue from three lactating bovines, and homogenates were pooled and aliquots were incubated with phosphate buffer, ^{125}I-insulin, and bovine serum albumin. Determination of degradation was made by precipitating remaining protein with TCA. Triplicate samples were counted and percentage of degradation was determined by gamma counting. Intra-assay variation was less than 5%. Tissue protein was determined by the Lowry method. Controls contained no tissue extract. This is representative of proteolytic activity from five studies on pooled tissue extracts.

inhibited activity of the protease. Sulfhydryl group modifiers increased the amount of proteolysis of radiolabeled insulin. Other protease inhibitors, nonspecific against insulin proteases (EDTA, 1,10-phenanthroline, leupeptin, phenylmethylsulfonyl fluoride, and antipain), had little or no effect on the activity. The apparent hydrated molecular mass, under reducing conditions, of the protein containing proteolytic activity was 23,000 daltons.

Because we were studying the effects of GH on insulin action, the presence of an insulin-specific protease prompted the obvious question as to whether GH altered this reaction. Various proteins were tested for their ability to inhibit the cell-free hydrolysis of ^{125}I-insulin (Figs. 6 and 7). Both growth hormone and prolactin inhibited the *in vitro* degradation of ^{125}I-insulin in bovine adipose tissue cell-free extracts. Histone protein also inhibited this activity to the same

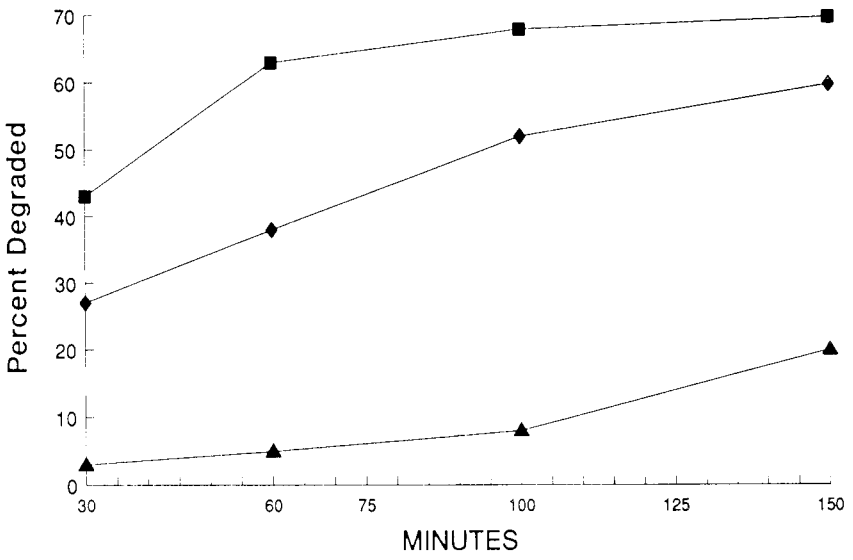

Figure 4. Degradation of ^{125}I-insulin by bovine adipose cell-free extracts and effect of pH. Adipose tissue cell-free extracts were prepared and activity was determined as described in the Legend to Figure 3 on pooled homogenate from three lactating bovines. Intraassay variation was less than 5%. Controls contained no tissue extract. ▲, pH 3.3; ♦, pH 5.0; ■, pH 7.8.

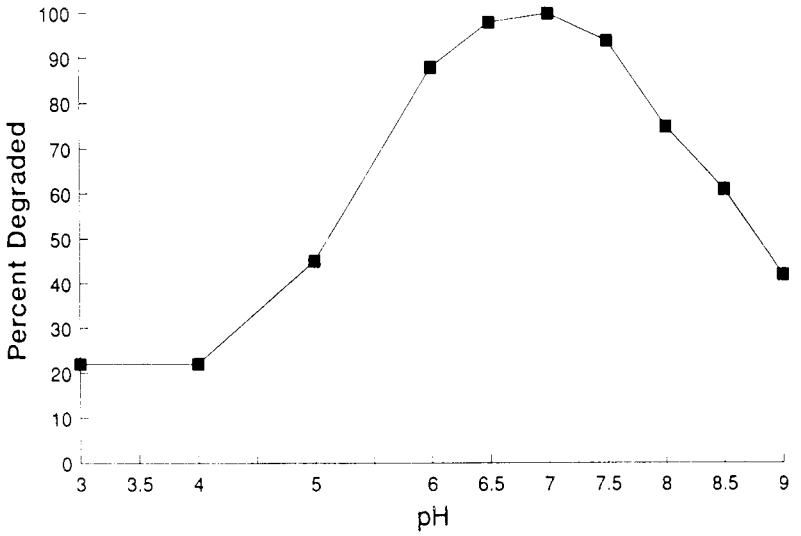

Figure 5. Effect of pH on degradation of ^{125}I-insulin by bovineadipose cell-free extracts. Adipose tissue cell-free extracts were prepared from three lactating bovines and activity was determined as described in Figure 3. Intra-assay variation was less than 5%. Controls contained no tissue extract.

extent. However, bovine serum albumin, egg albumin, lysozyme, and trypsin inhibitor did not affect the activity. Unlabeled insulin also decreased the activity of the protease against ^{125}I-insulin. The decrease in activity due to growth hormone and prolactin could not be accounted for stoichiometrically by dilution of radiolabeled protein. Bovine serum albumin, normally present in the assay at 100 times greater concentration than insulin, did not inhibit activity.

Discussion

Rates of lipogenesis in adipose tissue during lactation are partially dependent upon rates of milk production, energy intake, and resultant energy balance (26–28); however, there is wide variation even among animals in similar conditions. Previous studies with first lactation animals of average milk production (approximately 30 kg/day) and positive energy balance (26) determined rates of lipogenesis in the same range as that measured in the cows in the present study.

The metabolic effects of growth hormone on adipose tissue metabolism are complex and dependent upon species, physiological state, and nutritional status (33). One of the effects of growth hormone is the ability to partially inhibit the stimulatory effects of insulin in cultured adipose tissue from growing, pregnant, and lactating animals (9, 10, 12–16). The *in vivo* administration of GH to lactating dairy cattle may also decrease

Table II. Inhibition/Activation Analysis of ^{125}I-Insulin Proteinase in Bovine Adipose Tissue Cell-Free Extracts[a]

Reagent	Concentration (μM)	Proteinase Activity (% control)	
		30 min	60 min
Control	No addition	100	100
Phenylmethylsulfonyl fluoride	0.5	94.1	90.3
Leupeptin	2.0	91.6	98.6
Antipain	2.0	99.4	95.0
Pepstatin	3.3	100.0	98.9
Glutathione	2.0	181.3	161.2
Dithiothreitol	2.0	292.7	190.2
N-Ethylmaleimide	5.0		1.5
	1.0		1.2
p-Hydroxymercuribenzoic acid	1.0		0.0
	0.1		1.3
1,10-Phenanthroline	1.0		67.1
EDTA	10.0		85.4
	1.0		89.3

[a] Tissue was biopsied from three lactating primiparous bovines and homogenized as described in Materials and Methods. Proteinase activity was determined in triplicate under the conditions described in Table I and Materials and Methods. Control activity was 37.1 ± 0.64% and 49.1 ± 1.0% degraded at 30 and 60 min, respectively (mean ± SE from six replicates). Intra-assay variation was less than 5%.

lipid synthesis and increase the response of lipolysis to catecholamines. These effects are not the same across all physiological and nutritional states. For example, in early lactation, the effect on lipolysis may be greater than during later lactation (17, 33–35). These complex responses to growth hormone demonstrate that its regulatory actions are homeorhetic, that is, it can coordinate tissue metabolism differently within various physiological states (1, 33). This demonstrates the need to determine the qualitative and quantitative effects of these hormones in specific species in specific physiological states.

Growth hormone may be able to increase fatty acid release from bovine adipose tissue co-cultured with mammary tissues *in vitro* (18); it may also directly decrease insulin-stimulated lipogenesis during early lactation and cultured *in vitro* (19). These observations have not been confirmed. Our results confirm that GH does directly inhibit insulin-stimulated lipogenesis in adipose tissue from pregnant and lactating bovines. However, there was no clear indication that the response of adipose tissue to GH was different between 30 days antepartum and 60 days postpartum. The animals used in our study were only average milk producers that were in positive energy balance at 60 days of lactation. It is possible that tissue from animals of higher milk production may respond differently. In addition, no further effect was demonstrated at 100 μg/ml vs 10 μg/ml of GH, suggesting that this property of GH exists at physiological concentrations.

No clear cellular mechanism or mechanisms have as yet emerged that explain these effects of growth hormone. Adipose tissue has GH receptors (1, 2, 33); however, the response to GH does not appear to be an acute or rapid action, acting through the more typical

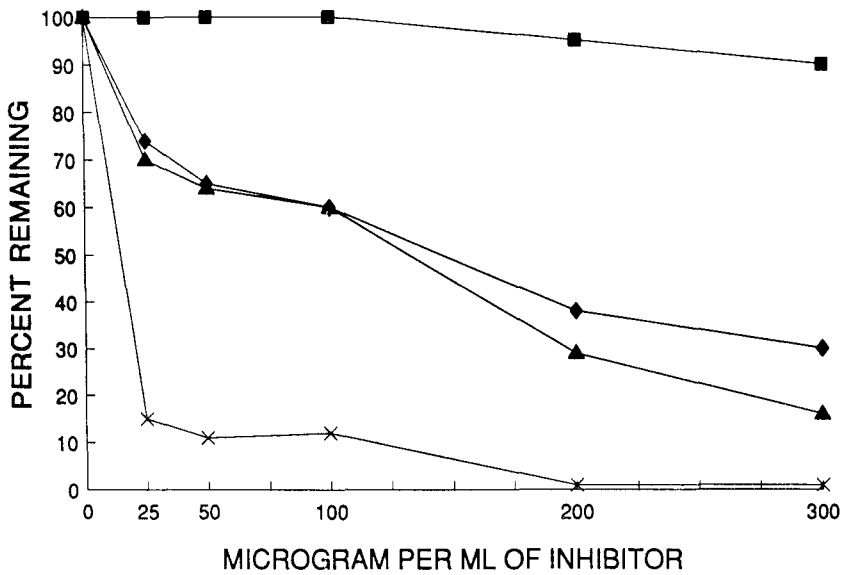

Figure 6. Effect of growth hormone, prolactin, and histone on protease activity against ^{125}I-insulin in adipose cell-free extracts from lactating bovines. Adipose tissue cell-free extracts were prepared from three lactating bovines and activity was determined as described in Figure 3. Proteins were dissolved in buffer and added to the mixture at the concentrations shown. Determination of degradation was made by precipitating remaining protein with TCA. ■, bovine serum albumin; ▲, bovine gH; bPrl ♦, bovine prolactin; X, insulin. Control activity in homogenates from three different cows on different days was 73.2 ± 3.94 (mean ± SE, among animals) percentage of insulin degraded. Intra-assay variation was less than 5%. Controls contained no tissue extract.

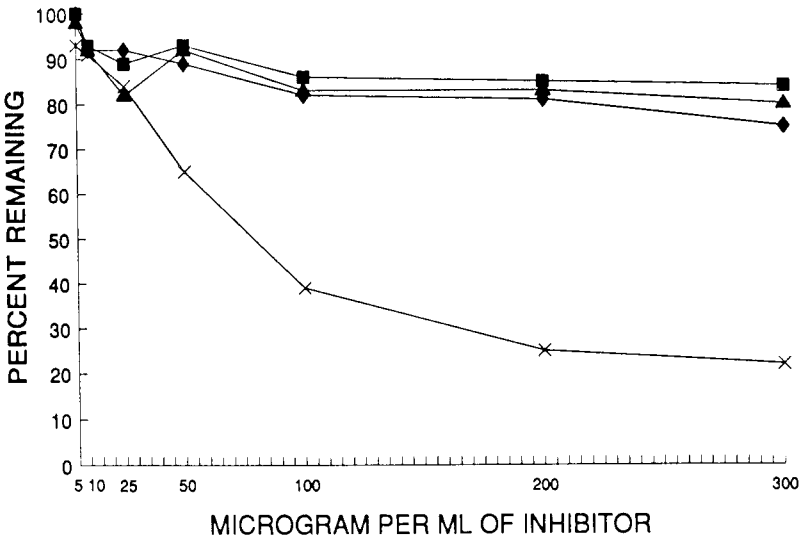

Figure 7. Effect of various proteins on protease activity against ^{125}I-insulin in bovine adipose cell-free extracts. Adipose tissue cell-free extracts were prepared from adipose tissue from three lactating bovines and activity was determined as described in Figure 3. Proteins were dissolved in buffer and added to the mixture at the concentrations shown. X, histone; ■, lysozyme; ▲, egg albumin; ♦, trypsin inhibitor. Determination of degradation was made by precipitating remaining protein with TCA. Intra-assay variation was less than 5%. Controls contained no tissue extract.

second messengers (e.g., cAMP and calcium). Rather, a longer term effect, requiring 24–48 hr for the full response, is required. Recently, it was shown that *in vivo* administration of GH to swine did not alter the number of insulin receptors nor the receptor kinase activity in adipose tissue *in vitro* (11).

Insulin effects on adipose tissue are varied, some (glucose uptake) acting very rapidly (5 sec) and requiring rapid signaling. Others, including increased protein synthesis and increased lipogenesis from carbohydrate, require increased enzyme synthesis and 24–48 hr for the full effect to be expressed. The latter phenomenon may require uptake of the hormone-receptor complex and subsequent degradation (20–22); however, a specific signaling mechanism is as yet unknown. Therefore, we began to study the possibility that bovine adipose tissue also possesses the ability to degrade insulin, and that GH may have some effect on this process.

Rat adipocytes have the ability to bind and internalize the insulin molecule as an insulin-receptor complex. We did not test internalization of hormones in our study. However, we did discover that bovine adipose tissue does indeed have the ability to degrade insulin. The majority of this activity is not considered to be lysosomal (20–22), a hypothesis supported by the high pH optimum of the protease activity in our study.

Bovine adipose tissue does not appear to possess proteolytic activity toward GH. Because our present assay measured only GH degradation to non-TCA-precipitable products, it is possible that some partial degradation occurred. However, upon testing this hypothesis, by molecular sieve chromatography postassay, it was not determined that any significant degradation of GH occurs in bovine adipose tissue under these conditions.

Although bovine adipose tissue may not rapidly degrade GH, it appears that GH may play a role in the degradation of insulin. We demonstrated, for the first time in any cell type, that GH and prolactin can inhibit the degradation of insulin. Because some proteins altered the activity while others did not, the inhibition is not likely just due to a dilution of ^{125}I-labeled protein. Histone protein also displayed such ability. Arginine-rich histones have been shown to possess some insulin-like properties in isolated rat adipocytes, such as stimulation of glucose incorporation and oxidation, while lysine rich histones do not (36). The physiological importance of these observations is unclear. However, if certain actions of insulin are carried out via internalization and metabolism to a histone-like peptide, then that may be a reason why histone could inhibit proteolysis of insulin.

The growth hormone and prolactin gene family of proteins possess a proline-induced "loop" near the N terminus of the molecule (37–39). Peptides of this composition have been shown to be inhibitors of insulin-specific proteases (39). Presently, we can only hypothesize that GH or part of it may inhibit insulin action via blocking proteolytic degradation of insulin and subsequent insulin action.

During lactation, adipose tissue metabolism is highly regulated by a combination of hormones. Growth hormone can partially inhibit the lipogenesis-stimulating action of insulin *in vitro* in adipose tissue from pregnant and lactating animals. We have demonstrated that this is a direct effect of GH on the tissue

during lactation. In addition, bovine adipose tissue extracts have the ability to rapidly degrade insulin but not growth hormone. The role of insulin internalization and degradation in metabolic regulation in adipocytes is not proven. It is entirely possible that the effects of GH and prolactin on insulin proteolysis are coincidental, and are not physiological phenomena *in vivo*. However, as both of these hormones have been reported to antagonize the effects of insulin in a wide variety of *in vivo* and *in vitro* conditions, it is possible to hypothesize that the effect of GH and prolactin on insulin action in adipose tissues is in part due to alteration of the normal internalization, processing, and subsequent actions of insulin.

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