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## Porcine leptin alters isolated adipocyte glucose and fatty acid metabolism<sup>☆</sup>

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### Abstract

This study examined if leptin can acutely affect glucose or fatty acid metabolism in pig adipocytes and whether leptin's actions on lipogenesis are manifested through interaction with insulin or growth hormone. Subcutaneous adipose tissue was obtained from ~55 kg crossbred barrows at the USDA abattoir. Isolated adipocytes were prepared using a collagenase procedure. Experiments assessed U-<sup>14</sup>C-glucose or 1-<sup>14</sup>C-palmitate metabolism in isolated adipocytes exposed to: basal medium (control), 100 nM insulin, 100 ng/ml porcine growth hormone, 100 ng/ml recombinant porcine leptin, and combinations of these hormones. Treatments were performed in triplicate and the experiment was repeated with adipocytes isolated from five different animals. Cell aliquots (250  $\mu$ l) were added to 1 ml of incubation medium, then incubated for 2 h at 37 °C for measurement of glucose and palmitate oxidation or incorporation into lipid. Incubation of isolated adipocytes with insulin increased glucose oxidation rate by 18% ( $P < 0.05$ ), while neither growth hormone nor leptin affected glucose oxidation ( $P > 0.5$ ). Total lipid synthesis from glucose was increased by approximately 25% by 100 nM insulin or insulin + growth hormone ( $P < 0.05$ ). Insulin + leptin reduced the insulin response by 37% ( $P < 0.05$ ). The combination of all three hormones increased total lipid synthesis by 35%, relative to controls ( $P < 0.05$ ), a rate similar to insulin alone. Fatty acid synthesis was elevated by insulin (32%,  $P < 0.05$ ) or growth hormone (13%,  $P < 0.05$ ). Leptin had no effect on fatty acid synthesis ( $P > 0.05$ ). Leptin reduced the esterification rate by 10% ( $P < 0.05$ ). Growth hormone and insulin could overcome leptin's inhibition of palmitate esterification ( $P > 0.05$ ).

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## 1. Introduction

Central administration of porcine leptin can reduce feed intake in hungry swine by up to 90% [1], while leptin expression may be altered by infection, fasting, or obesity in swine [2,3]. Leptin administration has been shown to reduce the fat mass in rodents [4,5], although peripheral effects in the pig are unknown. Several studies have suggested that lipolysis may contribute to this shift from fat accretion to fat depletion with leptin treatment [6,7]. However, the contribution of lipogenesis to this metabolic shift is unknown.

Experiments assessing the effects of leptin on lipogenesis with rodent adipocytes have produced mixed results, although the reasons for the diversity of responses (or lack thereof) have been difficult to identify [8]. The literature is evenly divided between studies that show no effect of leptin on carbohydrate metabolism of adipocytes and those studies that show leptin inhibits glucose conversion to lipid, while enhancing glucose oxidation [9–13]. Much of this research is confounded by an apparent interaction of leptin with insulin binding or insulin action [9,12,14]. This study examined if leptin can acutely affect glucose or fatty acid metabolism in pig adipocytes. Secondly, this study determined whether leptin's actions on lipogenesis are manifested through interaction with insulin or growth hormone mediated events.

## 2. Material and methods

Dorsal, subcutaneous adipose tissue was obtained from between the shoulder blades of ~55 kg crossbred (Yorkshire × Landrace × Hampshire) barrows, following an overnight fast, at the USDA-ARS abattoir following electrocution and exsanguination. Animal handling and care were according to the requirements and approval of the Beltsville Area Animal Care and Use Committee (Protocol # 98-030). Tissue was dissected free of any skeletal muscle and cut into cubes (~1 cm) and placed in warm (37 °C) Hanks salt solution prior to transfer to the laboratory. At the laboratory, tissue was washed and placed in fresh Hanks salt solution (37 °C). An ~5 g aliquot of the tissue were transferred to a 100 mm petri dish where it was minced into small pieces (~1 mm<sup>3</sup>). Isolated adipocytes were prepared according to the methods of Rodbell [15], as modified by Etherton et al. [16]. Approximately, 15 g of minced tissue was transferred to a 250 ml polypropylene flask containing a digestion buffer comprised of DMEM/F12 (Gibco, Grand Island, NY), 25 mM HEPES, 1.5% BSA (fatty acid free, Sigma A-6003, St. Louis, MO), pH 7.4 containing 10 mg collagenase/gm tissue (type 1 collagenase, Worthington Biomedical Corp., Lakewood, NJ). Two of these digestion flasks (30 g minced adipose total) were then incubated for 30 min at 37 °C in a shaking water bath (90 oscillations/min). A two-fold excess of wash buffer (DMEM/F12, 25 mM HEPES, 3% BSA, pH 7.4, 37 °C) was added to the digestion flask after 30 min of incubation. Flask contents were mixed and filtered through nylon mesh with 250 μm pores, to remove undigested tissue and large cell aggregates. The filtered cells were permitted to separate by flotation for 10 min and the infranatant below the floating adipocytes was removed with a siliconized pasteur pipet.

The adipocyte layer was washed three times with fresh wash buffer (37 °C) prior to use in incubations.

Glucose incubation medium was comprised of DMEM/F12, 25 mM Hepes, 3% BSA, 5.5 mM glucose, 1  $\mu\text{Ci/ml}$   $^{14}\text{C}$ -U-glucose (Moravек Biochemicals, Brea, CA), while palmitate incubation medium also included 1 mM sodium palmitate and substituted 0.5  $\mu\text{Ci/ml}$  1- $^{14}\text{C}$ -palmitic acid (Moravек Biochemicals, Brea, CA) for  $^{14}\text{C}$  glucose. Treatments included: basal medium (control), 100 nM porcine insulin (Sigma), 100 ng/ml porcine growth hormone (USDA-pGH-B-1, USDA-ARS, Beltsville MD), 100 ng/ml recombinant porcine leptin, and combinations of these hormones. Recombinant porcine leptin was prepared and acquired from Dr. Arieh Gertler [17]. Hormone concentrations were based upon previous *in vitro* experiments (leptin [7]; insulin [18–20]; growth hormone [19]). All treatments were performed in triplicate and the experiment was repeated with adipocytes isolated from five different animals.

A 250  $\mu\text{l}$  aliquot of cells was added to polypropylene 16  $\times$  100 test tubes (Sarstedt) containing 1 ml of incubation medium. Following addition of the cell aliquot, tubes were gassed with 95% air/5% carbon dioxide and then capped with rubber stoppers containing center wells. Tubes were then incubated for 2 h at 37 °C in a shaking water bath (90 oscillations/min).

Following 2 h of incubation, 0.5 ml 1N  $\text{H}_2\text{SO}_4$  was injected into the medium to kill the metabolic activity of the cells. Ten minutes later, 250  $\mu\text{l}$  of methylbenzothionium hydroxide (Sigma) was injected into the center wells. Carbon dioxide was captured during a 30-min incubation. Stoppers were then removed and the center wells were transferred to scintillation vials for counting. The medium was transferred to 16  $\times$  125 test tubes. Five milliliters of Dole's solution was added to incubation tubes, vortexed, and transferred to the test tubes. Lipid extractions were performed according to the method of deCingolani [21]. Incorporation of label into  $\text{CO}_2$ , total lipid and fatty acids following saponification was determined as described by Azain and Martin [22].

Fat cell size and number were determined through electronic quantification using the method of Hirsch and Gallian [23] as modified by Cartwright [24]. Duplicate, isolated fat cell samples were fixed in a solution containing 0.12 M osmium tetroxide in 50 mM collidine (2,4,6-trimethylpyridine) buffer for each cell isolate. Samples were fixed for at least 1 week at room temperature. Fixed adipocytes were rinsed with 0.9% NaCl through a 200- $\mu\text{m}$  nylon screen and then were collected on a 20- $\mu\text{m}$  nylon screen. Samples were analyzed on a Coulter electronic particle counter (model ZM; Coulter Electronics, Hialeah, FL). Data were then expressed as nanomoles of substrate utilized per  $10^6$  cells.

### 2.1. Statistical analysis

Data were subjected to analysis of variance with the detection of mean separation by Student–Newman–Keuls test (SigmaStat Software, SPSS Science, Chicago, IL). Significant differences were defined at the 95% confidence level. Due to the inherent variability of cell incubations, data (nanomoles utilized/ $10^6$  cells) are expressed as a percentage relative to basal activity (100%) for each series of incubations.

### 3. Results

Incubation of isolated pig adipocytes with insulin increased the glucose oxidation rate by 18% ( $P < 0.05$ , Fig. 1). Growth hormone had no direct effect on glucose oxidation ( $P > 0.5$ ). The combination of growth hormone and insulin increased glucose oxidation by 20% ( $P < 0.05$ ). Leptin had no effect on glucose oxidation ( $P > 0.05$ ). However, the combination of growth hormone and leptin increased the oxidation rate relative to control incubations ( $P < 0.05$ ).

Palmitate oxidation was quite low in pig adipocytes, representing less than 1% of the quantity of palmitate esterified and incorporated into lipid (Fig. 2). There were no detectable hormonal effects on palmitate oxidation ( $P < 0.05$ ).

The incorporation of carbon from glucose into total lipids was increased by approximately 25% by incubation with 100 nM insulin or insulin in combination with growth hormone ( $P < 0.05$ , Fig. 3). Growth hormone was also able to increase the rate of glucose conversion to lipid by 8% ( $P < 0.05$ ). Leptin exposure increased glucose conversion into lipid by 5% ( $P < 0.05$ ). The combination of leptin and growth hormone had no greater effect than the hormones individually. The combination of insulin and leptin reduced the response of the isolated adipocytes by 37%, relative to insulin alone ( $P < 0.05$ ), although the incorporation

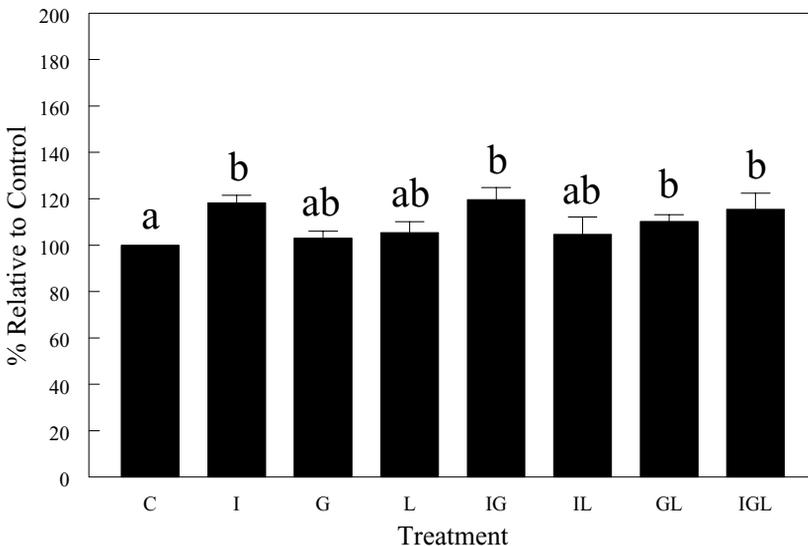


Fig. 1. Relative glucose oxidation in response to acute hormone exposure. Isolated pig adipocytes (250  $\mu$ l cells) were incubated  $\pm$  porcine insulin (100 nM);  $\pm$  porcine growth hormone (100 ng/ml); or  $\pm$  recombinant pig leptin (100 ng/ml) or combinations of these hormones in a medium containing 1  $\mu$ Ci U-<sup>14</sup>C-glucose/ml for 2 h, followed by collection of <sup>14</sup>CO<sub>2</sub> for analysis of glucose oxidation. Data are expressed relative to cultures incubated without hormone additions (control = 991  $\pm$  205 nmol/10<sup>6</sup> cells/2 h). Values not sharing a common superscript letter are different ( $P < 0.05$ ;  $n = 5$ ). C = control (basal medium), I = 100 nM insulin, G = 100 ng pig growth hormone per milliliter, L = 100 ng recombinant pig leptin per milliliter, IGL = 100 nM insulin + 100 ng pig growth hormone per milliliter + 100 ng recombinant pig leptin per milliliter.

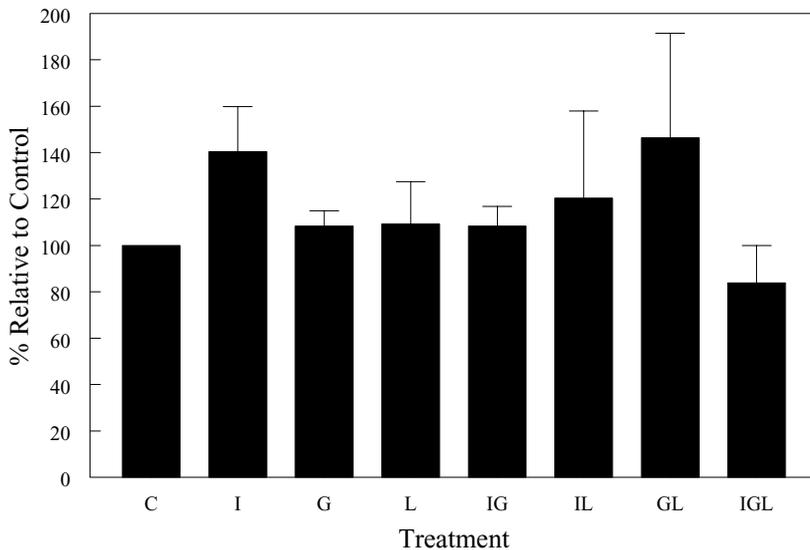


Fig. 2. Relative palmitate oxidation in response to acute hormone exposure. Isolated pig adipocytes ( $250 \mu\text{l}$  cells) were incubated  $\pm$  porcine insulin ( $100 \text{ nM}$ );  $\pm$  porcine growth hormone ( $100 \text{ ng/ml}$ ); or  $\pm$  recombinant pig leptin ( $100 \text{ ng/ml}$ ) or combinations of these hormones in a medium containing  $0.5 \mu\text{Ci } 1\text{-}^{14}\text{C}$ -palmitic acid/ml for 2 h, followed by collection of  $^{14}\text{CO}_2$  for analysis of palmitate oxidation. Data are expressed relative to cultures incubated without hormone additions (control =  $1.88 \pm 0.66 \text{ nmol}/10^6 \text{ cells}/2 \text{ h}$ ). No significant differences ( $P > 0.05$ ;  $n = 5$ ). C = control (basal medium), I =  $100 \text{ nM}$  insulin, G =  $100 \text{ ng}$  pig growth hormone per milliliter, L =  $100 \text{ ng}$  recombinant pig leptin per milliliter, IGL =  $100 \text{ nM}$  insulin +  $100 \text{ ng}$  pig growth hormone per milliliter +  $100 \text{ ng}$  recombinant pig leptin per milliliter.

rate was still greater than in controls ( $P < 0.05$ ). The combination of insulin, growth hormone, and leptin increased the glucose incorporation rate by 35% relative to controls ( $P < 0.05$ ), a rate similar to that produced by insulin alone.

The specific incorporation of glucose carbons into fatty acids was elevated by incubation with insulin (32%,  $P < 0.05$ , Fig. 4) or growth hormone (13%,  $P < 0.05$ ). Leptin had no effect on fatty acid synthesis ( $P > 0.05$ ). Insulin or growth hormone in combination with any other hormone had no greater effect than the individual hormones. Approximately, 79.1% of carbon incorporated within the total lipids was found within the fatty acid fraction.

The rate of fatty acid esterification was not changed by insulin ( $P > 0.05$ , Fig. 5). Growth hormone had no effect on the esterification process either ( $P > 0.05$ ). However,  $100 \text{ ng}$  pig leptin per milliliter reduced the esterification rate by 10% ( $P < 0.05$ ). Growth hormone and insulin could overcome leptin's inhibition of palmitate esterification ( $P > 0.05$ ).

#### 4. Discussion

Numerous studies have demonstrated that leptin can produce alterations in central mechanisms of feed behavior [4,5]. Initially, the reduction in feed intake produced by leptin

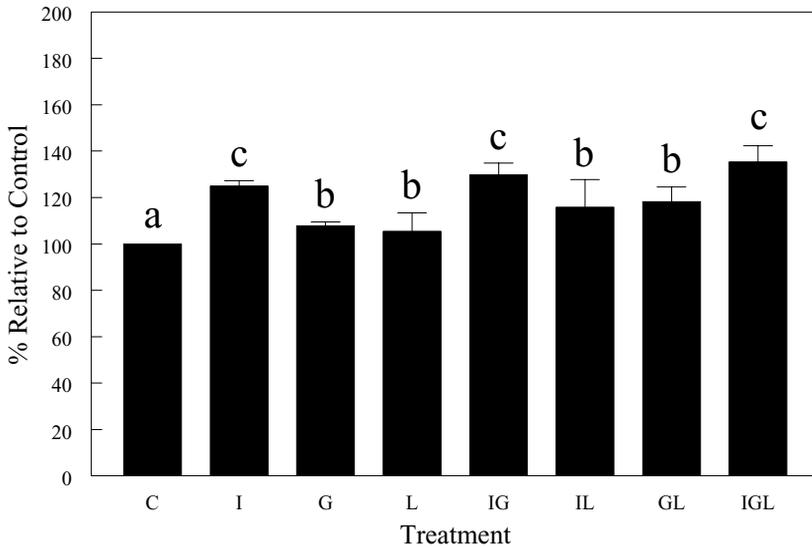


Fig. 3. Relative glucose conversion to total lipids in response to acute hormone exposure. Isolated pig adipocytes (250  $\mu$ l cells) were incubated  $\pm$  porcine insulin (100 nM);  $\pm$  porcine growth hormone (100 ng/ml); or  $\pm$  recombinant pig leptin (100 ng/ml) or combinations of these hormones in a medium containing 1  $\mu$ Ci U-<sup>14</sup>C-glucose/ml for 2 h, followed by extraction of total lipids as described in Section 2. Data are expressed relative to cultures incubated without hormone additions (control = 1272  $\pm$  373 nmol/10<sup>6</sup> cells/2 h). Values not sharing a common superscript letter are different ( $P < 0.05$ ;  $n = 5$ ). C = control (basal medium), I = 100 nM insulin, G = 100 ng pig growth hormone per milliliter, L = 100 ng recombinant pig leptin per milliliter, IG = 100 nM insulin + 100 ng pig growth hormone per milliliter + 100 ng recombinant pig leptin per milliliter.

treatment was thought responsible for the rapid loss of fat mass. However, recent studies have shown that many peripheral cell types express leptin receptors, including adipocytes [25,26]. This suggests that leptin may specifically affect adipocyte metabolic functions. Rouru et al. [27] have reported that leptin reduces glucose uptake in rodent adipose tissue. In the present study, there was no evidence of a change in glucose uptake as reflected by any change in glucose oxidation or fatty acid synthesis. The leptin concentration used in the present study has been previously shown by this lab to be effective for altering adipocyte metabolic activity [7]. However, no evidence can be presented from this study to indicate leptin reduces glucose uptake in the adipocyte under acute conditions. The observed slight increase in total lipid synthesis in the present study suggests that for leptin to reduce glucose uptake may require a more chronic exposure to leptin to indirectly affect glucose uptake and glycolysis.

Previous studies have demonstrated *in vivo* that acute leptin treatment does not alter lipogenesis [28]. However, Müller et al. [12] demonstrated that a 6-h incubation of isolated rat adipocytes was sufficient to demonstrate leptin's inhibition of lipogenesis. The present study was not able to demonstrate an effect of a 2-h incubation with porcine leptin (acute experiment). This difference in responsiveness between the isolated pig adipocyte incubations in the present study and the isolated rodent cell incubations mentioned above may be due to methodology, quality of the leptin preparation or perhaps be due to species

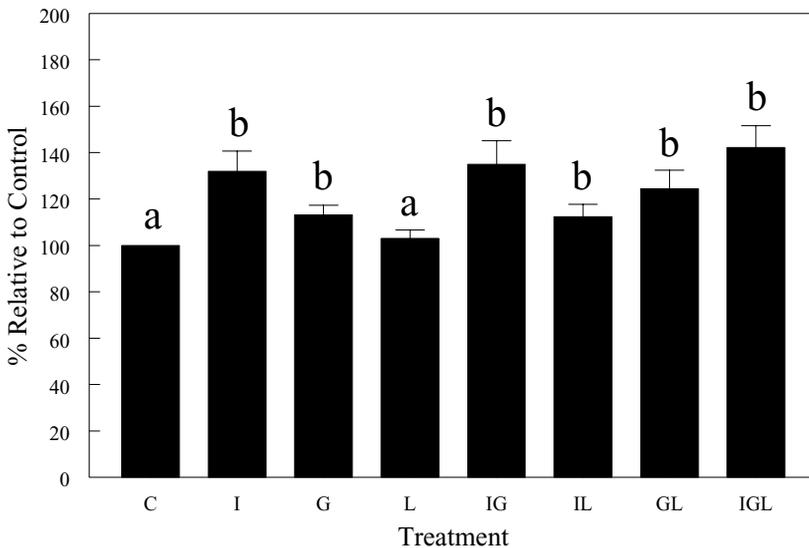


Fig. 4. Relative glucose incorporation into fatty acids in response to acute hormone exposure. Isolated pig adipocytes (250  $\mu$ l cells) were incubated  $\pm$  porcine insulin (100 nM);  $\pm$  porcine growth hormone (100 ng/ml); or  $\pm$  recombinant pig leptin (100 ng/ml) or combinations of these hormones in a medium containing 1  $\mu$ Ci U- $^{14}$ C-glucose/ml for 2 h, followed by extraction of fatty acids as described in Section 2. Data are expressed relative to cultures incubated without hormone additions (control = 1019  $\pm$  348 nmol/ $10^6$  cells/2 h). Values not sharing a common superscript letter are different ( $P < 0.05$ ;  $n = 5$ ). C = control (basal medium), I = 100 nM insulin, G = 100 ng pig growth hormone per milliliter, L = 100 ng recombinant pig leptin per milliliter, IGL = 100 nM insulin + 100 ng pig growth hormone per milliliter + 100 ng recombinant pig leptin per milliliter.

differences, or even time of incubation. Acute effects of leptin on the adipocyte are atypical [8]. However, chronic leptin exposure (>12 h) has demonstrated that leptin can alter lipogenic rates, typically by affecting insulin mediated events in adipocytes [8].

Insulin has previously been demonstrated to increase glucose oxidation and lipogenesis in pig adipocytes with varying levels of success [16,29,30,31]. The present study was able to demonstrate an 18% increase in glucose oxidation and a 32% increase in fatty acid synthesis, which is comparable to the observations of Mersmann and Hu [30]. Growth hormone (100 ng/ml) did not block this mild lipogenic response to insulin and in fact produced its own lipogenic response (13% increase) under these acute conditions. In contrast, chronic exposure to growth hormone results in a suppression of lipogenesis and insulin sensitivity in the pig [32]. The suppression in lipogenesis due to chronic growth hormone treatment appears to be through inhibition of fatty acid synthase transcription [33,34]. The acute insulin-like effect of growth hormone on lipogenesis has been well characterized in the rat adipocyte [35,36], although no such observation has previously been reported for the pig adipocyte.

Leptin significantly reduced insulin-stimulated total lipid synthesis in the present study. Several studies have now demonstrated that leptin can reduce insulin sensitivity [12,37] and thus affect adipocyte metabolism [8]. Müller et al. [12] reported that leptin could inhibit insulin-stimulated glucose uptake, lipogenesis, glycogen synthase activity and protein

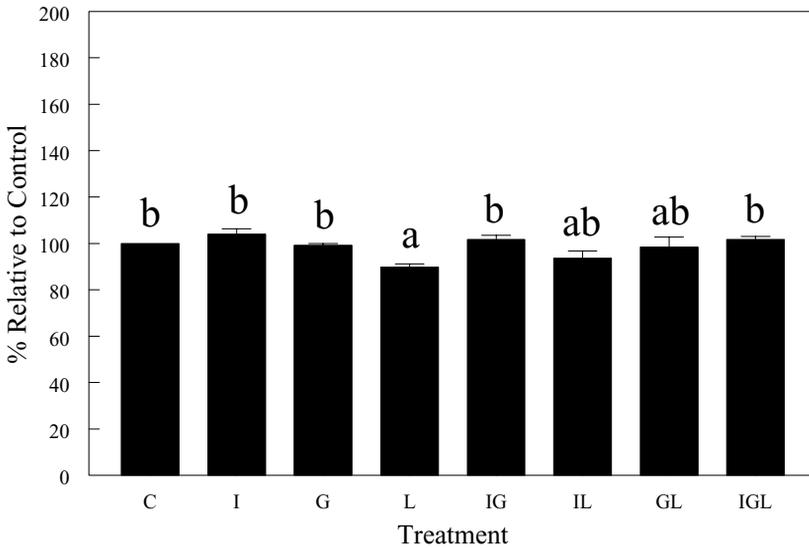


Fig. 5. Relative palmitate incorporation into total lipids in response to acute hormone exposure. Isolated pig adipocytes (250  $\mu$ l cells) were incubated  $\pm$  porcine insulin (100 nM);  $\pm$  porcine growth hormone (100 ng/ml); or  $\pm$  recombinant pig leptin (100 ng/ml) or combinations of these hormones in a medium containing 0.5  $\mu$ Ci 1- $^{14}$ C-palmitate/ml for 2 h, followed by extraction of total lipids as described in Section 2. Data are expressed relative to cultures incubated without leptin (control = 3174  $\pm$  738 nmol/10 $^6$  cells/2h). Values not sharing a common superscript letter are different ( $P < 0.05$ ;  $n = 5$ ). C = control (basal medium), I = 100 nM insulin, G = 100 ng pig growth hormone per milliliter, L = 100 ng recombinant pig leptin per milliliter, IGL = 100 nM insulin + 100 ng pig growth hormone per milliliter + 100 ng recombinant pig leptin per milliliter.

synthesis in rat adipocytes. William et al. [38] have also reported leptin inhibition of insulin-stimulated de novo fatty acid synthesis during a 6-h incubation. Elimam et al. [39] have reported that preincubation of human adipocytes with leptin can decrease insulin induced lipogenesis. Walder et al. [13] and Fukuda et al. [40] have reported that leptin inhibits maximum insulin binding in isolated rat adipocytes but does not affect receptor affinity. Cohen et al. [37] indicated that leptin induces dephosphorylation of IRS-1, thus antagonizing insulin's actions. Further studies are necessary to elucidate the how leptin binding impacts insulin signaling mechanisms in swine.

Metabolism of absorbed fatty acids by the porcine adipocyte is almost exclusively comprised of incorporation into triglyceride. William et al. [38] have reported that fatty acid oxidation in rat fat cells can be increased by up to 76%. However, very little fatty acid oxidation was detected in the pig adipocyte under the present incubation conditions; approximately, 1% of the carbon in palmitate was oxidized versus the amount that was incorporated into lipid. Etherton and Allen [41] reported that the rate of palmitate oxidation accounted for approximately 2.5% of the rate of palmitate esterification, in the presence of glucose-containing medium. It was impossible to interpret the results of the fatty acid oxidation experiments in the present study, as the activity was so small and thus so variable.

The esterification of fatty acids was unaffected by insulin or growth hormone. Insulin reduces fatty acid oxidation and stimulates esterification in rat adipocytes [42], but the limited

sensitivity of the pig adipocyte to insulin may preclude this phenomenon. Growth hormone administration has previously been demonstrated to not alter fatty acid esterification rates [43]. Incubation of pig adipocytes with leptin reduced the rate of palmitate esterification by 10.2%. This is a small but significant decrease in esterification rate. Both insulin and growth hormone could counteract the inhibitory actions of leptin on esterification, although they had no stimulatory action of their own.

Leptin has been reported to inhibit fatty acid esterification in rodent pancreatic islets [44] and skeletal muscle [45,46]. The present study extends this observation to porcine adipose tissue. It is uncertain if this inhibition of fatty acid incorporation into lipids is the consequence of inhibition of acyl CoA synthetase, diacylglycerol acyltransferase or other enzymes regulating triglyceride synthesis, or whether the inhibition is with glycerol phosphate acyltransferase. The role of leptin in regulating the metabolism of fatty acids through the metabolic pathway of esterification has not been characterized. The present data suggests that leptin can regulate the activity of at least one of the enzymes involved in fatty acid esterification.

This study demonstrates that acute leptin exposure can alter fatty acid utilization by porcine adipocytes, but not glucose conversion to fatty acids. However, total lipid synthesis from glucose was increased by leptin, implicating a change in glyceride glycerol synthesis. These metabolic effects are a direct response to leptin. This immediate response suggests that synthesis and secretion of additional autocrine/paracrine factors are not necessary prior to changes in fatty acid metabolism or glycerol synthesis, while an effect on glucose conversion to fatty acids, glucose oxidation or fatty acid oxidation require additional factors. If leptin affects insulin-stimulated glucose metabolism at the adipocyte, the present data suggest that effect may be indirect. However, the direct action of leptin to inhibit palmitate utilization by adipocytes for lipid synthesis suggests that leptin can function to partition energy away from adipose tissue.

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