

Porcine leptin inhibits lipogenesis in porcine adipocytes^{1,2}

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ABSTRACT: The present study examined whether recombinant porcine leptin alters lipid synthesis in porcine adipocytes. The stromal-vascular cell fraction of neonatal pig subcutaneous adipose tissue was isolated by collagenase digestion, filtration, and subsequent centrifugation. These cells were seeded on 25-cm² tissue culture flasks and proliferated to confluency in 10% (vol/vol) fetal bovine serum in Dulbecco's modified Eagle medium/F12 (DMEM/F12, 50:50). Cultures were differentiated using 2.5% pig serum (vol/vol), 10 nM insulin, 100 nM hydrocortisone. After 7 d of lipid filling, cultures were washed free of this medium, incubated overnight in DMEM/F12 containing 2% pig serum (vol/vol), and then used for experiments. Acute experiments assessed U-¹⁴C-glucose or 1-¹⁴C-palmitate metabolism in cultures exposed to porcine leptin (0 to 1,000 ng/mL medium) for 4 h. Chronic experiments used cultures incubated with 0 to 1,000 ng porcine leptin/mL medium for 44 h before measurements of U-¹⁴C-glucose and 1-¹⁴C-palmitate oxidation and incorporation into lipid. Another experiment examined whether chronic leptin treatment alters insulin responsiveness by including insulin (10 nM) with incubations containing leptin. Lep-

tin had no acute effects on glucose oxidation or conversion to lipid ($P > 0.05$). Acute leptin treatment decreased palmitate incorporation into lipids up to 45% ($P < 0.05$). Chronic leptin exposure decreased glucose oxidation (21%), total lipid synthesis (18%), and fatty acid synthesis (23%) at 100 ng/mL medium ($P < 0.05$). Insulin increased rates of glucose oxidation, total lipid, and fatty acid synthesis ($P < 0.05$); however, chronic exposure to 10 ng leptin/mL medium decreased the effectiveness of 10 nM insulin to affect these measures of glucose metabolism by approximately 18 to 46% ($P < 0.05$). Higher concentrations of leptin inhibited all effects of insulin on glucose metabolism ($P < 0.05$). Chronic exposure to leptin increased palmitate oxidation by 36% ($P < 0.05$). Chronic leptin exposure decreased palmitate incorporation into total lipids by 40% at 100 ng/mL medium ($P < 0.05$). Lipoprotein lipase activity was not affected ($P > 0.05$) by leptin. These data indicate that leptin functions to promote partitioning of energy away from lipid accretion within porcine adipose tissue by inhibiting glucose oxidation and lipogenesis indirectly, by decreasing insulin-mediated stimulation of lipogenesis, and by stimulating fatty acid oxidation while inhibiting fatty acid esterification.

Key Words: Adipocyte, Leptin, Lipogenesis, Metabolism, Swine

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J. Anim. Sci. 2003. 81:3008–3017

Introduction

Leptin is a small peptide secreted by fat cells that can affect reproduction, immune function, feed intake, and metabolism (Friedman and Halaas, 1998). Central administration of porcine leptin can decrease feed intake in hungry swine by up to 90% (Barb et al., 1998), and leptin expression may be altered by infection, fasting, or obesity in swine (Houseknecht et al., 1998; Ram-

say et al., 1998). Leptin administration has been shown to reduce the fat mass in rodents (Friedman and Halaas, 1998; Frühbeck et al., 1998), 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 (Wang et al., 1999b; Ramsay, 2000). 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 (Harris, 2000). Acute effects of leptin on the adipocyte metabolism are not commonly detected (Harris, 2000). Chronic leptin exposure (>12 h) has demonstrated that leptin can alter lipogenic rates, typically by affecting insulin-mediated events in rodent adipocytes (Harris, 2000).

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Received August 15, 2001.

Accepted July 23, 2003.

Measurement of insulin-sensitive metabolic pathways in the pig has been difficult due to relative insulin insensitivity of the pig adipocyte (Mersmann, 1989). The controlled environment of cell culture permits measurement of these pathways under chronic conditions and allows detection of insulin-sensitive metabolic activity (Ramsay et al., 1992). This study examined whether leptin can acutely affect glucose and fatty acid metabolism or whether chronic leptin exposure is necessary for a metabolic response. Secondly, this study determined whether leptin's actions on lipogenesis are manifested through interaction with insulin-mediated events.

Materials and Methods

Cell Culture

Dorsal subcutaneous adipose tissue was obtained from between the shoulder blades of 1- to 4-d-old, male crossbred pigs (Yorkshire \times Landrace) following electrocution and exsanguination. Primary cultures containing pig preadipocytes were prepared by methods previously published (Ramsay et al., 1989). Animal procedures were approved by the Beltsville Animal Care and Use Committee. Briefly, tissue was minced into sections of approximately 1 mm² with scissors and then incubated with 5 mL/g of tissue in a digestion buffer comprised of Dulbecco's modified Eagle medium/F12 (DMEM/F12, Gibco, Grand Island, NY), 100 mM HEPES, 1.5% BSA (fatty-acid free, A-6003, Sigma Chem., St. Louis, MO), pH 7.4, containing 2 mg/mL collagenase (Type 1, Worthington Biomedical Corp. Lakewood, NJ). A fivefold excess of digestion buffer (room temperature, excluding collagenase) was added to the digestion flask after 45 min of incubation at 37°C in a shaking water bath (90 oscillations/min). Flask contents were mixed and filtered through nylon screens with 250- and 20- μ m mesh openings to remove undigested tissue and large cell aggregates. The filtered cells were centrifuged at 500 \times g for 10 min to separate the floating adipocytes from the pellet of stromal-vascular cells. The stromal-vascular cells were then incubated with erythrocyte lysis buffer (0.154 M NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) at room temperature (Hauner et al., 1989) for 10 min, followed by centrifugation at 500 \times g for 10 min. The stromal-vascular cell pellet was washed with DMEM/F12, centrifuged, and resuspended in DMEM/F12 containing 10% FBS (vol/vol, Sigma).

Aliquots of the stromal-vascular fraction were removed, stained with Rappaport's stain, and counted on a hemocytometer. Cells were then diluted in DMEM/F12 containing 10% FBS (vol/vol), 10,000 U/L penicillin sodium, 100 mg streptomycin sulfate/L, 250 μ g amphotericin B/L (plating medium). Cells were seeded on 25-cm² tissue culture flasks at a density of 1.0 \times 10⁴ cells/cm² in plating medium. Cells were maintained at 37°C in a humidified, 5% CO₂ atmosphere.

Cells were maintained in plating medium until confluency (d 4 to 6 of culture), with medium changed every 2 d. At confluency, cultures were induced to differentiate and accumulate lipid using DMEM/F12 medium containing 2.5% pig serum (Sigma), 10 nM insulin, and 100 ng hydrocortisone/mL. After 7 d of lipid filling, cultures were washed free of this medium, incubated overnight in DMEM/F12 containing 2% pig serum, and then used for experiments. Each ¹⁴C-glucose experiment was repeated four times, with cell cultures derived from four separate pigs, using duplicate tissue culture flasks/treatment for each pig, and each ¹⁴C-palmitate experiment was repeated eight times using cells from eight pigs.

Experimental Design

The first experiment was designed to determine whether porcine leptin has acute effects on glucose or palmitate metabolism by porcine adipocytes derived from cell culture. A 4-h incubation period was selected because this is insufficient for transcription, translation, and post-translational modification of autocrine peptides, thus permitting analysis of leptin's direct effect on metabolism. To make the incubation medium, recombinant porcine leptin at concentrations of 0, 10, 100, or 1,000 ng/mL medium were added to Medium 199 (Gibco) supplemented with 25 mM HEPES, 3% BSA, and 1 μ Ci D-[U-¹⁴C]-glucose/mL (Moravek Biochemicals, Brea, CA) or 1 mM sodium palmitate and 0.5 μ Ci 1-[¹⁴C]-palmitic acid/mL (Moravek Biochemicals). Sodium palmitate was dissolved in 75°C water and then slowly dripped into the continuously stirred 12% BSA solution, diluting the BSA to 6%. Then 1-[¹⁴C]-palmitic acid in ethanol water (1:1) was added (<50 μ L) to this diluted 6% BSA solution, thus binding the fatty acid to the albumin. This 6% BSA-palmitate solution was then added 1:1 with 2 \times m199 containing 50 mM HEPES to produce the incubation medium. Basal medium (0 ng leptin/mL) served as a negative control. Duplicate flasks were incubated with 2 mL of these incubation media for 4 h with glucose and palmitate metabolism (oxidation and lipogenesis) measured during this period. Recombinant porcine leptin was prepared and acquired from Arieh Gertler (Raver et al., 2000).

The second experiment was designed to determine whether chronic exposure to leptin alters adipocyte metabolism. Chronic exposure (48 h in this experiment) provides time for leptin to stimulate the synthesis and secretion of other autocrine/paracrine factors from the adipocyte. These induced autocrine/paracrine factors then alter cellular metabolism. Therefore, chronic leptin treatment permits analysis of leptin's indirect effect on metabolism. Porcine leptin at concentrations of 10, 100, and 1,000 ng/mL medium were added to Medium 199 supplemented with 2% pig serum (vol/vol). Basal medium (2% pig serum [vol/vol], Medium 199) served as a negative control. Duplicate flasks were incubated with these media for 44 h, with medium changed after

approximately 24 h. At 44 h, medium was changed to 2 mL of the incubation medium from the first experiment, containing 0, 10, 100, or 1000 ng porcine leptin/mL, 25 mM HEPES, 3% BSA (wt/vol), and 1 μ Ci D-[U- 14 C]-glucose/mL or 1 mM sodium palmitate and 0.5 μ Ci 1-[14 C]-palmitic acid/mL. Flasks were incubated with these incubation media for 4 h; thus, the cultures were exposed to supplemental leptin for a total of 48 h.

The third experiment evaluated the potential role of leptin in altering insulin-regulated events in glucose metabolism by adipocytes. Porcine leptin at concentrations of 10, 100, and 1,000 ng/mL medium were added to Medium 199 supplemented with 2% pig serum (vol/vol). Basal medium (2% pig serum [vol/vol], Medium 199) served as a negative control. Duplicate flasks were incubated with these media for 44 h, with medium changed after approximately 24 h. At 44 h, medium was changed to 2 mL of incubation medium containing 0, 10, 100, or 1,000 ng porcine leptin/mL, 1 μ Ci D-[U- 14 C]-glucose/mL, 25 mM HEPES, 3% BSA (wt/vol), and 10 nM insulin, except for control incubations that did not receive insulin. Flasks were incubated with these test media for 4 h.

The fourth experiment evaluated the potential role of leptin in altering lipoprotein lipase activity in adipocytes. Porcine leptin at concentrations of 1, 10, 100, and 1,000 ng/mL medium or insulin at 10 ng/mL were added to Medium 199 supplemented with 2% pig serum (vol/vol). Basal medium (2% pig serum [vol/vol], Medium 199) served as a negative control. Wells were incubated with these media for 48 h with medium changed at 24 h.

Cell Culture Incubation

Following addition of radiolabeled medium, flasks were gassed for 1 min with 95% air:5% carbon dioxide and then capped with rubber stoppers containing center wells, according to procedures previously described (Ramsay et al., 1989). Following 4 h of incubation, 0.5 mL of 1 N H₂SO₄ was injected into the medium to kill the metabolic activity of the cells. Ten minutes later, flasks were placed vertically and 250 μ L of methylbenzethonium 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 removed and 5 mL of Dole's solution was added to each flask for extraction of lipids by the method of deCingolani (1972). Incorporation of label into CO₂, total lipid, and fatty acids following saponification was determined as described by Azain and Martin (1983).

Lipoprotein Lipase Activity

Following chronic exposure to leptin, culture medium was removed, wells were washed with Hanks salt solution and 1 mL of lipoprotein lipase (LPL) homogenizing buffer (50 mM NH₄OH, 50 mM NH₄Cl, and 1 U heparin/

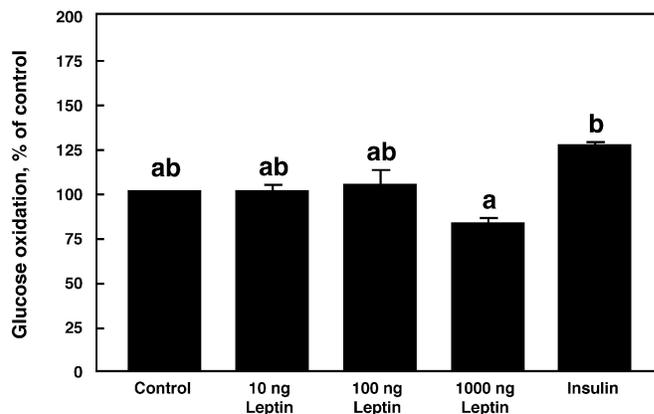


Figure 1. Relative percentage of glucose oxidation in response to acute porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin in a medium containing 1 μ Ci U- 14 C-glucose/mL for 4 h, followed by collection of 14 CO₂ for analysis of glucose oxidation. Data are expressed relative to cultures incubated without leptin (100% = 1,164 \pm 126 nmol of glucose incorporated/flask). Values that do not have a common letter differ ($P < 0.05$, $n = 4$).

mL buffer, pH 8.1) was added. Cultures were sonicated in tissue culture wells and then transferred to microcentrifuge tubes. Sonicated cultures were centrifuged (14,000 \times g, 30 min, 4°C), and the supernatant was used for assay of LPL activity according to the procedures of Nilsson-Ehle and Schotz (1976) as previously described (Ramsay et al., 1989).

Statistical Analysis

The experimental model for these experiments was a completely randomized design. Blocking was accomplished by converting data to percentages, relative to basal medium (0 ng/mL leptin) to account for culture-to-culture variation. Data were analyzed by one-way analysis of variance using SigmaStat software (SPSS Science, Chicago, IL). Mean separation was analyzed using a Student-Newman-Keuls test. Means were defined as significantly different at $P < 0.05$.

Results

Acute treatment with porcine leptin tended to reduce the relative rate of glucose oxidation by 18% ($P > 0.05$; Figure 1). Insulin (10 nM) tended to increase the oxidation rate above controls ($P > 0.05$). Chronic exposure to leptin decreased the relative rate of glucose oxidation by 21% at 100 ng leptin/mL medium ($P < 0.05$; Figure 2). A higher concentration of leptin (1,000 ng/mL) had no greater effect ($P > 0.05$), although the data suggest chronic leptin treatment decreased glucose oxidation in a dose-related manner. Insulin stimulated glucose

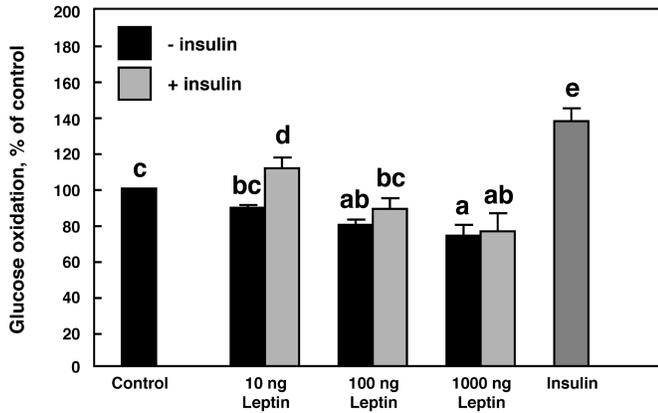


Figure 2. Relative percentage of glucose oxidation in response to chronic porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) for 44 h. This was followed by a 4-h incubation in a medium containing 1 μ Ci U- 14 C-glucose, the porcine leptin concentration corresponding to the chronic treatment \pm 100 nM insulin, and collection of 14 CO $_2$ for analysis of glucose oxidation. Data are expressed relative to cultures incubated without leptin (100% = 626 \pm 154 nmol of glucose incorporated/flask). Solid bars represent incubations without supplemental insulin, whereas hatched bars represent incubations supplemented with 100 nM insulin. Values that do not have a common letter differ ($P < 0.05$, $n = 4$).

oxidation 37% during the 4-h incubation period ($P < 0.05$).

Chronic exposure to leptin reduced the insulin responsiveness of glucose oxidation (Figure 2). Leptin (10 ng/mL) supplemented to the insulin-containing medium decreased insulin-stimulated glucose oxidation by 26% ($P < 0.05$). In the leptin-treated cells, insulin-stimulated glucose oxidation was reduced to control levels with higher media concentrations of leptin ($P < 0.05$); there was no suggestion of a dose relationship for leptin.

Acute treatment with 0 to 1,000 ng of porcine leptin did not affect palmitate oxidation ($P > 0.05$, Figure 3). Insulin (10 nM) did not alter the oxidation rate during acute exposure ($P > 0.05$). Chronic exposure to leptin increased the relative rate of palmitate oxidation by 36% at 1,000 ng leptin/mL medium ($P < 0.05$; Figure 4). A lower concentration of leptin (10 to 100 ng/mL) had no effect ($P > 0.05$). Addition of insulin during the period for measurement of palmitate oxidation (4-h incubation) had no effect compared to the control ($P > 0.05$).

Total lipid synthesis was unaffected by acute leptin treatment ($P > 0.05$; Figure 5). Insulin increased total lipid synthesis by 29% ($P < 0.05$). However, chronic leptin treatment reduced total lipid synthesis by \sim 20% at concentrations of 100 and 1,000 ng/mL ($P < 0.05$; Figure 6) relative to control incubations. Insulin-stimulated lipid synthesis was reduced by the presence of leptin (Figure 6). Leptin (10 ng/mL) reduced insulin-

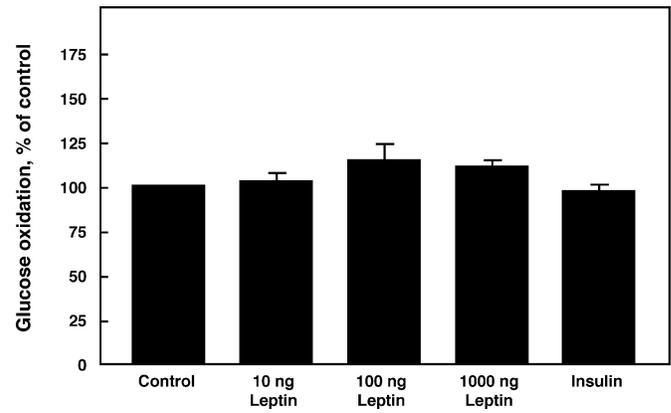


Figure 3. Relative percentage of palmitate oxidation in response to acute porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin in a medium containing 0.5 μ Ci 1- 14 C-palmitate/mL for 4 h, followed by collection of 14 CO $_2$ for analysis of palmitate oxidation. Data are expressed relative to cultures incubated without leptin (100% = 218 \pm 43 nmol incorporated/flask). No significant differences ($P > 0.05$, $n = 8$).

stimulated lipid synthesis from 130% of basal to 112% of basal ($P < 0.05$). Higher concentrations of leptin (100 and 1,000 ng/mL) reduced the insulin-stimulated lipogenesis to \sim 86% of basal lipogenesis (control incubations, $P < 0.05$), and eliminating any detectable effect of insulin.

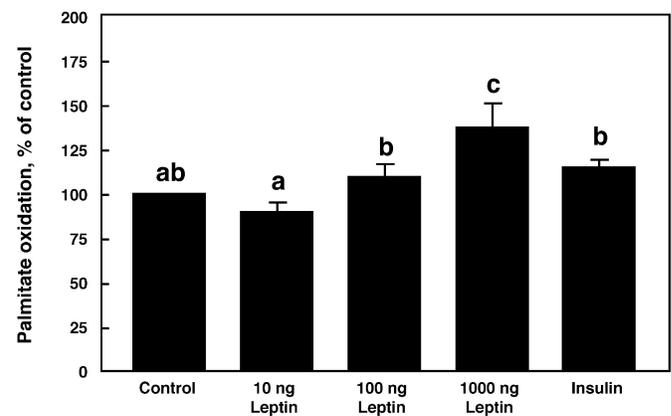


Figure 4. Relative percentage of palmitate oxidation in response to chronic porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) for 44 h. This was followed by a 4-h incubation in a medium containing 0.5 μ Ci 1- 14 C-palmitate/mL \pm porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin, followed by collection of 14 CO $_2$ for analysis of palmitate oxidation. Data are expressed relative to cultures incubated without leptin (100% = 227 \pm 49 nmol incorporated/flask). Values that do not have a common letter differ ($P < 0.05$, $n = 8$).

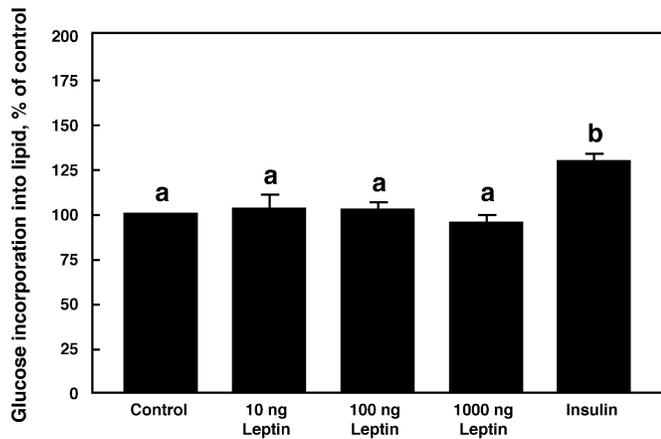


Figure 5. Relative percentage of glucose conversion to total lipids in response to acute porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin in a medium containing 1 μ Ci $U\text{-}^{14}\text{C}$ -glucose/mL for 4 h, followed by extraction of total lipids as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 266 \pm 61 nmol of glucose incorporated/flask). Values that do not have a common letter differ ($P < 0.05$, $n = 4$).

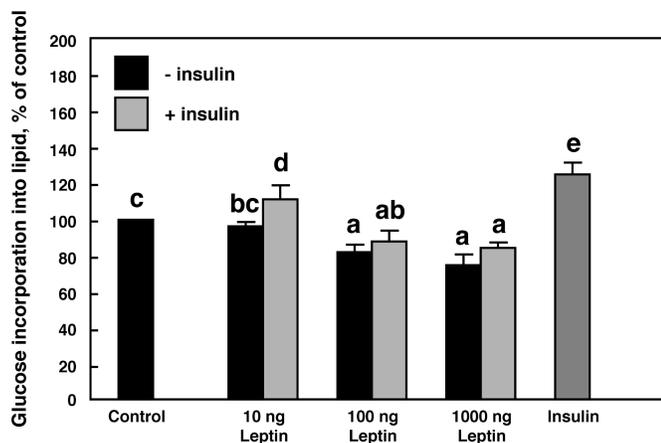


Figure 6. Relative percentage of glucose conversion to total lipids in response to chronic porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) for 44 h. This was followed by a 4-h incubation in a medium containing 1 μ Ci $U\text{-}^{14}\text{C}$ -glucose/mL, the porcine leptin concentration corresponding to the chronic treatment, \pm 100 nM insulin, followed by extraction of total lipids as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 153 \pm 18 nmoles of glucose incorporated/flask). Solid bars represent incubations without supplemental insulin, whereas hatched bars represent incubations supplemented with 100 nM insulin. Values that do not have a common letter differ ($P < 0.05$, $n = 4$).

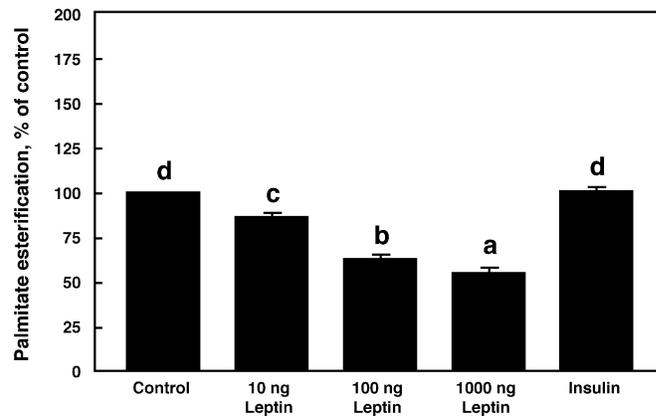


Figure 7. Relative percentage of palmitate incorporation into total lipids in response to acute porcine leptin exposure. Adipocyte-containing primary cultures were incubated \pm porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin in a medium containing 0.5 μ Ci $1\text{-}^{14}\text{C}$ -palmitate/mL for 4 h, followed by extraction of total lipids as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 13,796 \pm 1,335 nmol incorporated/flask). Values that do not have a common letter differ ($P < 0.05$, $n = 8$).

Esterification was reduced by acute leptin treatment ($P > 0.05$; Figure 7). A concentration of 10 ng leptin/mL of medium decreased palmitate incorporation into total lipids by 13.4% ($P < 0.05$). The highest level of inhibition was with 1,000 ng leptin/mL medium, a 45% ($P < 0.05$) reduction in palmitate incorporation into total lipids. Insulin did not affect the esterification rate ($P < 0.05$). Chronic leptin treatment reduced the relative esterification rate to a similar degree as acute leptin treatment (Figure 8). Exposure to 10 ng leptin/mL of medium for 48 h decreased the incorporation of palmitate into total lipids by 17% ($P < 0.05$). Higher concentrations of leptin decreased palmitate incorporation by approximately 42% ($P < 0.05$) relative to control incubations.

Fatty acid synthesis from glucose was not affected by acute exposure to leptin ($P > 0.05$; Figure 9), although insulin stimulated an 83% increase in fatty acid synthesis ($P < 0.05$). Chronic leptin treatment decreased fatty acid synthesis at medium concentrations of 100 and 1,000 ng/mL ($P < 0.05$, Figure 10); the data suggest that chronic leptin treatment decreased fatty acid synthesis in a dose-related manner. Insulin induced a 63% increase in fatty acid synthesis above control incubations ($P < 0.05$). Insulin-stimulated fatty acid synthesis was decreased with increasing concentrations of leptin ($P < 0.05$, Figure 10). Leptin at 10 ng/mL reduced insulin-stimulated fatty acid synthesis by 64%. Leptin (100 ng/mL) reduced insulin-induced fatty acid synthesis rate to the level of control incubations ($P < 0.05$), whereas 1,000 ng leptin/mL reduced the fatty acid synthesis rate to 74% of control incubations ($P < 0.05$).

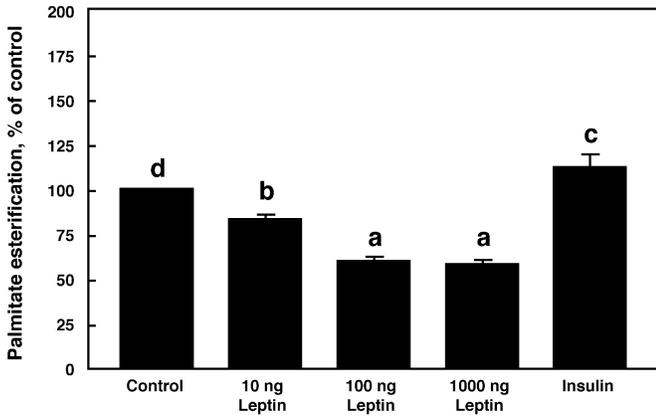


Figure 8. Relative percentage of palmitate incorporation into total lipids in response to chronic porcine leptin exposure. Adipocyte-containing primary cultures were incubated ± porcine leptin (10 to 1,000 ng/mL medium) for 44 h. This was followed by a 4-h incubation in a medium containing 0.5 μCi 1-¹⁴C-palmitate/mL ± porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin, followed by extraction of total lipids as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 13,764 ± 1,379 nmol incorporated/flask). Values that do not have a common letter differ (*P* < 0.05, *n* = 8).

Lipoprotein lipase activity was unaffected by chronic exposure to leptin (*P* > 0.05; Figure 11), although chronic exposure to insulin stimulated a 43% increase in LPL activity (*P* < 0.05).

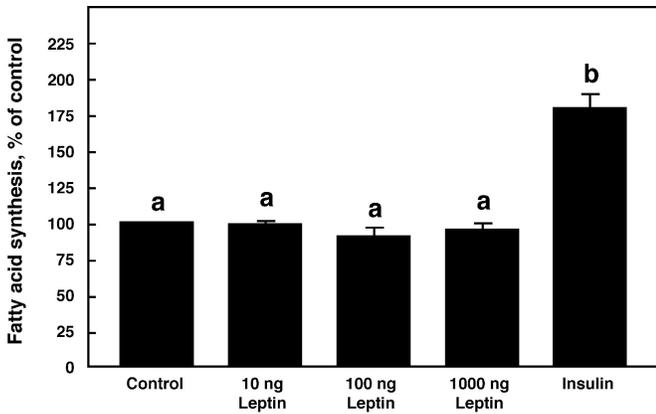


Figure 9. Relative percentage of glucose conversion to fatty acids in response to acute porcine leptin exposure. Adipocyte-containing primary cultures were incubated ± porcine leptin (10 to 1,000 ng/mL medium) or 100 nM insulin in a medium containing 1 μCi U-¹⁴C-glucose/mL for 4 h, followed by extraction of fatty acids as described in the Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 184 ± 40 nmol of glucose incorporated/flask). Values that do not have a common letter differ (*P* < 0.05, *n* = 4).

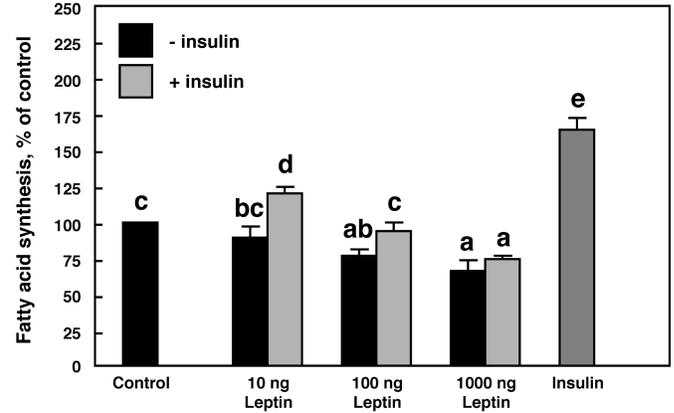


Figure 10. Relative percentage of glucose conversion to fatty acids in response to chronic porcine leptin exposure. Adipocyte containing primary cultures were incubated ± porcine leptin (10 to 1,000 ng/mL medium) for 44 h. This was followed by a 4-h incubation in a medium containing 1 μCi U-¹⁴C-glucose/mL, the porcine leptin concentration corresponding to the chronic treatment, ± 100 nM insulin. Fatty acid extraction was performed as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 122 ± 14 nmol of glucose incorporated/flask). Solid bars represent incubations without supplemental insulin, whereas hatched bars represent incubations supplemented with 100 nM insulin. Values that do not have a common letter differ (*P* < 0.05, *n* = 4).

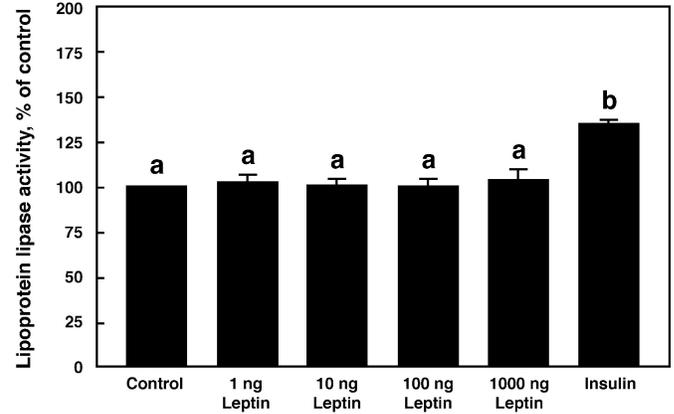


Figure 11. Relative percentage of lipoprotein lipase (LPL) activity in response to chronic porcine leptin exposure. Adipocyte-containing primary cultures were incubated ± porcine leptin (1 to 1,000 ng/mL medium) or 100 nM insulin for 48 h, followed by analysis for LPL activity as described in Materials and Methods. Data are expressed relative to cultures incubated without leptin (100% = 122 ± 14 nmol incorporated/well in 30 min). Values that do not have a common letter differ (*P* < 0.05, *n* = 4).

Discussion

Numerous studies have demonstrated that leptin can produce alterations in central mechanisms of feed behavior (Friedman and Halaas, 1998; Frühbeck et al., 1998). Initially, the reduction in feed intake produced by leptin 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 (Tartaglia et al., 1995; Bornstein et al., 2000). This suggests that leptin may specifically affect adipocyte metabolic functions.

Glucose oxidation was reduced by leptin treatment in the present study. This may be the result of leptin's inhibition of glucose uptake (Zhang et al., 1999). An acute effect of leptin on glucose oxidation was detected at the highest concentration tested (1,000 ng leptin/mL) in the present study, whereas a chronic effect was detected at 100 ng/mL. Leptin has been reported to stimulate the activities of pyruvate dehydrogenase and other enzymes of the Krebs cycle in skeletal muscle (Ceddia et al., 1999), but a similar effect in adipose has not been reported. As a decrease in glucose oxidation was reported in the present study, these data suggest that leptin may influence glucose uptake and/or utilization through glycolysis in porcine adipocytes. Rouru et al. (1999) have reported that leptin reduces glucose uptake in rodent adipose tissue. Although the 1,000 ng leptin/mL medium could reduce glucose oxidation, implying a direct action, the enhanced response to lower concentrations of leptin with chronic leptin treatment suggests that leptin may indirectly affect glucose uptake and glycolysis.

Palmitate oxidation was increased by leptin treatment in the present study, although only by very high concentrations (1,000 ng/mL) following chronic exposure. Wang et al. (1999) demonstrated that leptin up-regulates mRNA for peroxisome proliferator-activated receptor- α , carnitine palmitoyl transferase-1, and acyl CoA oxidase, suggestive of an increase in the capacity for intracellular fatty acid oxidation. Ceddia et al. (2000) reported that leptin (100 ng/mL) increased the rate of oleate degradation by up to 50% in rat adipocytes. That response by rat adipocytes was much greater than observed with pig adipocytes in the present study.

Similarly, the insulin response by rat adipocytes is much greater than for the pig adipocytes in the present study. Insulin reduces fatty acid oxidation in rat adipocytes and stimulates esterification (Harper and Saggeron, 1976). However, no effect of insulin was detected in the present study. Pig adipocytes have been characterized as having a limited insulin responsiveness (Mersmann, 1989), which may account for the absence of an effect. Whether the lack of an insulin or leptin response is a physiological difference between species or the result of differing methodologies cannot be determined. Further studies are necessary to determine

whether leptin can increase fatty acid oxidation by the porcine adipocyte.

Acute treatment with leptin did not affect glucose conversion into lipid, whereas chronic treatment with leptin reduced both total lipid and fatty acid synthesis from glucose. These data suggest that leptin may function through an intermediary molecule to alter glucose conversion to lipid, as a period sufficient for transcription and translation was necessary to produce a metabolic response, an adaptive response. Previous studies have demonstrated *in vivo* that acute leptin treatment does not alter lipogenesis (Lopez-Soriano et al., 1998). However, Müller et al. (1997) 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 4-h incubation with porcine leptin (acute experiment). This difference in responsiveness between the cell cultures in the present study and the isolated cell incubations mentioned above may be due to methodology, quality of the leptin preparation, or perhaps species differences. Fukuda et al. (1999) and Wang et al. (1999b) have reported that leptin can inhibit the expression of fatty acid synthase, and Fukuda and Iritani (1999) have reported that leptin can also inhibit ATP citrate lyase expression. Leptin's inhibition of these two key lipogenic enzymes may account for the significant reduction in lipogenesis observed in the present study.

As previously mentioned, leptin inhibits glucose uptake by adipose tissue (Rouru et al., 1999), which may certainly contribute to both the observed decrease in glucose oxidation and lipogenesis in the present study. Insulin can stimulate glucose uptake and utilization by adipocytes. Several studies have now demonstrated that leptin can reduce insulin sensitivity (Cohen et al., 1996; Müller et al., 1997) and thus affect adipocyte metabolism (Harris, 1998). Müller et al. (1997) reported that leptin could inhibit insulin-stimulated glucose uptake, lipogenesis, glycogen synthase activity, and protein synthesis in rat adipocytes. Walder et al. (1997) and Fukuda et al. (1999) have reported that leptin inhibits maximum insulin binding in isolated rat adipocytes but does not affect receptor affinity. Cohen et al. (1996) indicated that leptin induces dephosphorylation of insulin receptor substrate-1, thus antagonizing insulin's actions. However, more recent studies have demonstrated a complexity of interactions between the leptin receptor and insulin receptor signaling pathways (Szanto and Kahn, 2000). Further studies are necessary to elucidate the how leptin binding impacts insulin signaling mechanisms in swine.

In vivo experiments have demonstrated that leptin administration enhances glucose disposal and insulin sensitivity or responsiveness in rodents (Rouru et al., 1999; Sivitz et al., 1997). This is in direct contrast to the *in vitro* data presented here that demonstrates an inhibition of insulin responsiveness. This apparent conflict can be explained by differential tissue metabolic responses. Wang et al. (1999) reported that leptin has

differential effects in regulating glucose disposal among rat tissues. Using a hyperinsulinemic clamp, leptin treatment increased glucose utilization in extensor digitorum longus muscle and soleus muscle, while reducing glucose utilization in epididymal adipose tissue, by producing an insulin-resistant state in the adipocyte. Skeletal muscle is the major site of glucose disposal in the animal, and leptin enhances that utilization. Thus, leptin augments the partitioning of nutrients toward muscle and away from adipose tissue accretion.

These actions of leptin may not have any relationship to the obese state, wherein leptin resistance is observed. Rather, during the postprandial period leptin may function to partition nutrients away from adipose storage and toward utilization for oxidation or synthetic purposes by other tissues. Leptin has been demonstrated to stimulate glucose uptake in muscle (Cusin et al., 1998), as well as promote glycogen synthesis in muscle and hepatocytes (Aiston and Agius, 1999; Berti et al., 1997; Cusin et al., 1998).

Insulin has been demonstrated to regulate the expression of leptin in porcine adipocytes (Chen et al., 1999). The inhibition of insulin-mediated glucose metabolism in porcine adipocytes by leptin would suggest that leptin can feedback upon the adipocyte to regulate its own expression. Serum leptin levels rise in swine with increasing adiposity (Ramsay et al., 1998; Cameron et al., 2000). This may reflect increases in leptin secretion by enlarging adipocytes. This signal then can be integrated through the central nervous system to limit feed intake and thus limit further adipose accretion, and secondly this paracrine/autocrine leptin can feedback upon the adipocyte to limit its synthetic capacity and thus ability to enlarge. In other words, leptin may function to mediate energy balance/lipid accretion at the adipocyte. Perturbations in this regulatory cycle (environmental and physiological) may result in excess adipose accretion through elevation in consumption and adipose lipid accretion.

Leptin has been reported to inhibit fatty acid esterification in rodent pancreatic islets (Shimabukuro et al., 1997) and skeletal muscle (Muoio et al., 1999; Steinberg and Dyck, 2000). The present study extends this observation to porcine adipose tissue. It is uncertain whether 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 suggest that leptin can regulate the activity of at least one of the enzymes involved in fatty acid esterification.

Lipoprotein lipase is an enzyme that is typically elevated in the adipose tissue of animals with excessive adipose accretion or feed intake (Fielding and Frayn, 1998). Lipoprotein lipase is considered an important enzyme for regulating fatty acid uptake by adipocytes

(Fielding and Frayn, 1998). Peripheral leptin infusion did not alter LPL activity in adipose tissue of mice (Picard et al., 1998) or rats (Lopez-Soriano et al., 1998). However, chronic peripheral leptin treatment of rats was reported to produce a 90% increase in the level of LPL mRNA in brown adipose tissue (Siegrist-Kaiser et al., 1997). In the present study, chronic treatment of pig adipocytes *in vitro* did not alter LPL activity. This may be due to differences between brown and white adipose tissue. Alternatively, *in vivo* administration results in the interaction of leptin with the entire hormonal milieu. The *in vitro* experiment performed in this study only examined the chronic effect of leptin on LPL activity without assessing leptin's potential interactions with other hormones.

The metabolic effects of recombinant porcine leptin were assessed in a concentration range from 10 to 1,000 ng leptin/mL medium. This is beyond the physiological range for swine, 2 to 8 ng/mL (Barb et al., 2001; Cameron et al., 2000; Whisnant and Harrell, 2002). Experiments evaluating leptin's effects on metabolic activity have used various leptin preparations and methodologies. In rat adipocytes, Muller et al. (1997) required 32 ng of recombinant mouse leptin/mL medium to detect changes in adipocyte glucose metabolism and insulin sensitivity, whereas maximal inhibition of insulin's effects required 480 ng/mL. Aiston and Agius (1999) reported that glycogen synthesis in rat hepatocytes was maximally stimulated by 800 ng of mouse leptin/mL medium. In skeletal muscle, Ceddia et al. (1999) demonstrated that 1600 ng of mouse recombinant mouse leptin/mL medium stimulates glycogen synthesis twofold, whereas glucose oxidation can be increased 75% by only 16 ng/mL medium. These are some of the examples of the extreme variability in the metabolic response to leptin. The nonobese rat typically has serum leptin levels of between 5 and 10 ng/mL, whereas obese rodents may have serum levels approaching 50 ng/mL (Harris, 2000). Thus, many of these cited metabolic responses to recombinant leptin may be considered supraphysiological, if not pharmacological. The absence of a relative standard for recombinant leptin biological activity makes it difficult to determine whether this pharmacological response is the result of apparent differences in sensitivity between tissues or species, differences in experimental methodology, or varying quality between recombinant leptin preparations.

The data in the present study demonstrate that leptin inhibits glucose utilization and lipogenesis in porcine adipocytes. A portion of this inhibition may be due to antagonizing insulin binding and receptor signaling. These metabolic effects are primarily an indirect response to leptin. Following exposure to leptin, a period of time that permits synthesis and secretion of additional autocrine/paracrine factors is necessary before observation of a metabolic response. The data in the present study also demonstrate that leptin alters fatty acid utilization by porcine adipocytes. 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 before changes in fatty acid metabolism. This direct action of leptin to inhibit palmitate utilization and the indirect action of leptin to reduce glucose utilization by adipocytes for lipid synthesis suggests that leptin can function to partition energy away from adipose tissue. For example, with fasting, leptin may function to partition nutrients away from adipose storage and toward utilization for oxidation or synthetic purposes by other tissues. This partitioning effect of leptin may be useful in a pharmacological approach to reducing fat mass in swine.

Implications

Leptin is a hormone produced by adipose tissue that can affect feeding behavior, animal health, and reproduction. Studies in rodents have demonstrated that leptin can decrease fat mass. This experiment was designed to determine whether porcine leptin can alter lipid synthesis in the adipose tissue. These data indicate that leptin can indirectly inhibit glucose conversion to lipid and directly inhibit fatty acid incorporation by porcine adipocytes. These data also demonstrate leptin functions to promote the partitioning of energy away from lipid accretion within porcine adipose tissue, in part by decreasing insulin-mediated stimulation of lipogenesis by reducing fatty acid utilization. These actions of leptin may be useful in a pharmacological approach to decreasing fat mass in swine.

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