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The regulation of IGF-1 by leptin in the pig is tissue specific and independent of changes in growth hormone[1]

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Abstract

A combination of in vivo and in vitro experiments were performed to determine the extent to which exogenous leptin regulates serum growth hormone (GH) and insulin-like growth factor I (IGF-1) concentrations, and the abundance of IGF-1 mRNA in major peripheral tissues. Initially (Experiment 1), a recombinant human leptin analog was administered i.m. to young growing pigs (approximately 27 kg body weight) for 15 days at 0 (control), 0.003, 0.01 and 0.03 mg · kg^{-1} · day^{-1}. Although there was no sustained effect of leptin on serum GH, there was a reduction ($P < 0.02$) in serum IGF-1 at the intermediate dose that paralleled a decrease ($P < 0.09$) in hepatic IGF-1 expression. Leptin, at these doses, did not reduce feed intake ($P > 0.57$), nor was there an effect of leptin on dietary nitrogen retention ($P > 0.97$). In a second experiment, pigs were injected with vehicle or a higher dose of leptin (0.05 mg · kg^{-1} · day^{-1}) for 14 days. A third treatment group was injected with vehicle and pair-fed to the intake of the group treated with leptin. In this study, exogenous leptin resulted in a sustained increase in serum leptin ($P < 0.0001$) and reduction in feed intake of approximately 30% ($P < 0.0001$). Serum IGF-1 was depressed in both the leptin-treated and pair-fed groups, relative to the group allowed ad-libitum intake ($P < 0.01$). Furthermore, there was no difference among treatments in the relative abundance of IGF-1 mRNA in skeletal muscle ($P > 0.42$) or adipose tissue ($P > 0.26$), and liver mRNA abundance was actually increased ($P < 0.01$) by leptin, despite the lower feed intake. Finally, to determine whether leptin altered the secretion of IGF-1 by isolated pig hepatocytes, primary cultures were incubated with leptin for 24 to 48 hr (Experiment 3). Leptin (100 nM) caused a sharp reduction ($P < 0.0001$) in dexamethasone-induced IGF-1 secretion at 24 hr (47% reduction) and at 48 hr (40% reduction). Collectively, these data indicate that leptin may regulate hepatic IGF-1 production in the pig, independent of GH, but that hepatocyte sensitivity to leptin may be depend on dose and in vitro vs. in vivo conditions.

Keywords: Leptin; Pig; IGF-1; Growth hormone; Hepatocyte; Feed intake

1. Introduction

Leptin, the protein product of the *obese* gene, has been implicated in several neuroendocrine loops, including the growth hormone (GH)-insulin-like growth factor-1 (IGF-1) axis (reviewed by Heiman [1]. There is convincing evidence that leptin regulates GH secretion by the pituitary gland in some species [2–5]. Furthermore, exogenous leptin prevents the typical decline in serum GH concentrations in rats deprived of food, a result likely linked to the concomitant increase in steady state levels of the GH mRNA, and an attenuation of the starvation-induced decline in growth hormone releasing factor (GRF) mRNA [6].

Whereas leptin seems to stimulate GH release, *obese* gene expression in adipose tissue is attenuated by exogenous IGF-1 in hypophysectomized [7] and intact [8] rats. Similarly, the enhancement of *obese* expression by dexamethasone, in vitro, is markedly reduced when IGF-1 is included in the incubation medium [9], and IGF-1 depresses circulating leptin concentrations in humans with deficient concentrations of GH [10]. Although these findings are consistent with the placement of leptin within the GH-IGF-1 regulatory loop, it has been reported previously [11] that the abundance of IGF-1 mRNA in the adipose tissue of cattle

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treated with GH was highly correlated with the *obese* mRNA abundance, despite the typical increase in serum IGF-1 that accompanies GH administration [12]. Villafuerte et al. [13] reported similar results in that the abundance of the transcripts for leptin and IGF-1 were highly correlated across several adipose depots in rats. However, leptin did not stimulate IGF-1 expression in vitro, nor did IGF-1 enhance *obese* expression. The relationship between leptin and IGF-1 in the rat seemed to stem from the linkage of both proteins to adipocyte size, and perhaps other metabolic characteristics, rather than from a direct regulatory relationship between them.

To date, no studies have addressed the possibility that exogenous leptin influences serum IGF-1 status and the expression of IGF-1 in major peripheral tissues. Using the pig as the experimental model, the primary objective of the study reported herein was two fold. First, we sought to establish whether prolonged treatment with exogenous leptin would regulate the expression of IGF-1 in peripheral tissues and change serum concentrations of growth hormone and IGF-1. Furthermore, because of the anabolic actions of GH and IGF-1 in growing animals (reviewed by Russell et al. [14], we determined whether leptin administration alters dietary nitrogen retention. Finally, we tested the hypothesis that leptin would directly regulate IGF-1 secretion in isolated pig hepatocytes.

2. Materials & methods

2.1. Experiment 1

2.1.1. Animals and experimental design

The Animal Care & Use Committee, Purdue University, approved all animal care and handling procedures. Male castrates weighing approximately 27 kg were assigned to four treatment groups based on the dose of leptin (0, 0.003, 0.010, and 0.030 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}, designated as control, low, intermediate, and high doses, respectively). The experiment was conducted in three replicate groups over time, with 3 pigs per treatment in each replicate group ($n = 9$). Animals were weighed and assigned to treatments after being deprived of feed overnight. Thereafter, blood samples were obtained via jugular venipuncture for serum recovery, and each pig was placed in an individual metabolism crate in an environmentally controlled room to allow an assessment of nitrogen retention. A standard corn-soybean meal diet containing 18% crude protein and 0.65% lysine was offered at 4% of body weight (approximately 90% of the estimated ad libitum intake quantity). Half of the feed was provided in the morning (by 0900) and the remainder in the evening (about 1800).

A recombinant human leptin analog (LY355101) was provided by Eli Lilly & Co., Indianapolis, IN, and was demonstrated to be effective in the pig in a preliminary study in which feed intake was reduced in a dose-dependent manner [15]. The protein was reconstituted in a sterile glycerol-based phosphate buffer (pH 7.5), and was injected i.m. for a total of 15 days with half the daily dose given at each feeding. The leptin injection regimen was initiated on day 3. The first blood samples were obtained from all pigs 24 hr after the first injection, and on days 6 and 15 thereafter. On day 8, the pigs were reweighed so the quantity of leptin to be injected could be adjusted for change in body weight. Sample collection for the nitrogen balance portion of the experiment was initiated on day 9 and covered a 5-day period as described previously [16]. Analysis of nitrogen content of feeds, feces and urine samples was accomplished as reported previously [17]. Nitrogen retention (percentage basis) was calculated for each pig as follows:

$$N_r = \frac{N_i - (N_f + N_u) \times 100}{N_i}$$

where N_r = Nitrogen retention, N_i = Nitrogen intake, N_f = fecal Nitrogen, and N_u = urinary Nitrogen.

On day 17 (15 days of leptin treatment), and approximately 4 to 6 hr after the last injection, final blood samples were collected and the animals were killed by electrocution and exsanguinations and tissue samples were collected immediately, frozen in liquid nitrogen, and stored at −80°C pending RNA isolation.

2.1.2. Hormone assays

Both GH and IGF-1 were quantified using a double antibody RIA procedure reported previously [18, 19]. The assay standard was lyophilized recombinant human IGF-1 (hIGF-1, Austral Biological, San Ramon, CA). Rabbit anti-human IGF-1 IgG was used as the primary antibody (UB-AFB rabbit anti-hIGF-1, National Hormone and Pituitary Program). The secondary antibody was goat anti-rabbit IgG (ICN Pharmaceuticals, Aurora, OH). The intra-assay coefficient of variation was 7.7%. The standard for the GH assay was pure porcine growth hormone (USDA- pGH-B-1, USDA-ARS Animal Hormone Program, Beltsville, MD). The primary antibody was guinea pig anti- pGH IgG, and the secondary antibody was goat anti-guinea pig IgG. The intra-assay coefficient of variation was 6.3%. Porcine leptin concentrations in serum samples were also estimated using a double antibody RIA. The pig anti-leptin antibody was developed in rabbits immunized with recombinant (T7-tagged) porcine leptin and was affinity purified prior to use in the RIA. The intra-assay coefficient of variation was 16%. The concentration of the human leptin analog in the sera of leptin-treated pigs was determined at Linco Research, Inc., St Charles, MO. The commercially available kit is specific for human leptin, and has no cross reactivity with porcine leptin. The intra-assay coefficient of variation was 9%. Serum insulin was determined using a commercially available porcine-specific ELISA kit (ALPCO, Windham, NH) and the manufacturer's protocol.

2.1.3. Ribonuclease protection assays

Ribonuclease protection assays were performed to assess the relative abundance of the transcripts for IGF-1 in the liver, adipose tissue and skeletal muscle. Total RNA was extracted as described by Chomczynski and Sacchi [20]. The integrity of the RNA preparation was verified by visualization of the 18S and 28S bands with ethidium bromide after electrophoresis on 0.8% agarose gels. Construction of the riboprobes and nuclease protection assays have been described previously [11,21]. Thirty μg of total RNA were used for each assay. Total RNA was quantified using the ribogreen assay kit from Molecular Probes (Eugene, OR). Protected fragments were resolved on 5% acrylamide gels containing 8M urea and exposed to film. Signal intensity was quantified using a Digital Science Imaging System (V. 2.0.1, Kodak, New Haven, CT).

2.2. Experiment 2

Thirty barrows with a mean initial body weight of 53 kg were randomly assigned to three treatments ($n = 10$): a group fed ad libitum and injected with vehicle, a leptin-treated group and allowed ad libitum intake, and a third group pair-fed to the leptin-treated group and injected with vehicle. The leptin-treated group was injected daily (AM and PM) with leptin at a dose of 0.025 mg $\cdot$ kg^{-1} at each injection to achieve a dose of 0.05 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} for 14 days. Blood samples were collected prior to the commencement of treatments for determination of initial serum hormone and metabolite concentrations, and also on days 7 and 14 days. Animals were killed by electrocution and exsanguinations and tissue samples were collected and processed as described in experiment 1.

2.3. Experiment 3

2.3.1. Pig primary hepatocyte culture

Suckling piglets at approximately two weeks of age and 5 kg body weight were anesthetized by intraperitoneal injection of sodium pentobarbital (100 mg $\cdot$ kg^{-1} bodyweight). Liver cells were isolated by the two-step collagenase perfusion technique described by Seglen [22], with slight modifications [23,24]. Briefly, the liver was initially perfused with a pre-warmed (37°C) Ca^{2+}- free buffer containing 8.3 g NaCl, 0.5 g KCl, 2.4 g HEPES per liter at pH 7.4. This was followed by perfusion with a collagenase buffer (collagenase type 4, Sigma, St Louis, MO) containing 3.9g NaCl, 0.5g KCl and 24g HEPES per liter at pH 7.6. Liberated cells were sequentially filtered using cotton cheesecloth and a nylon mesh (200 μm) and the cell suspension was centrifuged twice at 50 *g*. Viable cells were plated on 60 mm Primaria tissue culture dishes at a density of 2-3 $\times$ 10^6 cells in an initial plating media (DMEM-Sigma D 5523 with Arginine) supplemented with 10% FBS, 100 nM dexamethasone and 100 nM porcine insulin. After 4 hr of initial plating, the cells were placed in a serum-free medium containing 100 nM dexamethasone, 1 nM porcine insulin and 0.1% BSA for 18 to 24 hr, after which cells the medium was changed to a treatment media (DMEM Sigma 5030), and supplemented with 2.02 g NaH_2CO_3, 2.38 g HEPES, 1g glucose per liter, 0.1% BSA and 1% antibiotic-antimycotic. Hepatocytes were cultured under different treatment conditions and designated as follows: control, without dexamethasone (dex) and leptin, leptin (with 100 nM leptin), dex with 100 nM dexamethasone and dex+leptin with 100 nM leptin and 100 nM dexamethasone) for 24 and 48 hr. At the termination of culture (after 24 or 48 hr of culture), media was aspirated and stored at −80°C prior to being assayed for the IGF-1 peptide.

2.3.2. Statistical analyses

For the in vivo experiments, all data were analyzed using a mixed model analysis of a split plot design [25]. For variables measured in serum samples collected over time (hormones and metabolites), Sattherthwaite's approximation of degree of freedom [26] was used to adjust for variation in sample size. For hormone and serum metabolite data, the main effects (treatment and day) and the interaction effects were tested using the treatment * replicate * day interaction as the error term. However, treatment * replicate was used as error term for performance variables and mRNA expression data. When protected by a significant F test, mean separation was accomplished using the least squares mean separation (pdiff) procedure [25]. For the in vitro study, analysis of variance was used. Treatment and time were the main effects and treatment $\times$ time was the interaction effect. Means were separated by least squares mean separation when protected by a significant F-test.

3. Results

3.1. Experiment 1

The effects of leptin administration on growth and related variables are shown in Table 1. There was no effect of leptin administration on the feed intake ($P > 0.57$) or growth rates of these pigs overall ($P > 0.70$) or during the weekly increments of the study. Likewise, neither the efficiency of weight gain (gain:feed) or nitrogen retention during the 5-day balance period were influenced by leptin ($P > 0.89$ and 0.97, respectively).

Serum hormone and metabolite concentrations are shown in Table 2. There was no significant effect of day for GH ($P > 0.11$) or IGF-1($P > 0.26$), nor was there a treatment by day interaction. However, GH was reduced approximately 20% ($P < 0.05$) by the low dose of leptin, whereas IGF-1 was decreased 24% ($P < 0.02$) in pigs receiving the intermediate dose of leptin. The insulin concentration was not influenced by leptin ($P > 0.29$), but was higher overall ($P < 0.0001$) on day 15 vs. previous days. There was no effect of leptin on concentrations on serum

Table 1
Feed intake, growth rate, and nitrogen retention in pigs injected with vehicle or a recombinant human leptin analog for 15 days

		Leptin Dose, mg · kg^{-1} · day^{-1}				
	Day	0	0.003	0.01	0.03	SEM[1]	P > F[2]
Mean Daily	3–8	1.11	1.08	1.10	1.10	0.05	0.70
Feed Intake (kg)	9–17	1.15	1.15	1.11	1.04	0.08	0.55
	3–17	1.14	1.12	1.10	1.07	0.06	0.57
Growth Rate (kg per day)	3–8	0.66	0.61	0.58	0.64	0.09	0.94
	9–17	0.67	0.67	0.68	0.53	0.10	0.21
	3–17	0.64	0.65	0.65	0.57	0.07	0.70
Gain:Feed	3–8	0.58	0.56	0.54	.58	0.09	0.98
	9–17	0.59	0.59	0.62	0.54	0.10	0.72
	3–17	0.56	0.58	0.59	0.55	.07	0.89
Nitrogen Retention (%)	9–13	60.99	61.89	61.89	61.12	1.97	0.97

[1] Pooled standard error.
[2] P > F treatment (n = 8–9).

urea nitrogen ($P > 0.22$) and there was no interaction of leptin with day ($P > 0.43$).

The expression data (relative mRNA abundance) for IGF-1 transcripts are summarized in Table 3. The effect of leptin on IGF-1 expression was dependent upon tissue and dose. The intermediate dose caused a 35% reduction ($P <$

Table 2
Serum hormone and metabolite concentrations in pigs injected with a recombinant human leptin analog for 15 days

		Leptin Dose, mg · kg^{-1} · day^{-1}						P > F for:		
Hormone	Day*	0	0.003	0.010	0.030	Day	SEM[1]	Trt[2]	Day	Trt*Day
GH[†] (ng/ml)	1	3.85	4.09	6.85	6.25	5.26	0.53			
	6	6.23	3.39	4.96	7.19	5.44	0.55			
	15	4.99	3.00	4.39	4.43	3.95	0.53	0.05	0.11	0.29
	Mean	5.02[b]	3.49[a]	5.40[b]	5.63[b]		0.33			
IGF-1 (ng/ml)	1	95.31	79.54	67.57	93.84	84.07	15.24			
	6	100.96	100.43	76.72	91.83	92.49	15.29			
	15	125.79	117.99	101.67	120.34	116.45	15.24	0.02	0.26	0.96
	Mean	107.36[b]	99.32[b]	81.99[a]	102.00[b]		8.42			
Insulin (ng/ml)	1	0.08	0.14	0.09	0.11	0.10[x]	0.02			
	6	0.11	0.09	0.06	0.08	0.09[x]	0.02			
	15	0.13	0.13	0.12	0.15	0.13[y]	0.02			
	Mean	0.11	0.11	0.09	0.11		0.01	0.29	0.0001	0.31
Leptin[3] (ng/ml)	1	3.30	3.75	3.65	4.26	3.79	1.12			
	6	3.10	4.24	3.25	3.00	3.31	1.17			
	15	3.38	3.55	3.8	4.67	3.65	0.96	0.61	0.65	0.88
	Mean	3.07	3.75	3.70	3.73		0.96			
Urea Nitrogen (mg/dl)	1	9.98	10.30	8.20	10.05	9.63	0.34			
	6	10.13	10.15	9.69	10.32	10.07	0.35			
	15	10.66	9.85	9.14	8.88	9.63	0.35	0.22	0.49	0.43
	Mean	10.26	10.10	9.01	9.75		+0.41			

* Refers to number of days on leptin treatment.
[†] Statistical significance is based on the logarithmic transformation of the GH data, which was necessitated by heterogeneity of variances.
[a,b]Means were separated using the pdiff procedure of mixed model when protected by a significant F-test for treatment (Dose). Means in the same row with different superscripts differ significantly ($P < 0.05$).
[x,y]Means were separated using the pdiff procedure of mixed model when protected by a significant F-test for Day. Means in the same column with different superscripts differ significantly ($P < 0.01$)
[1] Pooled standard error
[2] P > F for treatment (n = 8–9)
[3] Only the porcine leptin was measured in this assay

Table 3
Relative abundance of IGF-1 mRNA transcripts in selected tissues of pigs injected with vehicle or a recombinant human leptin analog for 15 days

	Leptin Dose, mg · kg^{-1} · day^{-1}				SEM[1]	P > F[2]
	0	0.003	0.010	0.030		
Tissue:						
liver[†]	52.86[a]	47.17[a]	34.26[b]	51.54[a]	4.3	0.09
muscle[m]	13.09	12.03	14.48	11.73	3.76	0.96
adipose	49.17[b]	60.64[a]	54.21[ab]	46.09[bc]	3.95	0.09

[a,b]Means with different superscripts significantly differ at P < 0.05
[†] Means differ at P < 0.09
[m] longissimus dorsi
[1] Pooled standard error
[2] P > F for treatment (n = 8–9)

0.09) in liver IGF-1 mRNA, whereas the low dose caused a 23% increase ($P < 0.09$) in adipose mRNA abundance. There was no effect of leptin on IGF-1 expression in longissimus muscle ($P > 0.96$).

3.2. Experiment 2

The daily feed intake pattern is presented in Fig. 1. Leptin depressed feed intake as early as 24 hr after the first injection (day1, $P < 0.09$). Though there was a transient lessening of the response on days 2 to 4, there was a sustained depression in feed intake in the leptin-treated pigs from day 5 on ($P < 0.001$). The weight gain and efficiency of gain data are presented in Table 4. The animals on all treatments remained in positive energy balance as reflected in their weight gain. Overall, the weight gain of the leptin-treated and pair-fed groups was 50 and 56%, respectively ($P < 0.0001$), as much as that of the control group allowed ad libitum intake. These groups also utilized their feed less efficiently for weight gain (36% and 30%, respectively) relative to the control group ($P < 0.01$).

The data on serum hormone and metabolite concentrations are shown in Table 5. The GH concentration was not different among treatments after 1, 7 or 14 days ($P > 0.26$). However, overall, IGF-1 was depressed by 12% and 18% in

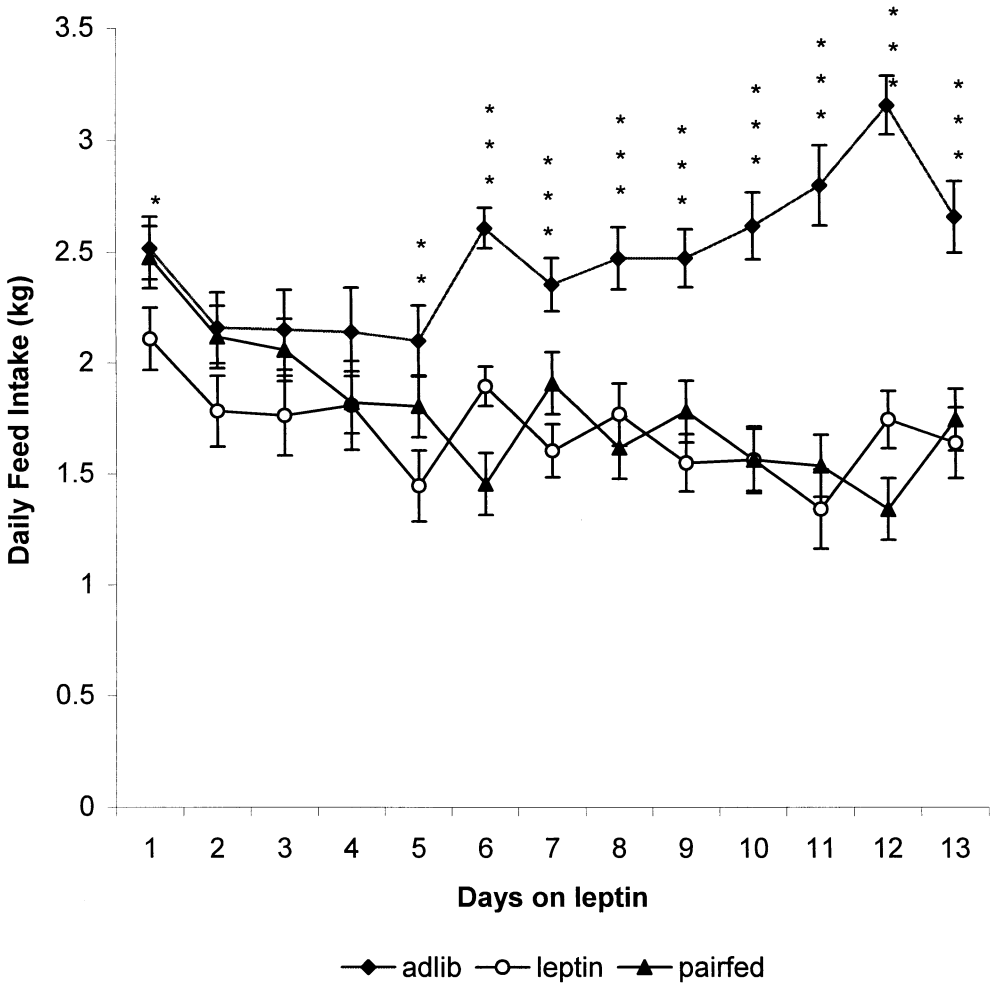

Fig. 1. Daily feed intake of pigs injected with vehicle or leptin (0.05 mg · kg^{-1} · day^{-1}). The ad libitum (adlib) group was allowed ad libitum intake and injected with vehicle twice daily for 14 days. The pair-fed group was injected with vehicle, and their intake was limited to that of the pigs injected with leptin. P values for mean separation: * P < 0.09; ** P < 0.02; *** P < 0.001.

Table 4
Growth rate and gain per unit of feed consumed (Gain:feed) in pigs injected with vehicle or recombinant human leptin analog for 14 days

		Treatment[1]				
	Day	Ad-libitum	Leptin	Pair-fed	SEM[2]	P > F
Growth Rate	1–7	0.99[b]	0.47[a]	0.61[a]	0.13	0.0001
(kg per day)	8–14	1.44[b]	0.67[a]	0.73[a]	0.24	0.0003
Mean	1–14	1.21[b]	0.57[a]	0.67[a]	0.11	0.0001
Gain:Feed	1–7	0.44[b]	0.24[a]	0.29[a]	0.03	0.0001
	8–14	0.52	0.35	0.49	0.09	0.20
Mean	1–14	0.48[b]	0.30[a]	0.39[a]	0.05	0.01

[1] The ad-libitum group was allowed ad-libitum intake and injected with vehicle twice daily for 14 days. The leptin group was injected with recombinant human leptin (0.05 mg · kg^{-1} · day^{-1} and also allowed ad libitum intake. The pair-fed group was injected with vehicle, and their intake was limited to that of the pigs injected with leptin:(n = 10 pigs per treatment).

[a,b]means with different superscripts are different at the corresponding treatment P values i.e., 0.0001 to 0.01.

[2] Pooled standard error

the leptin-treated and pair-fed pigs, respectively ($P < 0.01$), and there was no evidence of a treatment by day interaction ($P = 0.18$). Leptin administration caused a significant increase in serum leptin (approximately 300%, $P < 0.0001$). Serum urea nitrogen was reduced approximately 15% ($P < 0.02$) in the leptin-treated group relative to both the ad-libitum control group and the pair-fed pigs. The expression (mRNA abundance) data for IGF-1 are shown in Table 6. Expression was not influenced by leptin in adipose tissue or skeletal muscle ($P > 0.26$), but was increased about 50% ($P < 0.01$) in liver relative to both the control and pair-fed groups. *Obese* expression was not influenced by treatment ($P > 0$ .69).

3.3. Experiment 3

The concentration of IGF-1 in the hepatocyte culture medium is shown in Fig. 1. The secretion of IGF-1 was increased significantly by dexamethasone ($P < 0.0001$), but not leptin ($P > 0.88$). However, leptin did attenuate the stimulation of IGF-1 secretion by dexamethasone by 40 to 50% after 24 hr ($P < 0.06$) and after 48 hr ($P < 0.08$) of culture.

4. Discussion

There are few studies that have investigated the effects of exogenous leptin on GH and IGF-1, especially in non-

Table 5
Serum hormone and metabolite concentration in pigs injected with recombinant human leptin for 14 days

		Treatment[1]					P > F For:		
Hormone	Day	Ad-libitum	Leptin-treated	Pair-fed	Day Mean	SEM[2]	Trt	Day	Trt*Day
GH (ng/ml)	1	4.23	5.49	5.56	5.10	2.45			
	7	4.47	3.58	6.94	5.00	2.78			
	14	3.73	8.86	7.34	6.65	2.61	0.26	0.47	0.53
	Mean	4.14	5.98	6.62		1.57			
IGF-1 (ng/ml)	1	138.10	145.82	132.54	138.82	15.25			
	7	147.04	124.48	131.53	134.35	15.31			
	14	167.39	129.19	107.69	134.76	15.31	0.01	0.85	0.18
	Mean	150.85[a]	133.17[b]	123.92[b]		8.81			
Leptin[3] (ng/ml)	7	3.70	11.21	3.10	6.08	0.83			
	14	3.20	15.21	2.90	7.48	1.35	0.0001	0.22	0.24
	Mean	3.37[b]	13.31[a]	3.03[b]		1.25			
Urea N (mg/dl)	1	9.81	9.34	10.00	9.71	0.70			
	7	9.88	7.94	10.71	9.51	0.70			
	14	10.38	7.80	8.23	8.80	0.70	0.02	0.26	0.15
	Mean	10.02[a]	8.36[b]	9.64[a]		0.58			

[1] The ad libitum group was allowed ad libitum intake and injected with vehicle twice daily for 14 days. The leptin group was injected with recombinant human leptin (0.05 mg · kg^{-1} · day^{-1}) and also allowed ad libitum intake. The pair-fed groups was injected with vehicle, and their intake was limited to that of the pigs injected with leptin.

[2] Pooled standard error

[3] Sum of concentrations obtained in the pig leptin and human leptin RIAs

[a,b]Means were separated using the least squares mean separation when protected by a significant F-test for treatment. Means in the same row with different superscripts differ significantly at corresponding treatment P values: (n = 10 pigs per treatment).

Table 6
Relative abundance of IGF-1 mRNA transcripts in selected tissues of pigs injected with recombinant human leptin for 14 days

	Treatment[1]				
	Ad-libitum	Leptin-treated	Paid-fed	SEM[2]	P > F For Trt
IGF-1:					
Muscle[m]	0.70	0.50	0.48	0.1	0.42
Adipose	0.90	0.97	0.79	0.08	0.26
Liver	0.70[b]	1.0[a]	0.6[b]	0.1	0.01

[1] The ad libitum group was allowed ad libitum intake and injected with vehicle twice daily for 14 days. The leptin groups was injected with recombinant human leptin (0.05 mg kg^{-1} day^{-1} and also allowed ad libitum intake. The pair-fed group was injected with vehicle, and their intake was limited to that of the pigs injected with leptin: (n = 10 pigs per treatment).

[2] Pooled standard error

[a,b]Means with different superscripts significantly differ at $P < 0.05$

[m] longissimus dorsi.

rodent models. This is of particular interest because the regulation of GH production in rodents and humans differs considerably, and the pig may be more reflective of the latter. This is certainly true with respect to feed deprivation, which markedly diminishes GH secretion in rodents, and stimulates it in humans [27] and pigs [28]. The data obtained in the in vivo experiments reported herein indicate that leptin administration does not cause sustained changes in serum GH, even when a significant reduction in feed intake is achieved and maintained for an extended period, as happened in the second study. Leptin delivered centrally in the pig causes a transient increase in serum GH concentrations [3]. Although we cannot rule out a transient increase related to the timing of leptin administration, any such increase was not apparent in serum samples obtained several hours following the AM leptin injection on any of the designated sampling days. Although serum concentrations of leptin were clearly increased in the second study, it is possible that the level of leptin transported into the brain was insufficient to achieve a higher baseline concentration of GH.

The data presented herein indicate a potential complex relationship in which leptin targets the liver to regulate IGF-1 production. In the first study, leptin, at a relatively low dose, attenuated liver IGF-1 expression, and reduced serum IGF-1 concentrations, albeit in a dose-dependent manner. Furthermore, leptin attenuated the induction of IGF-1 release into the culture medium by dexamethasone in isolated pig hepatocytes. Taken together, these data seem to indicate that leptin regulates IGF-1 production in the liver. However, these findings must be reconciled with the lack of a response at the higher dose in Experiment 1, and an actual reversal in Experiment 2 in which leptin increased IGF-1 expression. It is possible that the higher doses of leptin, particularly when combined with an extended duration of administration (i.e., 2 weeks), invoked a desensitization response that negated the suppressive effect of leptin and eventually allowed hepatic IGF-1 expression to rebound. The suppressors of cytokine signaling (SOCS proteins) may block leptin signaling and promote leptin resistance in tissues expressing these genes [29]. Emilsson et al. [30] reported a marked induction (over 3-fold) of SOCS-3 expression in the liver of lean mice injected twice daily with leptin for several days, albeit at a higher dose than used in the present study. We have confirmed the expression of the SOCS-3 protein in isolated pig adipocytes and hepatocytes (unpublished results), and are working currently to establish the extent to which leptin regulates its own receptor and SOCS-3 expression. It is possible that the sensitivity of the pig hepatocyte to leptin is such that doses lower than those common to the rodent literature are sufficient to invoke SOCS-3 expression and attenuate leptin signaling, especially with prolonged administration.

The biological explanation for the attenuation of hepatic IGF-1 production by leptin is not clear, but may relate to the regulation of energy substrate utilization. This effect of leptin is consistent with that of interleukin 6 (IL-6) [40], another class I cytokine, and is perhaps reflective of similar JAK-STAT-mediated signaling. Hepatic production of IGF-1 is not essential to normal growth in mice [31], but is important for carbohydrate and lipid metabolism [32]. Leptin activates signaling pathways in the liver that overlap, but are yet distinct from, those common to insulin [33]. Thus, leptin may influence energy metabolism in part by regulating liver IGF-1 production.

Although not studied previously, several findings detailed in the literature indicate that leptin could influence nitrogen retention and muscle protein accretion. If leptin sustained an increase in serum GH, an increase in the serum IGF-1 concentration and in expression in liver and skeletal muscle would perhaps follow [3]. This is important because IGF-1 is a major anabolic growth factor that is highly correlated with protein accretion in skeletal muscle [34,35]. Secondly, in myotube cultures, leptin mimics the anabolic activity of insulin in that it activates the PI-3 kinase signaling pathway [36] and stimulates glucose transport [37]. In this study, we found no evidence that exogenous leptin influenced IGF-1 expression in skeletal muscle or improved nitrogen retention. Although serum urea nitrogen concentrations were reduced in Experiment 2 by leptin, this has been equated with the induction of a lipolytic response by

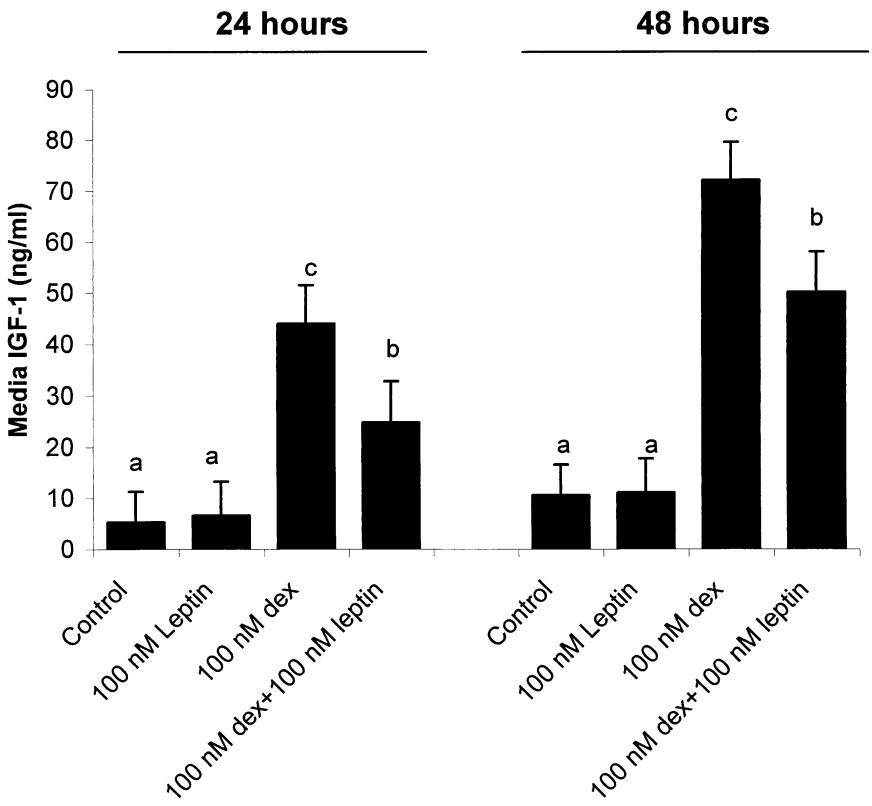

Fig. 2. Media IGF-1 concentration in hepatocytes treated with leptin and dexamethasone for 24 and 48 h. [a,b,c], Bars with superscript "a" differ from others at $P < 0.001$. Within each time period, bars with superscripts b and c differ from each other at $P < 0.06$ (24h) and at $P < 0.08$ (48h). Data represent means of 6 replicates.

leptin and the ensuing use of glycerol and fatty acids for energy, rather than amino acids [38]. In the absence of an enhancement of IGF-1 in the serum or expression in skeletal muscle, the lack of any effect of leptin on nitrogen retention is not surprising.

Although the literature is replete with documentation of the anorectic effect of leptin in rodent models, little is known regarding this role of leptin in the pig. Barb et al. [3] reported that leptin administered centrally suppressed feed intake in pigs in a short term study, and Wuethrich et al. [15] established that leptin administered peripherally reduces feed intake in a dose dependent manner. Herein, we confirm these previous findings (Experiment 2), and also show that the anorectic effect of leptin is sustained for at least 2 weeks in the pig. The efficacy of leptin diminishes within two weeks in rodents injected with substantially higher doses [39], but whether this is a species difference or consequence of dose remains to be determined.

In summary, these data indicate that prolonged leptin administration does not cause a sustained increase in circulating GH concentrations, nor does it alter nitrogen retention in young growing pigs. However, leptin acts directly on hepatocytes to suppress IGF-1 secretion, and influences liver IGF-1 mRNA abundance and circulating IGF-1 concentrations in an apparent dose-sensitive manner. (Fig. 2)

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