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Regulation of PPARγ but not *obese* gene expression by dietary fat supplementation

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Leptin, the product of the obese *gene, and peroxisome proliferator activated receptor gamma (PPARγ) are important regulators of energy metabolism, adipogenesis, and immune function. In rodent models, both genes seem to respond at the mRNA and/or protein levels to dietary fat consumption. To determine the effect(s) of dietary saturated and polyunsaturated fatty acids on the expression (mRNA abundance) of these genes, adipose tissue was obtained from pigs fed three different dietary fat sources. Corn-soybean meal diets containing no added fat (NO, control) or 10% beef tallow (BT), safflower oil (SO), or fish oil (FO) were fed ad libitum (*n = *12) for 12 weeks. The abundance of* obese*, PPARγ1, and PPARγ2 mRNA was quantified relative to 18S rRNA using ribonuclease protection assays. The gain:feed ratio was improved (*P < *0.05) 21% by all fats with a corresponding reduction (*P < *0.05) in feed intake. Relative to pigs fed NO, serum total cholesterol was increased (*P < *0.01) in pigs fed BT and triglyceride and nonesterified fatty acid concentrations were increased (*P < *0.01) by all supplemental fats. Serum insulin was increased (*P < *0.10) only by SO. Neither* obese *nor PPARγ1 mRNA abundance were responsive to added fat (*P > *0.15). However, the abundance of PPARγ2 mRNA was increased fourfold by SO compared with the NO diet. These data indicate that the abundance of* obese *mRNA is independent of dietary fat consumption per se, whether saturated or unsaturated, when feed consumption is reduced due to greater dietary caloric density. Furthermore, we provide evidence that expression of the PPARγ2 gene in porcine adipose tissue is selectively responsive to SO (presumably linoleic acid, 18:2n-6).* (J. Nutr. Biochem. 11: 260–266, 2000)

Keywords: porcine; PPARγ; leptin; fatty acids; dietary fat

Introduction

The peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and have been linked to myriad biological processes including glucose homeostasis, monocyte function, lipid metabolism, and adipocyte differentiation.[1–3] To date, there are three known PPARs: α, γ, and δ, with PPARγ having at least three isoforms that originate from the use of different promoters and alternative splice sites.[4,5] It is possible that there are no functional differences in the PPARγ isoforms. However, PPARγ2 has received much attention recently because it is highly expressed in adipose tissues compared with other tissues and is associated with adipocyte differentiation and lipid filling. Similarly, leptin, the product of the *obese* gene, is a key regulator of energy balance and metabolism in mammalian species[6,7] and is also an integral component of specific immunologic processes.[8,9] Although there is a paucity of information regarding endogenous ligands for the PPARγ proteins, some prostaglandins[10] and polyunsaturated fatty acids (PUFA), including 18:2n-6,[11] have been implicated as activators. As regards the *obese* gene, regulatory factors known to date include glucose, nonesterified fatty acids (NEFAs), cyclic adenosine monophosphate (cAMP), cortisol, insulin, and growth hormone. Furthermore, increased dietary fat consumption may increase *obese* mRNA and/or circulating leptin concentrations, even when adiposity is not changed.

Collectively, several findings indicate a possible biochemical linkage of PPARγ and leptin. Recent studies by

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Table 1 Ingredient compositions and calculated analyses of the growing (A–D) and finishing (E–F) diets

	A	B	C	D	E	F	G	H
Ingredient (%)								
Ground soft wheat	26.51	8.27	8.29	8.27	38.11	20.09	20.11	20.09
Wheat flour	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Wheat middlings	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal (48% CP)	21.35	29.61	29.58	29.61	9.50	17.62	17.60	17.62
Calcium carbonate	1.08	0.99	0.99	0.99	1.18	1.08	1.08	1.08
Dicalcium phosphate	0.54	0.60	0.60	0.60	0.57	0.63	0.63	0.63
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Bleachable fancy tallow (BT)		10.00				10.00		
Safflower oil (SO)			10.00				10.00	
Fish oil (FO)				10.00				10.00
Amino acids*	0.02	0.03	0.03	0.03	0.14	0.07	0.07	0.07
Vitamins and trace minerals†	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis (%)								
Crude protein	21.19	22.47	22.46	22.47	17.33	18.51	18.50	18.51
Calcium	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Available phosphorus	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.97	1.14	1.14	1.14	0.75	0.88	0.88	0.88
Energy	14.05	16.48	16.47	16.48	14.25	16.69	16.68	16.69
Lysine (g/MJ DE)	0.69	0.69	0.69	0.69	0.53	0.53	0.53	0.53

*Provided L-lysine (as L-lysine HCl, 98%), L-methione (as DL-methionine), and/or L-threonine as needed to meet needs. Ratio of digestible amino acids to lysine were: total sulfur amino acids, 0.65; threonine, 0.72; trytophan, 0.185.
†Provides vitamins and trace minerals such that the final diet contains (per kg): vitamin A, 6,500 IU; vitamin D_3, 660 IU; vitamin E, 22 IU; vitamin B_{12}, 19.8 μg; choline, 895 mg; niacin, 25 mg; pantothenic acid, 17 mg; riboflavin, 4.8 mg; copper, 222 mg; iodine, 0.7 mg; iron, 168 mg; manganese, 45 mg; selenium, 0.3 mg; zinc, 189 mg.

our group[12,13] have shown *obese* and PPARγ2 mRNA abundance in porcine adipose tissue to decrease concomitant with feed deprivation. In addition, despite greater feed intake and adipose mass, rats treated with thiazolidinediones (high affinity ligands of PPARγ) have reduced *obese* mRNA abundance relative to controls.[14] At the molecular level, Hollenberg et al.[15] have shown that activation of PPARγ by thiazolidinediones antagonizes C/EBPα, a positive regulator of *obese* gene transcription. Thus, activation of PPARγ may depress *obese* transcription. Given that some PUFAs influence PPARγ expression and activity, and that activation of this transcription factor may impact *obese* expression, the primary objective of this study was to determine the impact of dietary fatty acid supplementation on PPARγ and *obese* mRNA abundance in porcine adipose tissue.

Materials and methods

Diets and experiment design

Adipose tissue was obtained from control animals that were a part of a larger study designed to evaluate the effect of dietary fat source on immunologic response criteria in pigs challenged with lipopolysaccharide. The experiment protocol was reviewed and approved by the institutional (Purina Mills, Inc., St. Louis, MO USA) animal care and use committee. For the dietary treatments, corn-soybean meal diets containing 10% beef tallow (BT), safflower oil (SO), or menhaden fish oil (FO) were formulated to maintain a constant lysine to metabolizable energy (ME) ratio using a least-cost algorithm. Minima for other amino acids were determined by their ratio to lysine (i.e., "ideal protein" pattern). A corn-soybean meal diet devoid of added fat was used as the control diet (NO). Diet compositions and calculated analyses are shown in *Table 1* and dietary fatty acid profiles in *Table 2*.

Forty-eight pigs (progeny of Pig Improvement Company [PIC] Camborough females mated to line 63 males) were assigned to the four treatment groups (n = 12) based on initial body weight (approximately 23 kg). The pigs were confined in individual pens in a totally enclosed, environmentally regulated building for the duration of the study. Within the building, treatment was assigned to each replicate group of pens at random. Feed and water were provided ad libitum. At the completion of the study, blood samples were obtained by jugular venipuncture for serum and plasma recovery, and the pigs were sacrificed by exsanguination following mechanical stunning. Tissue samples were collected immediately and frozen in liquid nitrogen pending selected analyses.

Diet and tissue fatty acid determinations

Fatty acid profiles were determined for each diet and for selected tissues collected from three pigs fed each diet. Samples were prepared for gas chromatographic analysis using procedures detailed by the Association of Official Analytical Chemists.[16] Briefly, sample lipid was extracted with chloroform-methanol and saponified with alcoholic potassium hydroxide, and the free fatty acids extracted with hexane. After washing with water, the fatty acid preparation was dried with sodium sulfate and the fatty acids were esterified with methanol using boron trifluoride as the catalyst. The fatty acid methyl esters were dissolved in heptane and injected into the gas chromatograph equipped with a flame ionization detector. The percents of individual fatty acid methyl esters were calculated relative to the total.

mRNA extraction and ribonuclease protection assays

Total RNA extractions and ribonuclease protection assays were carried out as described previously.[12,13] The 18S rRNA subunit was used as the internal standard to correct for small RNA loading differences. Subcutaneous adipose tissue was collected from the

Table 2 Major fatty acids contained in the growing and finishing diets

	Growing				Finishing			
	NO	BT	SO	FO	NO	BT	SO	FO
ω-6 (%)								
18:2 Linoleic	56.11	11.71	69.45	14.85	56.41	11.43	70.62	13.17
20:2 Eicosadienoic	<0.10	<0.10	<0.10	0.35	<0.10	<0.10	<0.10	0.37
20:4 Arachidonic	<0.10	<0.10	<0.10	0.46	<0.10	<0.10	<0.10	0.46
ω-3 (%)								
Linolenic	4.50	1.06	1.13	2.39	4.28	0.96	0.91	2.20
Eicosapentaenoic	<0.10	<0.10	<0.10	6.98	0.30	<0.10	<0.10	7.43
Docosapentaenoic	<0.10	<0.10	<0.10	1.57	<0.10	<0.10	<0.10	1.58
Docosahexaenoic	<0.10	<0.10	<0.10	7.10	<0.10	<0.10	<0.10	7.32
Saturated (%)								
14:0 Myristic	0.21	2.57	0.16	6.75	0.42	2.45	0.13	7.08
16:0 Palmitic	17.47	22.83	8.81	20.24	17.41	21.99	8.57	20.34
20:0 Arachidic	0.25	0.14	0.33	0.17	0.17	0.13	0.31	0.26
Other (%)								
16:1 Palmitoleic	0.24	2.39	0.13	7.43	0.45	2.33	0.12	7.69
18:1T Elaidic	<0.10	3.92	<0.10	1.03	<0.10	4.78	<0.10	0.80
18:1C Oleic	15.55	30.25	13.91	10.02	15.47	30.18	13.43	10.46
18:1C Vaccenic	0.99	1.57	0.82	2.93	0.96	1.58	0.77	2.88
20:1 Eicosenoic	0.64	0.25	0.27	1.18	0.67	0.26	0.26	1.18

NO–no added fat. BT–beef tallow. SO–safflower oil. FO–fish oil.

depot located over the cervical spine. Composite samples of middle and outer adipose tissues were extracted.

Serum chemistry

Serum variables (glucose, cholesterol, and triglyceride) were quantified using an automated clinical chemistry analyzer (Hitachi Model 704 Hitachi, San Jose, CA USA). All assay kits were purchased from Sigma Chemical Co. (St. Louis, MO USA). NEFAs were measured using a kit purchased from Wako Chemical (Richmond, VA USA) and the manufacturer's protocol. Insulin concentrations were determined using a commercially available porcine insulin kit and protocol (ALPCO, Windham, NH USA).

Statistical analysis

The general linear models (GLM) procedure of SAS[17] was used to perform the analysis of variance (ANOVA). Treatment means were compared based on the least significant differences test (LSD)[18] when protected by a significant *F*-test. Heterogeneity of variances required that the insulin data be transformed logarithmically prior to ANOVA and LSD procedures.

Table 3 Adipose tissue fatty acid profiles in pigs fed selected fat sources for 12 weeks*

	NO	BT	SO	FO
ω-6 (%)				
18:2 Linoleic	10.59	9.06	46.58	9.37
20:2 Eicosadienoic	0.45	0.32	1.75	0.64
20:4 Arachidonic	0.23	0.17	0.33	0.32
ω-3 (%)				
18:3 Linolenic	0.72	0.68	0.57	1.53
20:5 Eicosapentaenoic	<0.10	<0.10	<0.10	3.46
22:5 Docosapentaenoic	<0.10	<0.10	<0.10	2.11
22:6 Docosahexaenoic	<0.10	<0.10	<0.10	4.84
Saturated (%)				
14:0 Myristic	1.45	1.64	0.72	2.18
16:0 Palmitic	25.87	21.27	14.75	20.72
20:0 Arachidic	0.19	0.12	0.20	0.15
Other (%)				
16:1 Palmitoleic	2.48	2.82	0.83	4.59
18:1C Oleic	37.10	40.73	21.78	23.48
18:1C Vaccenic	2.79	2.65	1.28	4.50
18:1T Elaidic	0.24	2.38	0.14	0.28
20:1 Eicosenoic	0.69	0.72	0.47	0.71

*Values are least squares means of three pigs selected at random from each dietary treatment group.
NO–no added fat. BT–beef tallow. SO–safflower oil. FO–fish oil.

Table 4 Growth performance of pigs fed different dietary fat sources during the growing-finishing phases (12 weeks)

	NO	BT	SO	FO	SEM	$P < F$*
Daily gain (g)	932[b]	968[b]	968[b]	882[a]	16	0.01
Feed intake (g)	2,395[b]	2,068[a]	2,045[a]	1,895[a]	63	0.01
Gain: feed	0.391[a]	0.472[b]	0.475[b]	0.472[b]	0.009	0.01

*Probability of a greater *F*-value. Least squares means ($n = 12$) in the same row not followed by a common letter differ ($P < 0.05$) using least significant differences procedure.
NO–no added fat. BT–beef tallow. SO–safflower oil. FO–fish oil.

Results

Marked changes in fatty acid composition in adipose tissue were achieved by adding specific fat sources to the diets (*Table 3*). Of particular importance to the objective of this study was the confirmation that feeding SO, which is approximately 80% 18:2n-6, enriched the adipose tissue content of this fatty acid approximately 3.5-fold. Likewise, feeding FO resulted in substantial increases in the total n-3 content; the predominant n-3 fatty acids were 18:3, 20:5, 22:5, and 22:6. Only negligible quantities of 20:3 and 22:2 (n-3) were detected. The pigs fed the BT diet had tissue fatty acid profiles quite similar to those of the pigs fed the NO diet.

Growth performance data are summarized in *Table 4*. FO reduced ($P < 0.01$) daily gain by 5 to 8% compared with pigs fed the other fats and the NO diet. All fat sources reduced ($P < 0.01$) feed intake relative to pigs fed the NO diet. The reduction in feed intake was reflected in an improved ($P < 0.01$) gain:feed ratio.

Serum variables are presented in *Table 5*. Total cholesterol was approximately 20% higher ($P < 0.01$) in the serum of pigs fed the BT diet. Serum triglyceride and NEFA concentrations were increased ($P < 0.01$) by all fat sources, and glucose concentrations were higher ($P < 0.01$) in pigs fed BT and FO versus pigs fed the diet devoid of added fat. There was also a tendency for insulin concentrations to be higher ($P < 0.10$) in the serum of pigs fed the SO diets than in those fed the NO diet.

The PPARγ and *obese* mRNA data are presented in *Figures 1* and *2*, respectively. Both isoforms of PPARγ were readily detected in adipose tissue of pigs from all dietary treatments. Although neither PPARγ1 nor *obese* mRNA abundance were responsive to added fat ($P > 0.15$), the abundance of PPARγ2 mRNA in the adipose tissue of pigs fed the SO diet was approximately fourfold greater ($P < .01$) than that of pigs fed the NO diet.

Discussion

The activation of PPARs by PUFAs[19,20] and the linkage of PPAR activation to adipogenesis[1–3] exemplify the regulatory attributes of fatty acids. These regulatory relationships are now of considerable interest in light of the potential for enrichment of tissues with specific fatty acids as a means of influencing metabolism and growth. The data presented reaffirm the well-established potential for loading porcine tissues with n-6 or n-3 fatty acids by dietary modification. Adding PUFA (SO or FO) to the diet resulted in adipose tissue fatty acid profiles that were unique to each respective fat source. In addition, de novo lipogenic activity, coupled with the incorporation of diet-derived fatty acids, resulted in adipose tissue fatty acid profiles in pigs fed the BT diet that were similar to those of pigs fed the diet devoid of added fat.

Given the linkage of PPARγ to fatty acid transport genes,[21,22] it might be anticipated that one or both of the isoforms evaluated would be upregulated in response to increased dietary fat intake. Indeed, our results indicate a fourfold greater expression of PPARγ2 in response to supplemental SO in porcine adipose tissue. Vidal-Puig et al.[23] reported a similar result: Mature mice fed a high-fat diet had a greater abundance of PPARγ2 mRNA in adipose tissue than did controls. However, Rousseau et al.[24] reported that PPARγ2 was not influenced in rat pups by a high-fat weaning diet relative to the carbohydrate-based control. It seems possible that stage of maturity is a factor in the accumulation of PPARγ2 mRNA caused by dietary fat.

Table 5 Concentrations of serum metabolites and insulin in pigs fed different dietary fat sources during the growing-finishing phases (12 weeks)

	NO	BT	SO	FO	SEM	$P < F$*
Cholesterol (mg/dL)	101[a]	123[b]	104[a]	102[a]	4.0	0.01
Glucose (mg/dL)	77.1[a]	92.7[b]	84.4[a,b]	91.3[b]	3.6	0.01
Insulin (ng/mL[†])	113[a]	171[a,b]	195[b]	162[a,b]	24	0.10
NEFA (mEq/dL)	0.0428[a]	0.072[b]	0.084[b]	0.074[b]	0.00772	0.01
Triglycerides (mg/dL[‡])	20.5[a]	36.9[b]	32.9[b]	35.7[b]	3.0	0.01

*Probability of a greater *F*-value. Least squares means ($n = 12$) in the same row not followed by a common letter differ ($P < 0.05$) using least significant differences procedure.
[†]Heterogeneity of variances required that insulin data be transformed logarithmically prior to statistical analysis. However, nontransformed least squares means and SEM are presented for convenience.
[‡]Based on triolein as the assay standard.
NO–no added fat. BT–beef tallow. SO–safflower oil. FO–fish oil.

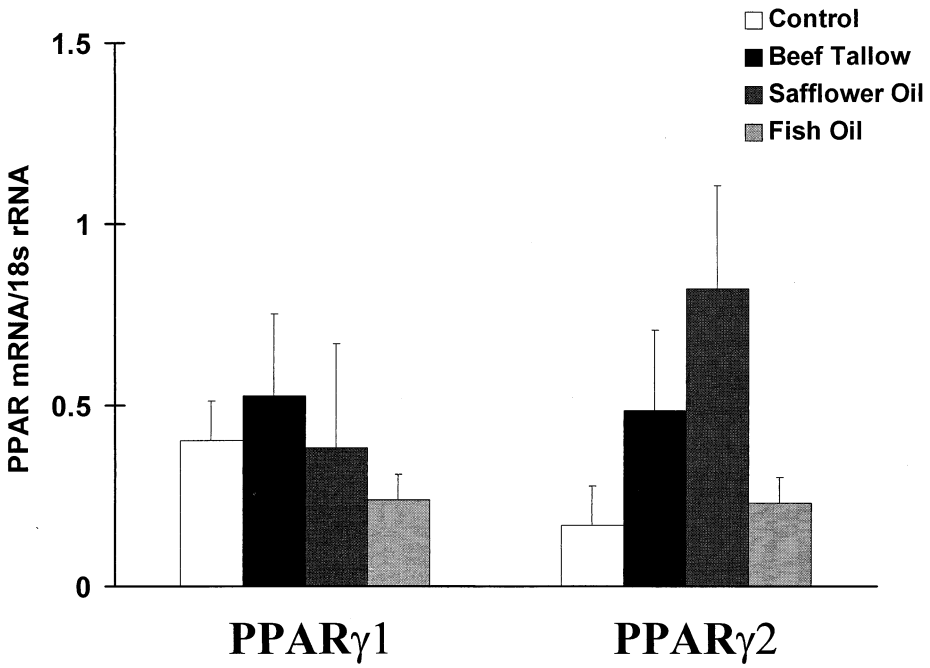

Figure 1 Effect of selected dietary fat sources on peroxisome proliferator activated receptor (PPAR) γ1 and PPARγ2 mRNA in subcutaneous adipose tissue. Growing-finishing pigs were fed corn-soybean meal-based diets containing no added fat (control) or 10% beef tallow, safflower oil, or menhaden fish oil for 12 weeks. Values represent least squares means (n = 12) and standard error. Expression of PPARγ1 was not influenced by dietary fat whereas expression of PPARγ2 was increased ($P < 0.01$) by safflower oil relative to the control diet.

The present results demonstrate for the first time that although PPARγ1 expression is not influenced by any of the fat sources used, PPARγ2 expression in the pig is markedly upregulated by SO (presumably by 18:2n-6) versus FO and BT, which are rich in n-3 and saturated fatty acids, respectively. The biochemical reason for this fatty acid specificity is not apparent but poses an intriguing question. Although characterization of the transcriptional roles of PPARγ is only beginning, it is tempting to speculate that each isoform will have unique targets. Transcription of the three PPARγ isoforms is under the control of distinct promoters[4,5] and the protein products of γ1 and γ2 differ structurally in that an additional 31 amino acids are placed at the N terminus of PPARγ2.[3] Furthermore, although PPARγ1 mRNA is the predominant form in porcine subcutaneous adipose tissue, restricting caloric intake or complete feed deprivation causes a change (reduction) only in PPARγ2 mRNA.[12] The molecular differences coupled with the fact that isoforms respond differentially to metabolic and dietary cues implies the possibility of unique regulatory targets for each isoform.

The upregulation of PPARγ2 mRNA occurred in parallel with the enrichment of the adipose tissue with 18:2n-6, an established ligand for PPARγ. Although activation of PPARγ per se was not evaluated in the present study, Frohnert et al.[22] identified a functional PPAR response element (PPRE) in the murine fatty acid transport protein gene and have demonstrated this gene to be upregulated in vitro in response to 18:2n-6. It is possible that greater intake

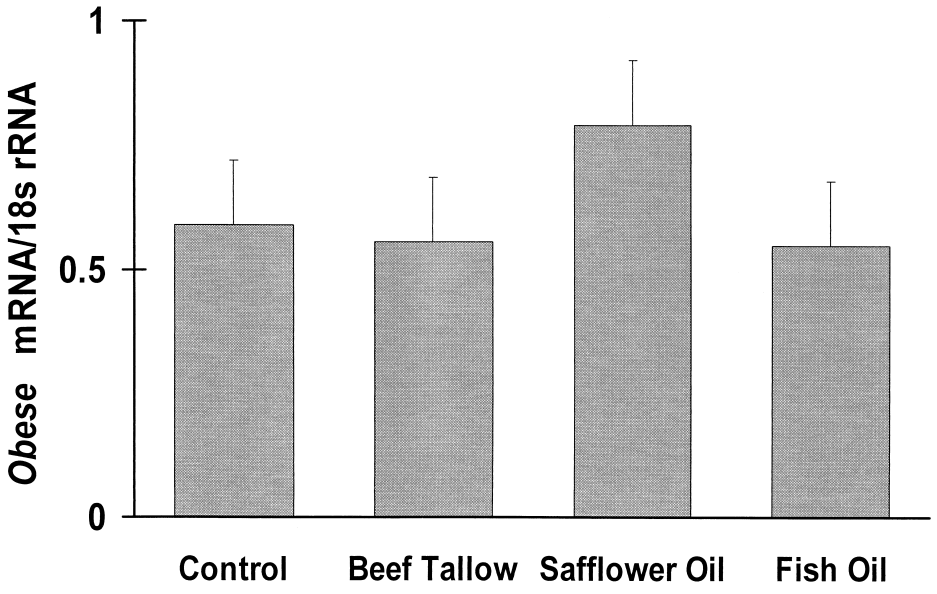

Figure 2 Effect of selected dietary fat sources on *obese* mRNA abundance in subcutaneous adipose tissue. Growing-finishing pigs were fed corn-soybean meal-based diets containing no added fat (control) or 10% beef tallow, safflower oil, or menhaden fish oil for 12 weeks. Values represent least squares means (n = 12) and standard error. Obese mRNA abundance was not influenced by dietary fat.

of 18:2n-6 causes an increase in PPARγ2 to afford adipocytes with a greater capacity to process this particular fatty acid. It should likewise be noted that insulin is a positive regulator of adipocyte PPARγ1 and PPARγ2 expression in vivo and in vitro[25] and insulin concentrations tended to be higher in pigs fed the SO diet than in those fed the NO diet. Finally, 18:2n-6 is readily elongated to form 20:4n-6, a major precursor for prostaglandin production. Some eicosanoids are potent regulators of PPARγ activity[11,26] and it seems possible that altered production of eicosanoids contributed to the greater expression of PPARγ2 in pigs fed SO.

The results reported herein show clearly that *obese* expression in the pig is unresponsive to diets containing appreciable levels of supplemental saturated (BT) or polyunsaturated (SO and FO) fats. Rodents consuming diets containing added fat often fail to regulate intake according to the greater caloric density of the diet. Studies in which expression of the *obese* gene and circulating leptin concentrations were evaluated indicate increased *obese* expression and/or circulating leptin concentrations.[23,24,27–30] We have shown previously[13] that *obese* gene expression in the pig is highly correlated with fat mass and is reduced by complete feed deprivation, but not by intake restriction. With respect to *obese* expression in the present study, two considerations are notable. First, all pigs consuming diets with added fat responded with improvements in the gain:feed ratio and corresponding reductions in feed intake that were anticipated based on the greater caloric density of these diets versus NO. Accordingly, differences in fat deposition would unlikely be of sufficient magnitude to drive an increase in *obese* mRNA abundance. Second, although pigs fed FO had slower growth rates and lower feed intake and were smaller at the completion of the study, these differences were likewise of lesser magnitude than what has been typically associated with differences in *obese* mRNA abundance in the pig.

Possible biochemical linkages between leptin and PPARγ have been suggested based on concomitant changes in these proteins or in the abundance of the transcripts that code for them. Our group previously determined that mRNA abundance for both PPARγ2[12] and the *obese* gene[13] decline in response to feed deprivation. Central administration of recombinant leptin causes a near doubling of PPARγ expression in rat adipose tissue.[31] Furthermore, ligand activators of PPARγ reduce *obese* expression in vitro[32] and in vivo,[14] a response that has been linked directly to PPARγ antagonism of C/EBPα,[15] a transcription factor that drives *obese* expression. Despite the possible linkages of these important adipocyte genes, we show clearly a marked increase in PPARγ2 expression with no change in that of the *obese* gene. It remains to be determined whether PPARγ1 is a constitutive regulator of *obese* transcription.

In summary, we have shown PPARγ1 and PPARγ2 mRNA abundance in porcine adipose tissue to be differentially regulated, with the latter being increased markedly in response to a diet enriched with linoleic acid. We have also shown *obese* mRNA abundance to be independent of dietary fat intake (when energy consumption is tightly regulated) and increased PPARγ2 mRNA abundance. Future characterizations of specific biochemical roles of these genes may identify novel targets for the manipulation of adiposity and related factors in mammals.

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