

Depression of Lipogenesis in Swine Adipose Tissue by Specific Dietary Fatty Acids¹

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ABSTRACT: The objective of this study was to document the influence of specific dietary fatty acids on rates of lipid synthesis and sensitivity to insulin in porcine adipose tissue. Weanling pigs were assigned to one of six groups, and each group was fed diets containing 10 g/100 g of added cornstarch or 10 g/100 g of added fatty acid. The fatty acid-enriched diets contained either a combination of 14:1 plus 16:1 (14:1/16:1 diet), 16:0, 18:0, 18:1, or 18:2 (*n*-6). With the exception of the cornstarch diet, all diets contained approximately 35% 14:0. Subcutaneous adipose tissue samples were collected at slaughter from the area overlying the first cranial vertebra. Fresh samples were incubated for 2 h in 20 mM glucose and 0, 10, 100 or 1,000 μ U/mL of porcine insulin. The smallest adipocytes were observed in adipose tissue from pigs

fed the 16:0 or 18:2 diets. Glucose incorporation into lipids was greater ($P < .05$) in adipose tissue from cornstarch-fed pigs than in adipose tissue from the other treatment groups. Lipogenesis was 67, 53, 35, 32, and 20% lower ($P < .05$) in adipose tissue from 16:0-, 14:1/16:1-, 18:0-, 18:2-, and 18:1-fed pigs, respectively, than in adipose tissue from the cornstarch-fed pigs. Insulin increased lipogenesis by 19% ($P < .05$) in adipose tissue from the cornstarch-fed pigs and by 15 to 40% ($P < .05$) in adipose tissue from the 14:1/16:1-fed pigs. Insulin did not stimulate lipogenesis ($P > .4$) in adipose tissue from pigs fed the 16:0, 18:0, or 18:1 diets. The data suggest that fatty acid chain length and unsaturation are determinants in the effects of dietary fat and insulin on de novo lipogenesis.

Key Words: Adipose Tissue, Porcine, Lipogenesis, Insulin, Adipocytes

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Introduction

Allee et al. (1971a) demonstrated that 10% dietary corn oil and 10% dietary beef tallow are equally effective in depressing lipogenesis in porcine adipose tissue, suggesting that unsaturated and saturated fatty acids were similar in their effects on de novo fatty acid biosynthesis. However, beef tallow is composed primarily of oleic acid [18:1(*n*-9)] and contains only 25 to 30% palmitic acid (16:0) (St. John et al., 1987). To our knowledge, the effects of specific fatty acids on de novo fatty acid biosynthesis in porcine adipose tissue have not been demonstrated.

High fat diets also reduce insulin responsiveness of muscle and adipose tissues (Anderson, 1981; Susini and Lamau, 1978; Salans et al., 1981; Grundleger and Thenen, 1982; Hissin et al., 1982; Sidery et al., 1990).

Harris (1992) demonstrated that, whereas a low fat diet (2% of energy) initially increased sensitivity, a high fat diet (61% of energy) decreased insulin sensitivity compared with a control diet (21% of energy from fat). Fatty acid saturation also influences the extent of insulin resistance. Diets high in saturated fatty acids (30% of energy from palm oil) increased insulin resistance in rats much more than diets high in either monounsaturated (30% of energy from olive oil) or polyunsaturated fatty acids (30% of energy from sunflower seed oil) (van Amelsvoort et al., 1988). With a reduction in fat from 30% of energy to 15% of energy intake, insulin sensitivity improved in all rats; however, the difference between the saturated fatty acid and polyunsaturated fatty acid diets was still apparent.

This investigation took advantage of the availability of several free fatty acid mixtures, including a unique combination of myristoleic acid (14:1) and palmitoleic acid (16:1). Our results indicate that dietary fatty acid composition has pronounced effects on overall lipogenesis; dietary fatty acid composition also affected insulin sensitivity of porcine adipose tissue.

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Table 1. Baseline diet composition^a

	Amount
Composition (g/100 g as fed)	
Sorghum	56.48
Soybean meal	29.00
Lysine HCl, 98.8%	.14
Lipid or cornstarch	10.00
Dicalcium phosphate	1.56
Limestone	.92
Vitamin mix ^b	.50
Trace mineralized salts ^c	.25
Salt	.25
Medication ^d	1.00
Calculated content, %	
Crude protein	18.00
Total lipids (cornstarch diet)	2.50
Total lipids (other diets)	12.50
Lysine	1.05
Calcium	.80
Phosphorus	.65

^aDiet exceeds all NRC requirements.

^bComposition of the vitamin mix: retinyl palmitate, 2.3 mg/kg; cholecalciferol, 6.4 µg/kg; all-*rac*- α -tocopheryl acetate, 44 mg/kg; menadione sodium bisulfate, 2.8 mg/kg; riboflavin, 7.7 mg/kg; *d*-pantothenic acid, 30 mg/kg; niacin, 33 mg/kg; choline, 287 mg/kg; cyanocobalamin, 44 µg/kg.

^cContributed the following (mg/kg of diet): Cu, 10; Fe, 100; I, .3; Mn, 15; Zn, 100; Se, .3.

^dProvided 55 mg of Carbadox per kg of diet.

Materials and Methods

Animals and Treatments. Weanling pigs were obtained from sows housed at the Texas A&M University Department of Animal Science. Procedures for the handling and sampling of animals were approved by the Texas A&M University Animal Care Committee. Each treatment group contained seven pigs. The pigs were weaned at approximately 28 d of age. Weanling pigs were fed a sorghum-based diet containing neither added fat nor cornstarch (Table 1) for 1 wk after weaning to allow acclimation to solid food and were then allotted to one of the six treatment groups. One of six littermates from each of seven sows was assigned to each diet group. Five groups received test diets containing 10 g of added fatty acids/100 g diet (by wet weight); one group received the control soybean meal diet containing cornstarch substituted by weight for the added fatty acids. The cornstarch diet, although not isocaloric to the diets containing added fatty acids, was included to allow comparison of these relatively high fat diets to a normal diet. All diets contained identical amounts of protein, lysine, roughage, minerals and medication (Table 1).

The cornstarch or fatty acids were mixed with the other dietary components weekly in a food mixer. The cornstarch diet was calculated to contain 2.5 g lipid/100 g of diet (as fed), whereas the other diets contained 12.5 g of lipid/100 g of diet (2.5 g/100 g endogenous lipid plus 10 g/100 g added fatty acids) (Table 1). The fats were fed as free fatty acids,

obtained from the Henkel Corporation (Cincinnati, OH). The pigs were allowed free access to the food, and fresh water was available at all times. Diets were fed for 35 d, and pigs were weighed weekly.

For the fatty acid-enriched diets, the predominant fatty acids were myristoleic and palmitoleic acids combined (14:1/16:1 diet; 24 and 12%, respectively, of the dietary fatty acids), palmitic acid (16:0 diet; 52%), stearic acid (18:0 diet; 51%), oleic acid (18:1; 47%), or linoleic acid (18:2 *n*-6; 38%) (Table 2). All fatty acids were obtained from the Henkel Corporation (Emery Group). The myristoleic-palmitoleic acid mixture (provided without charge) was Emery 889, a non-food-grade fatty acid mixture. Palmitic, stearic, oleic, and linoleic acids were Emersol food grade fatty acids (6343, 6353, 6321, and 315, respectively).

Because the 14:1/16:1 fatty acid mixture contained approximately 33% 14:0 (Table 2), myristic acid (Sigma Chemical, St. Louis, MO) was added to all other diets containing added fatty acids. In this manner, we were able to control for any effects of the 14:0 present in the 14:1/16:1 diet. Although we did not distinguish positional isomers for 18:1, sunflower oil was the source of this fatty acid mixture, and it is therefore presumed to consist predominantly of the *n*-9 isomer.

The fatty acid composition of the actual diets also is indicated in Table 2. The cornstarch diet contained a high percentage of 18:1 and 18:2; however, because the cornstarch diet contained only 2.5 g of total lipid/100 g of diet, the actual amounts of 18:1 and 18:2 were less than .6 and 1.1 g/100 of g diet, respectively. In contrast, the 18:1- and 18:2-enriched diets contained 5.7 g of 18:1/100 g of diet and 4.6 g of 18:2/100 g of diet, respectively.

Adipose Sample Collection. Pigs were transported to the abattoir (approximately 1 km) on the morning before slaughter and were slaughtered by accepted industry procedures. Adipose tissue samples were obtained at slaughter from the area overlying the first cranial vertebra and transported to the laboratory in Krebs-Henseleit bicarbonate buffer with 5 mM glucose (pH 7.4) at 37°C. Adipose tissue samples were incubated in vitro within 30 min after slaughter.

Fatty Acid Analysis. The fatty acid composition of subcutaneous adipose tissue was measured by gas-liquid chromatography as described previously (Sturdivant et al., 1992). One hundred milligrams of subcutaneous adipose tissue was extracted according to the method of Folch et al. (1957), and the extracted lipids were methylated according to a modified Morrison and Smith (1964) procedure. Subsequently, the fatty acid methyl esters were analyzed using a Packard Chrompack gas chromatograph (model 437a, Chrompack, Claritan, NJ) and separated on a stainless steel column (3 mm × 10 m) packed with Chromasorb W-Aw 80/100. Injector and detector temperatures were 225°C and 215°C, respectively. Oven temperature was 180°C initially. After 8 min,

Table 2. Fatty acid composition of diets

Fatty acid	Diet group					
	Baseline	14:1/16:1	16:0	18:0	18:1	18:2
Composition of fatty acid mixtures, g/100 g of total identifiable fatty acids ^a						
14:0	— ^b	33.4	.5	ND ^c	1.8	.1
14:1	—	30.2	.1	ND	1.4	ND
16:0	—	5.4	94.7	4.0	3.8	3.3
16:1	—	14.6	.2	ND	6.7	.2
18:0	—	ND	4.4	94.8	.2	.8
18:1	—	13.3	ND	ND	78.4	24.9
18:2(<i>n</i> -6)	—	.7	ND	ND	5.2	63.4
18:3(<i>n</i> -3)	—	.1	ND	ND	.2	7.2
Composition of diets, g/100 g of total lipid in diet ^d						
14:0	.21	25.8	31.1	28.8	28.2	31.3
14:1	.03	23.8	ND	ND	.78	ND
16:0	14.2	7.7	51.6	6.0	5.7	6.0
16:1	.54	11.9	ND	ND	3.9	.03
18:0	2.5	.48	3.2	50.9	.69	.87
18:1	27.1	.65	4.2	4.8	46.7	19.0
18:2(<i>n</i> -6)	50.0	17.5	7.3	8.9	13.1	37.6
18:3(<i>n</i> -3)	6.1	12.1	.05	.51	1.6	5.3

^aComposition of fatty acid mixtures as received.

^bMeasurement not applicable.

^cND = not detectable.

^dFatty acid composition of the total lipids of the diets (as fed). The baseline diet (with no added fat) was calculated to contain 2.5% lipid, whereas the other diets contained 12.5% (2.5% endogenous lipid plus 10% added lipid). Myristic acid was added to the 16:0, 18:0, 18:1, and 18:2 diets to equal the amount of 14:0 observed in the 14:1/16:1 diet (approximately 33%).

the oven temperature was increased by 10°C/min to a final temperature of 210°C. The flow rate of the carrier gas (nitrogen) was 22 mL/min. To each sample, a known amount of an internal standard (methyl laurate) was added. A standard of known composition was analyzed to verify the identity of the fatty acids in the samples. Fatty acid peaks determined by gas chromatography were then used to calculate amounts of fatty acids according to calculations described by Slover and Lanza (1979).

Tissue Incubations. Fresh adipose tissue was minced with scissors, and samples (50 to 100 mg) were incubated in 3 mL of incubation medium containing

Krebs-Henseleit bicarbonate buffer (pH 7.4), 20 mM glucose, 1 μ Ci of [U-¹⁴C]glucose, and 0, 10, 100 or 1,000 μ U/mL of porcine insulin. Vials were gassed for 1 min with 95% O₂:5% CO₂, capped, and incubated for 2 h in a shaking water bath at 37°C. At the end of the incubation period, reactions were terminated by addition of 3 mL of 5% trichloroacetic acid. Explants were rinsed with .154 M NaCl and Krebs-Henseleit bicarbonate buffer to remove free lipid and unincorporated substrate. The neutral lipids were extracted using the Folch et al. (1957) procedure as modified by Mersmann (1987). Samples were evaporated to dryness and resuspended in 10 mL of scintillation cocktail, and

Table 3. Final body weights, average feed intakes, and average feed:gain ratios of swine fed the test diets for 35 days

Measure	Diet group						SD ^b
	Baseline ^a	14:1/16:1	16:0	18:0	18:1	18:2	
Overall (35 d)							
Final wt, kg	22.0	23.8	22.3	22.3	21.5	23.3	2.73
Feed intake, kg/d	.83	.75	.79	.85	.65	.92	NA ^c
Feed:gain ratio	2.12	1.66	1.99	2.12	1.66	2.15	NA
Last 7 d							
Feed intake, kg/d	1.27	1.14	1.34	1.51	.92	1.11	NA
Feed:gain ratio	3.62	1.55	3.05	3.44	2.70	2.57	NA

^aValues are means for seven animals per treatment group.

^bStandard deviation.

^cNA = not applicable. Pigs were pen-fed, so data for feed intake could not be analyzed statistically.

Table 4. Fatty acid composition of adipose tissue of swine fed the test diets for 35 days

Tissue fatty acid	Diet group						SD ^b
	Baseline ^a	14:1/16:1	16:0	18:0	18:1	18:2	
	% total lipids						
14:0	1.3 ^e	8.1 ^{c,d}	9.1 ^c	9.0 ^c	7.4 ^d	7.5 ^d	1.50
14:1	.1 ^e	6.0 ^c	.5 ^d	.4 ^d	.3 ^d	.2 ^e	.05
16:0	25.7 ^d	20.3 ^e	32.1 ^c	26.7 ^d	25.3 ^d	18.7 ^e	1.45
16:1	5.7 ^d	14.6 ^c	6.5 ^d	5.2 ^d	6.8 ^d	3.0 ^e	.52
18:0	13.2 ^c	6.8 ^d	9.8 ^{c,d}	13.8 ^c	11.4 ^c	7.2 ^d	.82
18:1	42.2 ^c	34.4 ^d	30.5 ^{d,e}	34.5 ^d	36.8 ^{c,d}	26.5 ^e	3.80
18:2(<i>n</i> -6)	10.1 ^d	8.3 ^e	10.3 ^d	9.0 ^{d,e}	9.3 ^{d,e}	32.1 ^c	1.30
18:3(<i>n</i> -3)	1.5 ^d	1.4 ^d	1.0 ^d	1.1 ^d	1.8 ^d	4.2 ^c	.30
20:4(<i>n</i> -6)	.3	.2	.2	.2	.7	.6	.11
	g/100 g adipose tissue						
Total	57.7	55.7	57.4	58.7	62.2	58.4	4.62

^aValues are means for seven animals per treatment group.

^bStandard deviation.

^{c,d,e}Within a row, means without a common superscript differ ($P < .05$).

radioactivity was counted with a scintillation counter. Incorporation of ¹⁴C-labeled glucose into neutral lipids was calculated as glucose incorporated per 2-h incubation period per 10⁶ cells.

Adipose Tissue Cellularity. Procedures outlined by Etherton et al. (1977) as modified by Prior (1983) were used to determine adipocyte cellularity. Subcutaneous adipose tissue samples were frozen at -25°C and sliced in 1-mm-thick sections to facilitate tissue fixation. Fixed cells were filtered through 240- μ m, 64- μ m, and 20- μ m nylon mesh screens using .01% Triton in .154 M NaCl. The cells from the 20- μ m screen were counted and sized by a Coulter Counter (model ZM) equipped with a channelizer (model 256, Coulter Electronics, Hialeah, FL), using a 280- μ m aperture. The cells from the 64- μ m screen were counted on the same equipment with a 400- μ m aperture. Adipocyte sizes were divided into 10- μ m intervals; those counted with the smaller aperture were observed in channels between 20 and 60 μ m, and those counted with the larger aperture were in channels ranging from 60 to 190 μ m. Cells occurring in the 60- μ m channel from both apertures were summed.

Statistical Analyses. The data were analyzed using the GLM procedure of SAS (1986). The model included diet, insulin concentration, diet \times insulin concentration, and animal within diet. Insulin effects on rates of lipogenesis from glucose were tested by a paired *t*-test. Data for the adipose tissue fatty acid composition were analyzed by analysis of variance with diet as the main effect. Means were separated with Scheffe's multiple comparisons test using a predetermined significance of $P < 0.05$ for all comparisons (Steel and Torrie, 1980).

Results

Animal Performance. It was not possible to analyze feed intake data statistically because the pigs were pen-fed. Overall and during the last 7 d of the study, feed intake and feed:gain seemed lower in pigs fed the 14:1/16:1, 18:1, or 18:2 diets, i.e., the unsaturated fatty acid diets (Table 3). This had no apparent effect on animal performance, because there was no difference ($P = .22$) in final body weight after 35 d on trial, nor was there a diet \times time interaction ($P = .69$).

Fatty Acid Composition of Subcutaneous Adipose Tissue. Adipose tissue from pigs fed the cornstarch diet contained substantially less 14:0 (1.3%) than that from pigs fed the test diets (7.4 to 9.1%) (Table 4). The concentration of 14:1 was elevated from barely detectable to 6% of total fatty acids in pigs fed the 14:1/16:1 diet. The 16:0 diet increased, and the 18:2 diet decreased, the percentage of 16:0. The 18:0 diet had no effect ($P > .05$) on adipose tissue fatty acids, whereas the 18:1 diet increased 18:1 only relative to the 18:2 diet. The highest percentage of 18:1 was observed in adipose tissue from the cornstarch-fed pigs (Table 4). The percentages of 18:2 and 18:3 were significantly higher in the adipose of pigs fed the 18:2 diet, relative to the other treatment groups. There was no difference ($P > .05$) in total fatty acid content of the subcutaneous adipose tissue among treatment groups (Table 4).

Cellularity. Only adipose tissue from 18:1-fed pigs contained fewer ($P < .05$) cells per gram than adipose tissue from cornstarch-fed pigs (Figure 1). Adipose tissue from the 16:0-fed pigs contained more cells per gram ($P < .05$) than adipose tissue from 18:1-, 18:2-, or 14:1/16:1-fed pigs. Adipocytes from the 18:0- and 14:1/16:1-fed pigs were larger ($P < .05$) than

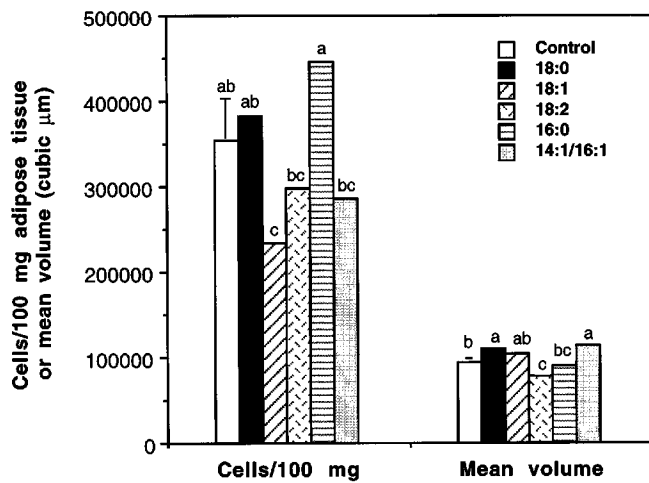


Figure 1. Cellularity of adipose tissue from pigs fed either cornstarch- or fatty acid-enriched diets. Each bar represents the mean for seven pigs per treatment group. The average standard errors of the mean for each measure (cells/gram or mean volume) are affixed to the symbols for the cornstarch-fed group. *a,b,c*Treatment groups with common superscript letters do not differ.

those from cornstarch-fed pigs, whereas adipocytes from 18:2-fed pigs were smaller (Figure 1).

Two general distributions of adipocytes were observed among treatment groups (Figure 2). Adipocytes from cornstarch-fed pigs and from those fed 18:0, 18:1, or the 14:1/16:1 mix exhibited essentially biphasic distributions, with peak diameters at approximately 30 and 70 to 80 μm (Figure 2a). The smaller population of adipocytes represented a greater proportion of the cells, and the larger population was greatly diminished, in adipose tissue from pigs fed the 16:0- and 18:2-enriched diets (Figure 2b).

Lipogenesis. Lipogenesis was greater ($P < .05$) in adipose tissue from cornstarch-fed pigs than in all other treatment groups (Figure 3). Lipogenesis was lower in tissue from pigs fed the 16:0 diet than in adipose tissue from pigs from the 18-carbon fatty acid diets. Lipogenesis in adipose tissue from the 14:1/16:1-fed pigs was intermediate between rates observed in pigs fed the 16:0 and 18-carbon fatty acid-enriched diets. Insulin induced a small increase ($P < .05$) in lipogenesis in adipose tissue from pigs fed the cornstarch diet or diets containing either 18:2 or 14:1/16:1.

Discussion

Fatty Acid Composition. Absorption of the dietary fatty acids was not measured. However, plasma fatty acid concentrations were measured and have been reported separately (Smith et al., 1992). Because

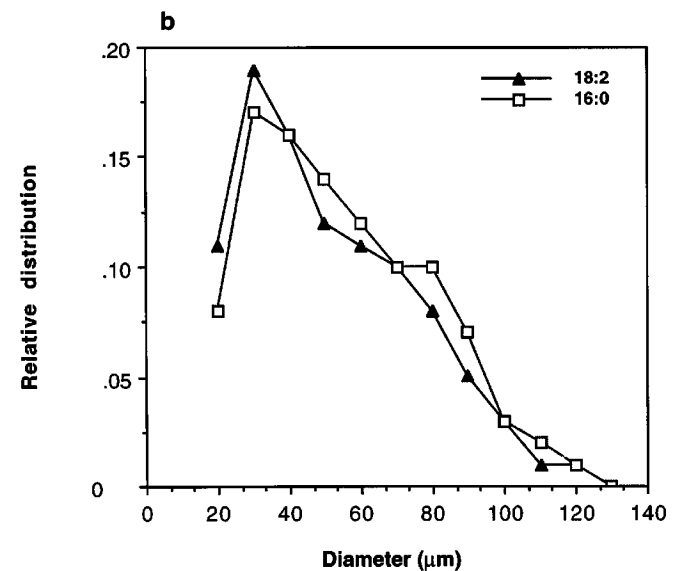
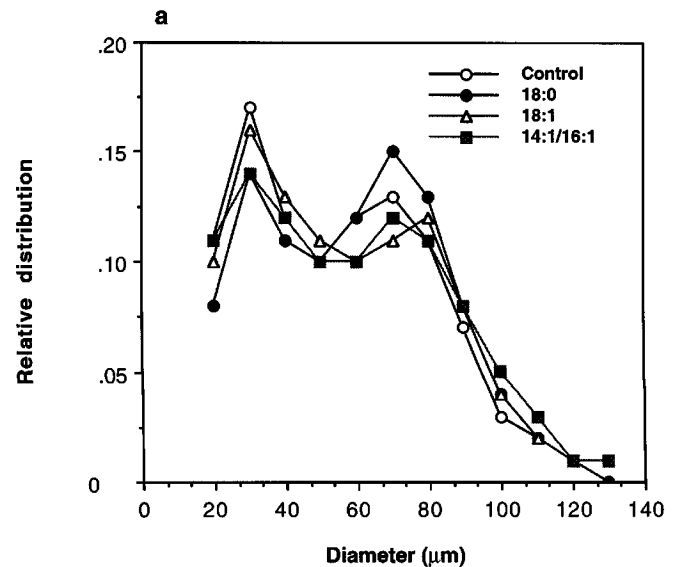


Figure 2. Adipocyte diameter distributions for pigs fed either cornstarch- or fatty acid-enriched diets. a) Distributions for pigs fed the cornstarch-, 18:0, 18:1, or 14:1/16:1-enriched diets. b) Distributions for pigs fed the 18:2 or 16:0 diets. Each data point is the mean for seven pigs per treatment group.

concentrations of circulating fatty acids indicate their availability to adipose tissue, the plasma fatty acid data from Smith et al. (1992) are summarized here (all indicated differences are significant, $P < .05$).

Allee et al. (1971a) reported that the addition of 13% beef tallow to the diets of pigs doubled the concentration of plasma fatty acids. Similarly, the addition of 14:0 to the diets increased plasma 14:0 from four- to eightfold (from .09 mmol/L in the baseline group). Plasma 16:0 was elevated only in those pigs fed the 16:0-enriched diet (from 1.37 in the baseline group to 3.46 mmol/L in the 16:0-fed pigs). Plasma 18:0 was completely unaffected by diet (e.g.,

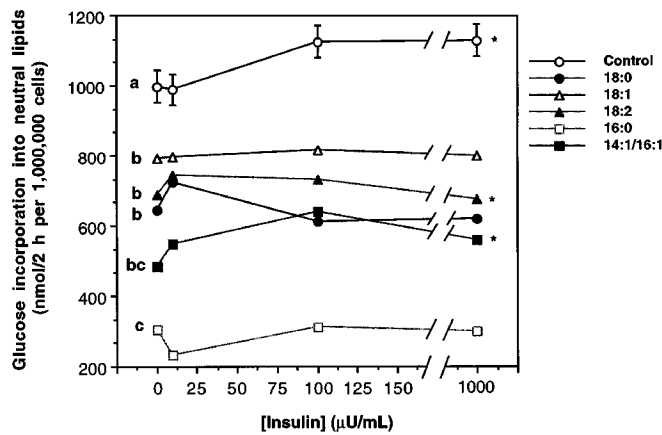


Figure 3. Effects of dietary fats and of insulin in medium on lipogenesis from glucose in porcine subcutaneous adipose tissue. Adipose tissue from pigs fed a cornstarch- or fatty acid-enriched diet was incubated 2 h in medium containing 20 mM glucose, 1 μ Ci of [U- 14 C]glucose, and either 0, 10, 100, or 1,000 μ U/mL of porcine insulin. Each data point is the mean for seven pigs per treatment group. The average pooled standard error of the mean for each treatment group is affixed to the symbols for the cornstarch-fed group. ^{a,b,c}Treatment groups with common letters do not differ (data pooled across insulin concentrations). *Indicates significant insulin effect (paired *t*-test, relative to baseline value within each treatment group).

2.05 mmol/L in the baseline group vs 1.36 mmol/L in the 18:0-fed pigs). Plasma 14:1 was undetectable in the baseline group and was elevated to .21 mmol/L in the pigs fed the 14:1/16:1 mixture, and 16:1 was elevated only by the 14:1/16:1 diet (from .2 mmol/L to 1.21 mmol/L). Consumption of the 18:1 diet approximately doubled the plasma concentration of 18:1 (to 4.69 mmol/L), and the 18:2 diet more than tripled the plasma 18:2 (from 1.32 to 5.01 mmol/L). Except for the 18:0-containing diet, the diets containing added fatty acids also increased the plasma 18:2 (to approximately 3 mmol/L).

The effects of the dietary fatty acids on adipose tissue fatty acid composition were consistent with their elevations in plasma. For example, 14:0 was greater in adipose tissues from all pigs fed added fatty acids than in the baseline group, and the highest percentage of 16:0 was observed in those pigs fed diets containing 16:0. Compared with the baseline group, the pigs fed the 18:0-enriched diet exhibited an increase only in 14:0. This is consistent with the inability of dietary 18:0 to elevate the plasma concentration of 18:0. The modest increase in 18:1 in adipose tissue of pigs fed the 18:1-enriched diet seems unusual in light of our previous demonstration that diets containing elevated 18:1 can markedly increase adipose tissue 18:1 (St. John et al., 1987; Klingenberg

et al., 1995). However, our previous investigations incorporated diets in which the 18:1 concentrations were at least 60% of the total dietary lipid content. The concentration of 18:1 used in the present study (47% of total fatty acids) was not markedly greater than the concentration typically observed in porcine adipose tissue (St. John et al., 1987; Klingenberg et al., 1995).

Cellularity. Mersmann et al. (1975) and Allen (1976) reported a biphasic distribution for adipocytes from market weight pigs (greater than 100 kg live weight). Allen (1976) suggested that the biphasic distribution was due either to a reinitiation of hyperplasia or to multiphasic periods of differentiation from preadipocytes and subsequent lipid filling. The pigs used in the present investigation were considerably smaller (approximately 25 kg by the end of the feeding trials), and the biphasic distribution of adipocytes observed in these animals suggests that one or both of the mechanisms suggested by Allen (1976) may have been occurring in the younger pigs.

Allee et al. (1971a) demonstrated a linear decrease in lipogenesis from glucose in porcine adipose tissue as dietary corn oil was increased from 4 to 13%. However, backfat thickness was not different across levels of dietary corn oil, indicating that a portion of the adipose tissue hypertrophy was due to uptake of dietary lipids. In contrast, the lower value for the larger population of adipocytes we observed in the 16:0- and 18:2-fed pigs suggests that these dietary fatty acids reduced the magnitude of lipid filling in adipocytes.

The lower number of larger adipocytes from pigs fed the 16:0-enriched diet is consistent with the nearly 80% reduction in lipogenesis caused by the 16:0 diet. The 18:2 diet may have increased the fragility of the cells, causing loss of larger adipocytes during osmium fixation. However, the 16:0 diet also eliminated the larger population of adipocytes even though it would not have increased the fragility of the cells. Similarly, the 14:1/16:1 diet contained nearly 30% 18:2 plus 18:3, yet adipose tissue from pigs fed the 14:1/16:1 diet exhibited a substantial proportion of larger adipocytes, particularly for those adipocytes with diameters greater than 100 μ m. These results seem to rule out cell fragility as a basis for the lesser mean cell volume in pigs fed the 18:2 diet.

Dietary Fat and Lipogenesis In Vitro. The lower level of lipogenesis elicited by the dietary fats, relative to the cornstarch diet, was as demonstrated in early studies with rats (Hill et al., 1958) and pigs (Allee et al., 1971a,b). We included the cornstarch-fed group to indicate the lipogenic rate of pigs of the same age and weight that were fed a typical low fat diet. Allee et al. (1971a,b) included sand in the fat-enriched diets as a nonnutritive diluent to make the diets isocaloric to a cornstarch-enriched diet, yet demonstrated nearly identical depressions in lipogenesis by dietary fats as

we observed in this investigation. We conclude that fatty acids per se were responsible for the effects on lipogenesis caused by the dietary fatty acids. Several investigators have indicated that dietary fats suppress fatty acid biosynthesis by depressing the expression of genes encoding lipogenic enzymes (Herzberg and Rogerson, 1987; Clarke et al., 1990; Shillabeer et al., 1990).

The 18:0 diet increased neither plasma nor adipose tissue 18:0 concentration. The concentration of 14:0 was elevated (relative to the cornstarch diet) in plasma and adipose tissue by the 18:0 diet, reflecting its addition to the 18:0 diet. We have demonstrated stearoyl-coenzyme A desaturase activity in porcine intestinal mucosa (Klingenberg et al., 1995), which would convert any absorbed 18:0 to 18:1 and minimize our ability to elevate plasma 18:0 with increased dietary 18:0. However, the ineffectiveness of dietary 18:0 in eliciting corresponding changes in plasma and adipose tissue concentrations probably was caused by relatively poor absorption of 18:0. Ockner et al. (1972) demonstrated that dietary saturated fatty acids, once released from triacylglycerols, are absorbed more slowly than unsaturated fatty acids, which has been confirmed by several recent investigations. Brink et al. (1995) and Renaud et al. (1995) indicated that 18:0 in the *sn*-1,3 positions of dietary triacylglycerols are poorly absorbed, particularly in the presence of elevated dietary calcium (Brink et al., 1995). A recent study in piglets (Innis et al., 1993) indicated that this also applies to 16:0 in the *sn*-1,3 position. Thus, feeding 18:0 as the free fatty acid may have caused poor absorption.

Because the 18:0-enriched diet resulted in lower lipogenesis but was apparently poorly absorbed, we conclude that the added 14:0 (which was almost 30% of total dietary fatty acids) was responsible for this effect. What is unusual is that the 18:2-enriched diet was no more effective than the 18:0 diet in decreasing the rate of lipid synthesis from glucose. In rat liver and adipose tissue, 18:2 is more effective in reducing lipogenesis than either saturated (Herzberg and Rogerson, 1987; Wilson et al., 1990) or monounsaturated fatty acids (Musch et al., 1974). Herzberg and Rogerson (1988) demonstrated that feeding corn oil to rats decreased lipogenic enzyme activities and lipogenesis in vitro more effectively than a tallow diet, and neither was as effective as menhaden oil (enriched with *n*-3 fatty acids). Shimomura et al. (1990) demonstrated that a safflower oil-enriched diet resulted in less body fat accumulation in rats than a tallow-enriched diet. Our results are consistent with those of Allee et al. (1971a), who demonstrated that 13% corn oil and 13% tallow were equally effective in decreasing fatty acid biosynthesis from glucose in porcine adipose tissue.

Herzberg and Rogerson (1987) demonstrated that diets enriched with ethyl-palmitate did not reduce

hepatic lipogenesis in diabetic rats, whereas ethyl-linoleate reduced lipogenesis by nearly 30%. In this study, lipogenesis clearly was decreased to the greatest extent in those pigs fed the 16:0-enriched diet. The 14:1/16:1-enriched diet produced results that were intermediate between those caused by the 18-carbon diets and the 16:0 diet. These results were reflective of the composition of the 14:1/16:1 diet, which contained nearly 69.2% 14- plus 16-carbon fatty acids (vs 82.7% in the 16:0 diet) and 29.6% 18:2 plus 18:3 (vs 42.9% in the 18:2 diet).

Insulin Responsiveness of Porcine Adipose Tissue. A consistent response of porcine adipose tissue to insulin has been difficult to demonstrate. Mersmann (1989), in a thorough set of investigations, reported that 3,000 μ U/mL of insulin stimulated lipogenesis in vitro by as little as 55% and as much as 144%. Mersmann (1989) suggested that the variation observed in his and other investigations may result from a combination of "pig genetics, husbandry, or seasonal effects."

In a study with older pigs (80 to 127 kg live weight), Walton and Etherton (1986) demonstrated a 50% stimulation of lipogenesis by insulin. Rule et al. (1987) reported a 10% stimulation of glucose incorporation into total lipids by insulin, whereas Mersmann and Hu (1987) observed a 30% stimulation of lipogenesis by insulin. Mersmann and Hu (1987) and Rule et al. (1987) used adipose tissue from young pigs (20 to 50 kg live weight), as did the present investigation, and the 15% stimulation by insulin in the present investigation for adipose tissue from the cornstarch-fed pigs is consistent with the earlier reports.

Certain dietary fats reduce or abolish insulin responsiveness in rat adipocytes (Hissen et al., 1982; Harris, 1992). Additionally, polyunsaturated fatty acids more effectively reduce insulin sensitivity of rat adipose tissue than monounsaturated fatty acids (van Amelsvoort et al., 1988). Thus, porcine adipose tissue again differs from rat adipose tissue in that insulin sensitivity was maintained in the 18:2-fed pigs but was abolished in pigs fed diets enriched with 18:1.

Because adipose tissue from animals fed the 14:1/16:1- and 18:2-enriched diets retained insulin sensitivity, it can be concluded that the addition of 14:0 to the 16:0-, 18:0-, and 18:1-enriched diets was not responsible for the loss of insulin sensitivity in adipose tissue from animals fed those diets. The availability of the 14:1/16:1 fatty acid mixture provided us with a novel opportunity to test the effects of shorter-chain monounsaturated fatty acids on lipogenesis and insulin sensitivity in adipose tissue. Without this test group, we would have concluded that, in porcine adipose tissue, dietary saturated (16:0 or 18:0) and monounsaturated (18:1) fatty acids abolish insulin responsiveness, whereas polyunsaturated fatty acids (18:2) do not. However, the greatest response to insulin was observed in adipose tissue from the 14:1/

16:1-fed pigs; this indicates that the combination of monounsaturations plus shorter fatty acid chain length resulted in an unusual biological effect. Thus, the 14:1/16:1 diet was nearly as effective as the 16:0 diet in decreasing overall rates of fatty acid biosynthesis, but behaved like the 18:2 diet in its inability to reduce insulin sensitivity.

In summary, we have demonstrated that supplementing the diets of pigs with 10% fat, regardless of type, reduces fatty acid biosynthesis from glucose as compared with biosynthesis in pigs fed diets supplemented with 10% cornstarch. Furthermore, the various fatty acids differed in their effects in decreasing lipogenesis and (or) insulin responsiveness. To our knowledge, the current study represents the first report of the interaction between dietary fatty acids and insulin in modulating lipid synthesis from glucose in porcine adipose tissue. The use of dietary free fatty acids rather than naturally occurring triglycerides provided somewhat different results from previous investigations in other animal models, but has provided additional insight into the interaction between fatty acid chain length and degree of unsaturation. This and previous research (Allee et al., 1971a) strongly indicate that porcine adipose tissue and rat adipose tissue respond differently to specific dietary fatty acids.

Implications

This investigation provided results that may have relevance to human medicine and animal production. By controlling the fatty acid composition of their diets, noninsulin-diabetics may in part ameliorate their reduced insulin sensitivity. For swine production, our results suggest that selecting dietary feeds higher in shorter-chain fatty acids may influence the extent of body fat accumulation, due to both decreased overall lipogenesis and reduced sensitivity to insulin.

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