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Influence of dietary fat on beta-adrenergic receptors and receptor-controlled metabolic function in porcine adipocytes

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In order to measure the effect of dietary fat on adipocyte plasma membrane function, 5-week-old pigs were fed either a low-fat diet, a high-fat diet with tallow (saturated fat), or a high-fat diet with corn oil (unsaturated fat) for approximately 5 weeks. Pigs fed the three diets gained the same amount of weight. Adipocytes isolated from the subcutaneous fat depot were larger in pigs fed the two high-fat diets than in pigs fed the low-fat diet. The fatty acid composition of the crude adipocyte membrane fraction (ghosts) was markedly different between the dietary groups. The affinity for the beta-adrenergic receptor was the same in ghosts from pigs fed the three diets. The number of receptors per cell was less in ghosts from pigs fed the low-fat diet compared with pigs fed either high-fat diet; receptor number per unit surface area was not different, implying that receptor number was related to adipocyte size. Activity of the membrane-bound enzyme, 5'-nucleotidase was the same in ghosts from pigs fed the three diets. Isoproterenol-stimulated adipocyte lipolytic activity tended ($P < 0.1$) to be greater in pigs fed the high-saturated fat diet than in pigs fed the low-fat diet, whereas it was intermediate in pigs fed the high-unsaturated fat diet. Although there were marked differences in fatty acid composition of adipocyte ghosts obtained from pigs fed diets with different fatty acid compositions, there was no alteration in membrane-bound receptor function, nor in membrane-bound enzyme function, and only marginal differences in receptor-controlled metabolism. (J. Nutr. Biochem. 6:302–309, 1995.)

Keywords: swine; lipolysis; adrenergic receptors; dietary fat; adipocytes

Introduction

In nonruminant mammals including the pig, the fatty acid composition of the diet is reflected to a large extent in the fatty acid composition of cellular neutral lipids and phospholipids.[1,2] We have examined the influence of dietary fatty acids on the porcine adipocyte plasma membranes because of our continuing interest in the function and control of the plasma membrane–bound beta-adrenergic receptor(s). Previously, we fed young postweaning pigs (5-week-old) a high-fat tallow (saturated) or high-fat corn oil (unsaturated) diet.[3] After approximately 5 weeks of feeding, the membrane fraction had a fatty acid composition that tended to reflect the dietary fat. However, the beta-adrenergic receptor ligand affinity measured in the membranes was not different between the two dietary groups. Furthermore, the receptor-controlled lipolytic function of adipose tissue was not different between the dietary groups. Thus, in these young pigs, a major change in the fatty acid composition of adipocyte membranes did not influence the

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function of adipocyte beta-adrenergic receptors. In contrast to our observations, Nicolas et al.,[4] observed differences in adipocyte ligand binding to the receptor and in adipocyte adenylate cyclase activity in older pigs fed a low-fat compared with a high-fat safflower oil diet. Because of our interest in young mammals and the divergent results between the Nicolas studies and ours, we repeated our experiments and added a third low-fat diet group. We measured ligand binding to the beta-adrenergic receptor(s), 5′-nucleotidase activity (representing another plasma membrane-bound functional protein), lipolytic function of adipocytes, and lipid composition of the membrane fraction used for receptor studies.

Methods and materials

Methods will be briefly outlined because they are similar to those used in the previous study.[3] Four-week-old crossbred pigs were obtained from a local breeder, brought to the animal care facility, and placed in three group pens with ad libitum access to the basal diet (*Table 1*) and water. There were six nonlittermate pigs per pen. After 1 week, each pen was fed ad libitum a diet that contained approximately 18% protein and 3% fat supplemented with either 15% cellulose (low-fat diet), or 15% corn oil (high-fat unsaturated diet), or 15% tallow (high-fat saturated diet). The two high-fat diets were prepared by mixing 85 parts of the basal diet (*Table 1*) with 15 parts corn oil or 15 parts beef tallow. The low-fat diet contained 84 parts of the basal diet, 1 part corn oil, and 15 parts cellulose (Alphacel; ICN Biochemicals, Inc., Costa Mesa, CA, USA). The diets were approximately isonitrogenous and contained approximately the same concentration of vitamins and minerals. The two high-fat diets were isoenergetic but the low-fat diet contained less energy than the high-fat diets. The low- and high-fat unsaturated diets were each supplemented with 164 mg of cholesterol/kg of diet; cholesterol was dissolved in the corn oil before addition to the diet. The amount of cholesterol added was assumed to be that in a kilogram of the high-fat tallow diet (109 mg of cholesterol/100 g of tallow).[5] The fatty acid composition of the three diets is indicated in *Table 2*. Fatty acids in the low-fat diet were contributed primarily by the corn and corn oil in the diet plus any residual lipid in the extracted soybean meal.

Table 1 Basal diet composition

Substances	Amount (g/kg diet)
Components	
Ground corn	608
Soybean meal (44% protein)	350
Vitamin premix[a]	2
Mineral premix[b]	2
Choline chloride	2
Dicalcium phosphate	24
Ground limestone ($CaCO_3$)	8
Iodized salt	4
Calculated, as-fed basis	
Protein (%)	20.6
Fat (%)	2.6

[a]Supplies the following (per kg of diet): retinyl palmitate, 2.904 mg; cholecalciferol, 17.6 μg; all-rac-α-tocopherol acetate, 70.4 mg; menadione, 3.52 mg; vitamin B_{12}, 26.4 μg; riboflavin, 5.28 mg; niacin, 28.16 mg; D-pantothenic acid, 21.12 mg; biotin, 88 μg; and thiamin, 2.2 mg.
[b]Supplies the following (μg/g of diet): Cu (as cupric oxide), 10; Fe (as ferrous sulfate heptahydrate), 160; Mn (as manganese oxide), 20; Zn (as zinc oxide), 100; and $CaCO_3$ used as carrier (0.30% of diet).

Table 2 Fatty acid composition of diets[a]

Fatty acid	Diet: Low-fat	High-fat unsaturated	High-fat saturated	SD
14:0			1.46	
14:1			0.37	
16:0	15.64[c]	13.92[b]	20.21[d]	0.25
16:1			2.96	
17:0			0.60	
17:1			0.60	
18:0	3.57[c]	2.29[b]	10.74[d]	0.16
18:1(n-9)	19.22[b]	21.91[c]	37.93[d]	0.48
18:2(n-6)	58.20[d]	59.56[c]	23.87[b]	0.79
18:3(n-3)	3.36[d]	1.83[c]	1.66[b]	0.05
24:0		0.49	0.44	0.04

[a]Diets were analyzed in triplicate. Data are indicated as the mean and the pooled SD (g/100g of fatty acids).
[b,c,d]Values within a row with different superscripts are significantly different ($P < 0.05$); values with the same superscript or no superscript are not different ($P > 0.1$).

The experimental diets were fed for 4 to 5.5 weeks; the feeding period was not constant because the only available housing was in group pens. However, pigs were killed two/day such that each three consecutive animals killed represented one from each dietary group; thus, one pig from each dietary group was fed the diet for the same time period (within 1 day because the logistics for kill did not allow handling of tissues from more than two pigs/day). Weights were obtained at day 28 of feeding, and pen feed intake was measured to day 28. Pigs were killed by captive bolt gun followed by exsanguination. Blood was collected for serum lipid analysis. Subcutaneous fat was removed from the dorsal neck region and transported to the laboratory as indicated.[6] The animal protocol was approved by the Baylor College of Medicine Animal Care and Use Committee.

Adipose tissue was sliced and digested with collagenase to prepare isolated adipocytes. Adipocytes were washed and suspended in Krebs–Ringer bicarbonate buffer containing 5.6 mM glucose; adipocytes isolated from 5 g of tissue slices were suspended in 15 mL total volume. Adipocytes were used to measure the lipolytic rate, fixed in osmium for cell size determination, and suspended in hypotonic medium containing 7.5 mM EGTA and 0.2 mM phenylmethylsulfonyl fluoride (as proteinase inhibitors) to prepare a crude membrane fraction (adipocyte ghosts). Methods for adipocyte isolation and ghost preparation have been detailed.[7] Lipolysis was measured by incubation of 0.5 mL of cells in a total volume of 3.0 mL of Krebs–Ringer bicarbonate buffer containing 5.6 mM glucose, 0.56 mM ascorbate, and 4% bovine serum albumin. Incubation was for 120 min at 37°C with gyrorotatory agitation at 66 rpm. Incubations were with no additions to the medium (basal) and in the presence of 10^{-3} M theophylline (Theo), 10^{-5} M (−)-isoproterenol (Iso; 10 μM Iso completely saturates the lipolytic response in porcine adipocytes),[6,8] or Iso + Theo. The reaction was terminated by cooling on ice, then the medium below the floating adipocytes was removed and stored at −20°C until extraction and quantification of fatty acids by nonaqueous titration (storage time <3 weeks).

Methods for measuring lipolysis[6] and for fatty acid determination[8] have been described. Cells (0.5 mL) were fixed in osmium for cell size determination with a particle counter; particles >20

μm were included in the sample. Methods for cell size determination have been described.[3,9]

Equilibrium ligand binding to beta-adrenergic receptor(s) was measured by incubation of adipocyte ghosts for 30 min at 25°C with ^{3}H-dihydroalprenolol (DHA; New England Nuclear); after incubation, the ghosts were trapped on filters and washed, then radioactivity was determined by liquid scintillation counting. Saturation curves for DHA were used to determine receptor affinity and number; competition curves for isoproterenol were generated in the presence of 2.5 nM DHA to determine the affinity of isoproterenol for the receptor. Ligand binding data were analyzed with a computer program to yield a measure of the receptor affinity (K_d or concentration at half saturation for DHA and K_i or the isoproterenol concentration to inhibit the binding of 2.5 nM DHA 50%) and the receptor number (B_{max} or the amount of DHA bound at saturation). Detailed methods for ligand-binding have been described.[7,10] Protein was measured by a modified[11] Lowry method[12] on trichloroacetic acid precipitated samples. The number of receptors (B_{max}) was expressed per milligram of ghost protein. The receptor number was extrapolated to a cell basis assuming a 100% yield of ghost protein during ghost preparation by lysis of cells. (Porcine adipocyte ghosts do not contain 100% of the total cellular beta-adrenergic receptor ligand-binding activity; the ghosts contain a variable amount of the receptor that averages approximately 75% of the total.[13] At this time there is no marker for porcine adipocyte plasma membranes to correct for beta-adrenergic receptor recovery.[13]) Receptor number was extrapolated to a surface area basis using the average cell diameter. The 5′-nucleotidase activity was measured in the ghost fraction by liberation of ^{3}H-adenosine from ^{3}H-adenosine 5′-monophosphate[14] as previously described.[13]

Adipocyte ghosts were extracted with chloroform:methanol (2:1, vol/vol), and the extracts were washed by the method of Folch et al.[15] Inorganic phosphorus was determined on the lipid extract using the Bartlett procedure;[16] the phosphorus values were converted to micrograms of phospholipid using an average phospholipid MW of 775. Cholesterol was determined on the lipid extract using an enzymatic method after chemical hydrolysis of esters according to Tercyak;[17] the method uses 2,4-dibromophenol and is sensitive from 0.5 to 40 μg of cholesterol. Fatty acid composition was determined on the diets, the isolated adipocytes, and the adipocyte ghost preparations. Diets, isolated adipocytes, and adipocyte ghosts were extracted with hexane:isopropanol, transesterified with HCl:methanol, and the methyl esters were analyzed by capillary gas chromatography with flame ionization detection; the identity of chromatographic peaks was established by comparison of retention times with those of authentic standards. We did not have standards for several of the longer-chain fatty acids so that double-bond position or cis-trans orientation could not be determined by gas chromatography. Details of the fatty acid analysis procedure have been presented.[3]

Serum total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were measured with a Ciba-Corning 550 Express autoanalyzer (Ciba-Corning Diagnostics, Gilford Systems, Oberlin, OH). Statistical analysis was by one-way analysis of variance (three diet groups) with mean separation by least significant difference when the F-test was significant.

Results

Animals

The three groups of pigs weighed the same initially, at day 28 of the test, and at the time of kill (*Table 3*). Feed intake (kg/day/pen of 6 pigs) to day 28 of feeding was 6.42 and 6.96 for the pigs fed unsaturated and saturated high-fat diets, respectively; pigs fed the low-fat diet consumed more feed (8.06 kg/day/pen) as expected because the energy content of the low-fat diet was less than that of the two high-fat diets.

Table 3 Weight and adipocyte size in pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Variable	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
Weight (kg)				
Initial	10.4	10.1	9.9	1.7
28 days	24.1	24.5	25.0	4.5
Kill	28.7	27.6	29.4	4.3
Cell number (per g × 10^{-7})[b]	1.4[d]	0.8[e]	0.9[e]	0.1
Cell diameter (μm)	46.8[d]	53.6[e]	54.9[e]	3.3
Cell volume ($\mu m^3 \times 10^{-3}$)[c]	54.1[d]	81.8[e]	87.3[e]	14.6

[a]Values are represented as mean with pooled SD.
[b]1.4 = 14,000,000 cells/g.
[c]54.1 = 54,100 μm^3; calculated from average cell diameter for each pig.
[d,e]Values in the same row with different superscripts are different ($P < 0.05$); values with the same superscript or no superscript are not different ($P > 0.1$).

Adipocyte diameter was the same in pigs fed the two high-fat diets and was greater than that in pigs fed the low-fat diet (*Table 3*). The diameter differences represent a >50% increase in cell volume for cells from high-fat fed pigs compared with low-fat fed pigs. Although there were no measurements of body composition, one might speculate that because of the larger cell size, the pigs fed the high-fat diets deposited more fat than the pigs fed the low-fat diet even though weight gain was the same in all groups.

The fatty acid composition of the two high-fat diets reflected the composition of the fat added at 15% (*Table 2*); the saturated diet (tallow) had greater 16:0, 18:0, and 18:1 fatty acids and less 18:2 fatty acid than the unsaturated diet (corn oil). The fatty acid composition (percentage) of the low-fat diet resembled that of the high-fat unsaturated diet because the main fat sources were the ground corn in the diet and the corn oil added as a carrier for the added cholesterol; the low-fat diet had a greater percentage of 18:3 fatty acid than either of the other two diets because the extracted soybean meal contains an estimated 1.1% fat residue that would contribute the 18:3 fatty acid. The fatty acid composition of the adipocyte total lipid extract (mostly depot lipid) to a large extent reflected the fatty acid composition of the diet fed (*Table 4*). For example, the pigs fed the high-fat saturated diet had a high percentage of 18:0 and 18:1 fatty acids and a lower percentage of 18:2 fatty acids relative to the pigs fed the high-fat unsaturated diet. The pigs fed the low-fat diet had a fatty acid pattern that was somewhat intermediate between the saturated and unsaturated high-fat diets, as reflected for example in the percentage of 18:1 and 18:2 fatty acids. The 16:0 percentage of fatty acid in adipose tissue from pigs fed the low-fat diet was greater than the diet percentage, presumably because fatty acid was synthesized de novo in this group with 16:0 fatty acid being the primary product of biosynthesis. At

Table 4 Fatty acid composition of adipocytes isolated from pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Fatty acid	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
14:0	1.62[b]	0.65[c]	1.44[d]	0.10
15:0	0.10[b]	0.07[c]	0.14[d]	0.01
16:0	21.55[d]	14.30[c]	20.47[b]	0.97
16:1(n-7)	3.85[b,e]	1.66[c]	3.56[b,f]	0.59
17:0	0.93[b]	0.57[c]	1.01[d]	0.12
17:1	0.47[b]	0.13[c]	0.68[d]	0.08
18:0	9.63[b]	5.79[c]	10.20[d]	0.96
18:1(n-9)	35.17[b]	29.81[c]	49.22[d]	1.67
18:2(n-6)	23.77[b]	43.74[d]	10.67[c]	2.12
18:3(n-3)	0.92[d]	0.85[b]	0.55[c]	0.06
19:0	0.08[c]	0.13[d]	0.10[b]	0.01
19:1	0.10[c]		0.13[b]	0.01
20:0	0.14[d]	0.12[b]	0.11[c]	0.01
20:1(n-9)	0.59[b]	0.49[c]	0.92[d]	0.08
20:2(n-6)	0.85[b]	1.15[d]	0.42[c]	0.13
20:3(n-6)	0.16[b,f]	0.17[b,e]	0.12[c]	0.02
20:4(n-6)	0.43[b,e]	0.42[b,f]	0.27[c]	0.03
Saturated	34.05	21.63	33.47	
Monounsaturated	40.18	32.09	54.51	
Polyunsaturated	26.13	46.22	12.01	

[a]Samples were analyzed in triplicate. Data are presented as mean with pooled SD (g/100g of fatty acids).
[b,c,d,e,f]Values in the same row with different superscripts are different (b,c,d = $P < 0.05$; e,f = $P < 0.1$); values with the same superscript or no superscript are not different ($P > 0.1$).

least some of the 18:0 and 18:1 fatty acids would be expected to arise from chain elongation and desaturation of 16:0 fatty acid in the low-fat group.

Serum total cholesterol tended to be lower in pigs fed the low-fat diet than in pigs fed either high-fat diet (*Table 5*) even though all diets had the same calculated concentration of cholesterol (low-fat and high-fat corn oil diets were supplemented with cholesterol to the level calculated in the high-fat tallow dict). The elevated cholesterol concentration in serum from pigs fed both high-fat diets was reflected in the HDL cholesterol but not in the low density lipoprotein (LDL) cholesterol (data not shown) concentrations. The serum triglyceride concentration was increased in pigs fed high-fat compared with low-fat diets.

Table 5 Serum lipid analysis of pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Variable	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
Cholesterol	2.00[d]	2.60[e]	2.83[e]	0.57[b]
HDL cholesterol	0.85[d]	1.38[e]	1.40[e]	0.24[c]
Triglyceride	0.37[d]	0.82[e]	0.75[e]	0.24[c]

[a]Values indicated as mean (mmol/L) with pooled SD. [b]$P < 0.10$; [c]$P < 0.05$.
[d,e]Values in the same row with different superscripts are different; values with the same superscript are not different ($P > 0.1$).

Composition of ghosts

Protein concentration in the adipocyte ghosts from pigs fed the low-fat diet was greater than in ghosts from pigs fed either the unsaturated or saturated high-fat diets (*Table 6*). This result was expected because more (smaller) cells were represented per gram of tissue in the pigs fed the low-fat diet (*Table 3*); smaller cells have more relative surface area and thus more plasma membrane than large cells and also contribute more intracellular membrane to the ghost fraction. The phospholipid concentration (extrapolated from an inorganic phosphorus concentration and expressed per milligram of ghost protein) of the lipid extracts from adipocyte ghosts was not different between dietary groups (*Table 6*). The cholesterol concentration (expressed per milligram of ghost protein) was greater in ghosts from pigs fed the high-fat unsaturated diet compared with the high-fat saturated diet or the low-fat diet. However, the phospholipid to cholesterol ratio of the ghosts was not different between dietary groups.

The fatty acid composition of the ghosts (*Table 7*) in many instances qualitatively reflected the composition of the diet. However, the quantitative percentage of an individual fatty acid in the ghosts in many instances did not faithfully reflect the composition of the diet or of the total adipocyte extract. Pigs fed the high-fat unsaturated diet had the highest 18:2 and lowest 16:0 and 18:0 fatty acid percentages, a reflection of the diet composition. Other membrane fatty acid percentages did not correspond to the diet composition. For example, pigs fed the low-fat diet had slightly more 16:0 fatty acid than those fed the high-fat saturated diet, whereas the 18:0 and 20:4 fatty acid percentages were similar to pigs fed the high-fat saturated diet and the 18:1 and 18:3 fatty acid percentages were similar to those fed the unsaturated diet. The 18:2 fatty acid percentage in ghosts from pigs fed low-fat diets was intermediate between that in the ghosts from pigs fed the saturated or unsaturated diets.

Table 6 Phospholipid and cholesterol content of adipocyte ghosts isolated from pigs fed diets containing low-fat with unsaturated or saturated fats[a]

Variable	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
Protein	2.00[b]	1.27[c]	1.41[c]	0.27
Phospholipid	455	518	500	67
Cholesterol	47.8[b]	54.4[c]	45.6[b]	5.7
Phospholipid/ cholesterol	9.5	9.5	11.0	1.2

[a]Values are mean (mg of ghost protein/mL of ghost suspension and μg of phospholipid or cholesterol/mg of ghost protein) with pooled SD.
[b,c]Values in the same row with different superscripts are different ($P < 0.05$); values with the same superscript or no superscript are not different ($P > 0.1$).

Table 7 Fatty acid composition of adipocyte ghosts isolated from pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Fatty acid	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
14:0	0.89[b]	0.59[c]	1.07[d]	0.10
16:0	16.20[d]	12.40[c]	15.40[b]	1.34
16:1(n-7)	2.61[b]	1.34[c]	2.60[b]	0.41
17:1	0.41[c]	1.16[b]	0.65[c]	0.25
18:0	19.35[b]	13.50[c]	20.65[b]	1.76
18:1	26.60[c]	24.97[c]	35.25[b]	1.86
18:2(n-6)	26.24[b]	41.48[d]	17.50[c]	2.11
18:3(n-3)	0.76[b]	0.72[b]	0.54[c]	0.06
20:1(n-9)	0.44[c]	0.42[c]	0.60[b]	0.06
20:2(n-6)	1.02[b]	1.26[d]	0.55[c]	0.11
20:4(n-6)	5.18[b]	3.02[c]	5.85[b]	0.82
24:0	0.52[b]	0.33[c]		0.04
Saturated	36.96	26.82	37.12	
Monounsaturated	30.06	27.89	39.10	
Polyunsaturated	33.20	46.48	24.44	

[a]Samples were analyzed in triplicate. Data are presented as mean with pooled SD (g/100g of fatty acids).
[b,c,d]Values in the same row with different superscripts are different (b,c,d = $P < 0.05$; values with same superscript or no superscript are not different [$P > 0.1$]).

Table 8 β-adrenergic receptor number and binding affinity in adipocyte ghosts isolated from pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Variable	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
K_d[b], nM	3.7	2.6	2.9	1.4
K_i[c], nM	282	342	305	177
B_{max}[d]				
fmol/mg of protein	150.8[f]	202.3[g]	200.5[g]	36.4
fmol/10^6 cell	16.1[f]	24.6[g]	24.5[g]	5.9
fmol/μm^2	2.4	2.7	2.6	0.8
Nucleotidase[e]	379	439	467	161

[a]Data expressed as mean with pooled SD.
[b]K_d is the equilibrium dissociation constant for binding of DHA to ghosts.
[c]K_i is the competitive binding equilibrium dissociation constant for isoproterenol competing with 2.5 nM DHA.
[d]B_{max} is the maximum number of binding sites determined from equilibrium binding; expressed per milligrams of ghost protein, or extrapolated to 10^6 cells or per unit surface area (μm^2) as indicated in the Methods and Materials section.
[e]5′-nucleotidase units are nmol per 60 min/milligram of protein.
[f,g]Values in the same row with different superscripts are different ($P < 0.05$); values with the same superscript or no superscript are not different ($P > 0.1$).

Functionality

The affinity of the ghost beta-adrenergic receptor(s) for DHA (K_d) was not different between dietary groups (*Table 8*). The affinity of the receptor(s) for isoproterenol (K_i) also was not different between dietary groups. The number of receptors was less in the low-fat than in the two high-fat groups when expressed per milligram of ghost protein or extrapolated to a per cell basis (*Table 8*). There was no difference in receptor number expressed per unit surface area between dietary groups. The activity of 5′-nucleotidase was measured as a purported plasma membrane protein not connected to the beta-adrenergic receptor; it was not different between the dietary groups (*Table 8*).

The lipolytic rate of isolated adipocytes was measured in the presence of the beta-adrenergic agonist, isoproterenol, as an indication of a metabolic pathway regulated by the adipocyte beta-adrenergic receptor(s). The basal lipolytic rate and the rate stimulated by isoproterenol plus theophylline were not different between dietary groups (*Table 9*). The lipolytic rate stimulated by theophylline also was not different between dietary groups; this rate was highly variable between animals, as reported previously.[3] The isoproterenol-stimulated lipolytic rate tended ($P < .01$) to be less in adipocytes from pigs fed the low-fat diet compared with pigs fed the high-fat saturated diet; adipocytes from pigs fed the high-fat unsaturated diet tended to have intermediate isoproterenol-stimulated lipolytic rates (*Table 9*).

Discussion

Young pigs fed high-fat diets supplemented with tallow (saturated fat diet) or corn oil (unsaturated fat diet) had similar adipocyte beta-adrenergic receptor binding affinity for both DHA and isoproterenol (*Table 8*) as observed previously.[3] The binding affinity for receptors from pigs fed a low-fat diet was not different from the affinities observed in the two high-fat fed groups (*Table 8*). This result was different from observations of Nicolas et al.,[4] who observed an adipocyte beta-adrenergic receptor K_d approximately two times greater in pigs fed a low-fat compared with a high-fat unsaturated diet (sunflower oil). There were differences between the Nicolas et al.[2,4] experiment and our experiments.[3] Nicolas et al. fed pigs from 25 to 100 kg body weight whereas we fed pigs from <10 kg to approximately 25 kg. Nicolas used intact male pigs and we used castrated male pigs. The growth rate of the Nicolas pigs appeared to be slower than for our pigs; they indicate they fed pigs for 6 months (from 25 to 100 kg body weight), whereas our

Table 9 Lipolysis in adipocytes from pigs fed diets containing low-fat or high-fat with unsaturated or saturated fats[a]

Lipolysis	Diet			SD
	Low-fat	High-fat unsaturated	High-fat saturated	
Basal	0.09	0.11	0.10	0.05
Theophylline (THEO)	0.54	0.95	2.08	1.52
Isoproterenol (ISO)	1.94[b]	2.73[b,c]	3.21[c]	0.92
ISO + THEO	4.31	4.89	5.36	1.06

[a]Data expressed as mean μequivalents fatty acid released/(120 min/10^6 cells) with pooled SD.
[b,c]Values in the same row with different superscripts are different ($P < 0.1$); values with the same superscript or no superscript are not different ($P > 0.1$).

pigs weighed 25 kg at 10 weeks of age and we project they would have weighed 100 kg at 6 months of age (the same gain as the Nicolas pigs but in 3.5 months). Our unsaturated high-fat diet was prepared with corn oil and theirs with sunflower oil; the fatty acid composition of these two diets was slightly different (*Table 2*).[2,3] The basal diet used by Nicolas et al.[2] was composed of barley, wheat, and soybean meal, whereas our basal diet was composed of corn and soybean meal (*Table 1*). Finally, the pigs used by Nicolas were of the Large White breed and our pigs were a cross-bred composite breed composed predominantly of the Duroc, Hampshire, Landrace, and Yorkshire (the American Large White) breeds.

We observed fewer receptors per adipocyte in pigs fed the low-fat compared with either high-fat diet (*Table 8*); the receptor number per unit surface area was constant across dietary groups. Maintenance of a constant receptor number per unit surface area would ensure the capacity of the cell to respond to adrenergic stimuli. Because there is less absolute surface area in a small cell than a larger cell, small cells have fewer receptors per cell. The receptor number was also less in the low-fat fed pigs than in the high-fat fed pigs when expressed per milligram of ghost protein. The ghost protein concentration was lower in the two high-fat than in the low-fat fed group. As adipocytes enlarge by filling with lipid, there is less organelle protein contributed per gram of tissue; thus the receptor number expressed on a milligram of protein basis was probably lower in the low-fat fed group because there was more organelle protein relative to plasma membrane protein in the ghost fraction of these preparations than in those from the two high-fat fed groups. In our previous experiment,[3] adipocytes from pigs fed the unsaturated diet were smaller and had fewer receptors than those from pigs fed the saturated diet; in the present experiment, adipocytes from pigs fed the low-fat diet were smaller and had fewer receptors than adipocytes from pigs fed either of the two high-fat diets. Combining the results of our two experiments, we conclude that receptor number is dependent primarily on adipocyte size rather than on the diet or the fatty acid composition of the membranes. Nicolas et al.[4] again observed different results than this laboratory in that receptor number was greater per adipocyte in membranes isolated from pigs fed the low-fat diet compared with membranes from pigs fed the high-fat, unsaturated diet; adipocytes from pigs fed low-fat diets had more receptors even though the cells were smaller.

As observed previously[3] there was no difference in any lipolytic rate between pigs fed high-fat saturated or high-fat unsaturated diets (*Table 9*). There was a tendency ($P < 0.1$) for the isoproterenol-stimulated lipolytic rate to be less in adipocytes from pigs fed the low-fat diet compared with those fed the high-fat saturated diet; the pigs fed the low-fat diet had the lowest number of receptors per cell but there was no difference in the receptor affinity (*Table 8*). Nicolas et al.[4] did not measure lipolysis. They observed a greater isoproterenol-stimulated adenylate cyclase activity in adipocyte ghosts from pigs fed the high-fat unsaturated diet (lower receptor number but slightly greater receptor affinity) compared with the low-fat (greater receptor number but slightly lower receptor affinity); the adenylate cyclase observations were significant only in the perirenal depot and not in the subcutaneous depot, the depot measured by us. The receptor-driven functional changes observed by Nicholas were minimal, and at best we observed minimal changes in receptor-driven function (no difference in Mersmann et al.,[3] and a marginal change in *Table 9*). It is possible that the lipolytic sensitivity may have been altered with variation in adipocyte receptor number. We did not measure lipolytic sensitivity to isoproterenol in these or previous experiments[3] but only the lipolytic response at a saturating concentration of isoproterenol.[8] Lipolytic sensitivity to isoproterenol in porcine adipocytes is highly variable; the ED_{50} = 0.7 to 32.6 nM for 12 pigs of the same breed, fed the same diet, and of similar weight (18 to 28 kg).[8] Consequently, given that most cells have a large excess of receptors such that stimulation of approximately 10 to 20% will yield a maximal physiological response, we assumed changes in receptor number would not be detectable by measurement of changes in lipolytic sensitivity.

We did not find any difference between the 5′-nucleotidase activity in adipocyte membranes from pigs fed low-fat, high-fat unsaturated, or high-fat saturated diets (*Table 8*). Nicolas et al.[18] also did not observe any difference in 5′-nucleotidase activity in adipocyte membranes from pigs fed low-fat or high-fat unsaturated diets.

When expressed per milligram of ghost protein, we observed the same phospholipid concentration in all dietary groups and more cholesterol in the group fed the high-fat unsaturated diet (*Table 6*); however, the phospholipid to cholesterol ratio was not different in any of the three dietary groups. Nicolas et al.[2] observed more phospholipid (but only in the perirenal depot and not the subcutaneous depot) and cholesterol per milligram of membrane protein in the pigs fed the low-fat compared with the high-fat unsaturated diet. The cholesterol to phospholipid ratio was not different between dietary groups in their studies.

The fatty acid composition of the adipocyte ghost fraction (*Table 7*) was quite different between the two high-fat fed groups of pigs, as observed previously.[3] The membrane fatty acid composition in the pigs fed the low-fat diet resembled that of the pigs fed the high-fat unsaturated diets for some fatty acids, it resembled the composition of pigs fed high-fat saturated diets for other fatty acids, and it was intermediate between the two high-fat fed groups for yet other fatty acids. Fatty acid composition of cell membranes is expected to be altered by diet and this has been demonstrated in pigs.[1] However, the percentage of an individual fatty acid is different in different organs and may or may not quantitatively reflect the composition of the diet or of the triglyceride fraction from the organ being analyzed; for some fatty acids and some organs the patterns may not be qualitatively similar. This type of observation, made for many species, organs, and organelles reflects the complexity of fatty acid chain elongation and desaturation mechanisms, the transport of lipids to tissues, the specificity of biosynthetic enzymes to incorporate fatty acids into phospholipids, and the specificity to incorporate particular phospholipids into individual organelle membranes. Although the three groups of pigs in our experiments had quite different adipocyte ghost fatty acid compositions, this did not result in changes in the total phospholipid concentration of the ghosts or in the ratio of phospholipid to cholesterol.

It should be noted that brush border membranes isolated from the jejunal mucosa of the same pigs reported in this manuscript had markedly changed fatty acid composition that generally reflected the diet composition; there were no differences in the phospholipid or cholesterol concentration in these membranes (M. Dudley, unpublished observations). Activities of the brush border enzymes, lactase, and sucrase-isomaltase were markedly greater in the brush border membranes from pigs fed the high-fat saturated diet compared with pigs fed the low-fat or the high-fat unsaturated diets. Thus, in the gut in contrast to the adipose tissue, the fatty acid composition of membranes affects enzyme function. We do not know whether dramatic alteration of membrane fatty acid composition is accompanied by unaltered function in other tissues or whether this is a unique observation for adipose tissue in young pigs; it is relatively certain that the lack of change in adipocyte membrane functions is not restricted to the beta-adrenergic receptor because 5′-nucleotidase activity also was not affected by diet composition.

In our experiments with young pigs, the very different fluidity expected in membranes (not measured) with such different fatty acid composition did not markedly affect membrane function as reflected in the affinity of the beta-adrenergic receptor for DHA or isoproterenol or in 5′-nucleotidase activity. The only differences we observed between the dietary groups were a greater ghost cholesterol concentration in the pigs fed the high-fat unsaturated diet and a tendency for a greater isoproterenol-stimulated lipolytic rate in adipocytes from pigs fed the high-fat saturated diet. As recently indicated,[19] changes in membrane fatty acid composition or even cholesterol concentration may not affect the microenvironment around a particular membrane-bound protein. Thus, in spite of alteration of the anisotropic responses of fluorescent dyes incorporated into the membranes (used as a measure of membrane fluidity) when the lipid composition of the membranes is altered, the function of a particular membrane-bound protein may not be altered because its specific placement in the membrane is such that the lipid microenvironment is not changed or the changes do not alter the movement of the protein enough to alter function.

Finally, we have not discussed our data in reference to beta-adrenergic receptor subtypes. Porcine adipocyte beta-adrenergic receptors are not clearly delineated into beta1-, beta2-, or beta3-adrenergic receptors. Studies using a dose *x* response design with doses to saturating concentrations and measuring the lipolytic response[8] or ligand binding[10,20] with purportedly receptor subtype-specific agonists and antagonists have not been able to decipher the subtypes present. Multiple binding sites are present (using competitive ligand binding techniques) but the data do not allow specification of a particular receptor subtype.[20] In regard to the beta3-adrenergic receptor that is predominant in adipose tissue from some species,[21] the evidence for porcine adipocytes is equivocal: (1) The present study used DHA, a radioligand incapable of detecting beta3-adrenergic receptors. Our previous studies[3,10] used iodocyanopindolol, a radioligand that detects all receptor subtypes; however, the maximal concentration was 1 nM (not great enough to saturate rodent beta3-adrenergic receptors). (2) The purported beta3-adrenergic receptor-specific agonist, BRL 37,344, binds to porcine adipocyte membranes but does not stimulate lipolysis (Scott Mills, Purdue University, personal communication). (3) The beta1- and beta2-adrenergic antagonist but beta3-adrenergic agonist, CGP 12,177, has some agonistic activity with porcine adipocytes, stimulating lipolysis at maximal response to about 20% of the isoproterenol-stimulated maximal response (Mersmann, unpublished data). (4) There was only one detectable binding site for ^{3}H-CGP 12,177 using concentrations from 100 pM to 100 nM and for iodocyanopindolol using concentrations from 25 pM to 3 nM. Specific binding was totally saturable at low concentrations of each radioligand (Mersmann, unpublished data). (5) Propranolol was used at 10 μM to measure nonspecific binding in the CGP 12,177 and iodocyanopindolol experiments. The nonspecific binding increased linearly across all concentrations of both radioligands suggesting that propranolol is an effective ligand for whatever receptor subtypes are present (Mersmann, unpublished data). (6) Using competitive ligand binding techniques and concentrations of competitive ligand from 100 pM to 1 mM, (±)-propranolol and (−)-isoproterenol both completely antagonized the binding of 100 pM iodocyanopindolol (approximately the K_d)[10] and of 2 nM iodocyanopindolol (a concentration that would detect rodent beta3-adrenergic receptors). At both low and high iodocyanopindolol concentrations, propranolol was more potent than isoproterenol, indicating that propranolol is an appropriate ligand for whatever receptor subtypes are present in porcine adipocyte membranes (Mersmann, unpublished data).

In contrast to rat adipocyte membranes, in porcine adipocyte membranes there was no low-affinity binding site (beta3-adrenergic receptor) for either CGP 12,177 or iodocyanopindolol, and propranolol was equally effective and more potent than isoproterenol as a competitive ligand. Recently, transcripts for beta3-adrenergic receptors were demonstrated in porcine adipose tissue mRNA with a mouse probe obtained from genomic DNA (R.L. McNeel and H.J. Mersmann, unpublished data; transcripts for beta1- and beta2-adrenergic receptors were also demonstrated). None of the studies with porcine adipocytes or adipocyte membranes is able to quantify the proportion of beta-adrenergic receptor subtypes or the response of the tissue to stimulation of specific receptor subtypes.

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References

1 Wall, K.M., Diersen-Schade, D., and Innis, S.M. (1992). Non-essential fatty acids in formula fat blends influence essential fatty acid metabolism and composition in plasma and organ lipid classes in piglets. *Lipids* **27**, 1024–1031

2 Nicolas C., Demarne, Y., Lecourtier, M.J., and Lhuillery, C.

(1990a). Specific alterations in different adipose tissues of pig adipocyte plasma membrane structure by dietary lipids. *Internation J. Obesity* **14,** 537–549

3 Mersmann, H.J., McNeel, R.L., Morkeberg, J.C., Shparber, A., and Hachey, D.L. (1992). Beta-adrenergic receptor-mediated functions in porcine adipose tissue are not affected differently by saturated vs. unsaturated dietary fats. *J. Nutr.* **122,** 1952–1959

4 Nicolas C., Lacasa D., Guidicelli, Y., Demarne, Y., Agli, B., Lecourtier, M.J., and Lhuillery, C. (1991). Dietary (n-6) polyunsaturated fatty acids affect beta-adrenergic receptor binding and adenylate cyclase activity in pig adipocyte plasma membrane. *J. Nutr.* **121,** 1179–1186

5 USDA (1978). Composition of foods. Agriculture Handbook No. 8-4, p. 15, 29, Human Nutrition Information Service, Hyattsville, MD, USA

6 Mersmann, H.J., and Hu, C.Y. (1987). Factors affecting measurements of glucose metabolism and lipolytic rates in porcine adipose tissue slices in vitro. *J. Anim. Sci.* **64,** 148–164

7 Mersmann, H.J. and McNeel, R.L. (1992a). Beta-adrenergic receptor binding in crude porcine adipose tissue plasma membranes. *J. Anim. Sci.* **70,** 781–786

8 Mersmann, H.J. (1992). Regulation of porcine adipose tissue lipolytic activity by adrenergic agonists and antagonists. *Comp. Biochem. Physiol.* **102C,** 407–411

9 Mersmann, H.J. and MacNeil, M.D. (1986). Variables in estimation of adipocyte size and number with a particle counter. *J. Anim. Sci.* **62,** 980–991

10 Mersmann, H.J. and McNeel, R.L. (1992b). Ligand binding to the porcine adipose tissue beta-adrenergic receptor. *J. Anim. Sci.* **70,** 787–797

11 Peterson, G.L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem.* **83,** 346–356

12 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

13 Mersmann, H.J., McNeel, R.L., and Heath, J.P. (1993). Distribution of beta-adrenergic binding in fractionated porcine adipocytes. *Int. J. Biochem.* **25,** 1121–1131

14 Avruch, J. and Wallach, D.F.H. (1971). Preparation and properties of plasma membrane and endoplasmic reticulum fragments from isolated rat fat cells. *Biochim. Biophys. Acta* **233,** 334–347

15 Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509

16 Bartlett, G.R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.* **234,** 466–468

17 Tercyak, A.M. (1991). Determination of cholesterol and cholesterol esters. *J. Nutr. Biochem.* **2,** 281–292

18 Nicolas C., Demarne, Y., Lecourtier, M.J., and Lhuillery, C. (1990b). Dependence between 5' nucleotidase activity and phosphatidylcholine content in pig adipocyte plasma membrane. Absence of relationship with membrane physical state. *Comp. Biochem. Physiol.* **96B,** 195–199

19 Lee, A.G. (1991). Lipids and their effects on membrane proteins: evidence against role for fluidity. *Prog. Lipid Res.* **30,** 323–348

20 Mersmann, H.J., Akanbi, K., Shparber, A., and McNeel, R.L. (1993). Binding of agonists and antagonists to the porcine adipose tissue beta-adrenergic receptor(s). *Comp. Biochem. Physiol.* **106C,** 725–732

21 Lafontan, M. and Berlan, M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* **34,** 1057–1091