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Effect of Obesity per se on Plasma Lipid and Aortic Responses to Diet in Swine (42068)

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Abstract. Thirty-two genetically lean and obese Yorkshire × Duroc crossbred castrated male pigs were divided within genetic line into two groups at 7 weeks of age. Eight pigs within each line were fed a diet low in fat and cholesterol (maize-soybean meal diet fortified with minerals and vitamins). The other group was fed a similar diet containing added beef tallow (11%) and dried egg yolk (1%). All pigs were fed *ad libitum* for 4 months when one-half of each group was slaughtered. All other pigs were continued on their respective diets at a restricted level of intake for an additional 5 months at which time the protein source of two pigs in each diet group within each genetic line was changed from soybean meal to casein. After an additional 6 months on their respective diets (16 months total duration of experiment) these pigs were slaughtered. Blood samples were taken monthly or bimonthly for total plasma cholesterol and triglycerides. At slaughter, the aorta was opened, stained with Sudan IV, and the stained area traced and measured planimetrically. Only a moderate rise occurred in plasma cholesterol and triglycerides of pigs fed high fat–high cholesterol diets. Genetically obese pigs were no more susceptible to diet-induced hypercholesterolemia and to the percentage of the surface area of the aorta stained with Sudan IV than were lean pigs. It is concluded that obesity per se is not necessarily associated with development of atherosclerosis in pigs and that innate ability to metabolize high dietary cholesterol is of greater importance than body fatness in determining the response to diet.

Obesity, dietary cholesterol, and saturated fat intake are all considered important factors related to the high incidence of cardiovascular disease in humans. The pig has become a widely used model for use in studies of human atherosclerosis (1–3). A population of genetically obese and lean pigs (Yorkshire × Duroc pigs derived from inter se matings of obese and lean purebreds selected for 20 generations for high or low backfat thickness) has been shown (4) to have similar plasma cholesterol, triglyceride and glucose levels, and normal glucose tolerance. Such pigs offer a unique tool to ascertain the importance of obesity per se as a component of atherosclerosis. Evidence presented by Keys (5) suggests that moderately overweight humans in the U.S. population may not be more susceptible to atherosclerosis than individuals at the recommended mean body weight. Nutritional requirements with humans reported in the literature seldom address the effect of desirable body weight on the response to dietary treatment.

The purpose of the present experiment was to determine the plasma lipid response and the development of aortic plaques in genetically obese and lean pigs fed a standard diet (low fat–low cholesterol) or a diet containing tallow and egg yolk (high fat–high cholesterol).

Materials and Methods. Thirty-two castrated male Duroc × Yorkshire pigs representing a lean and an obese population developed by selection for 20 generations within the parented breeds for high or low backfat (6) (16 obese and 16 lean) were assigned randomly within genetic groups at about 50 days of age to one of two dietary treatments, a low fat–low cholesterol all plant diet or a similar diet containing 11.0% beef tallow and 1.0% dried egg yolk (Table I). All pigs were penned separately and fed *ad libitum* for 4 months at which time one-half of each group (8 lean and 8 obese pigs fed each diet) was slaughtered. All other pigs were continued on their respective diets fed at 1.82 kg per animal daily for 1 additional year. At Month 10 of the experiment the protein source of two pigs in each diet group within each genetic line was changed from soybean meal to casein. After an additional 6 months on

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TABLE I. COMPOSITION OF DIETS

Ingredient	International feed number[a]	Low fat (%)	High fat (%)
Corn, No. 2, yellow dent	4-02-931	70.6	53.6
Soybean meal[b]	5-04-604	25.0	30.0
Dicalcium phosphate	6-01-080	2.4	2.4
Ground limestone	6-02-632	0.8	0.8
Iodized salt	6-04-151	0.4	0.4
Vitamin premix[c]	—	0.2	0.2
Choline chloride[d]	—	0.2	0.2
Trace mineral premix	—	0.4	0.4
Beef tallow	4-08-127	—	11.0
Dried egg yolk	—	—	1.0
		100.0	100.0
Ether extract fat, %		3	14
Cholesterol, mg/kg[e]		0	367

[a] National Research Council. U.S. Canadian Tables of Feed Composition, National Academy Science, Washington, D.C., 1982.

[b] At 12 months of age one-half of the pigs fed low fat and one-half of the pigs fed high fat diets within each genetic line were fed casein replacing soybean meal as the main protein source to slaughter at 18 months of age. Crude casein (85% protein) was fed at 14 and 16.5% of the low fat and high fat diets, respectively, at the complete expense of soybean meal. Corn was increased to 81.6 and 67.1% of low fat and high fat diets into which casein was substituted.

[c] Supplies the following (units/kg diet): vitamin A, 5280 IU; vitamin D_3, 704 IU; vitamin E, 70.4 IU; vitamin K, 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; thiamin, 2.2 mg.

[d] Supplies the following (ppm): Cu (as cupric oxide), 10; Fe (as ferrous sulfate heptahydrate), 160; Mn (as manganese oxide), 20; Zn (as zinc oxide), 100; $CaCO_3$ used as carrier (0.30% of diet).

[e] Tallow contains 95 mg and dried egg yolk 2630 mg of cholesterol per 100 g.

their respective diets (16 months total duration of the experiment) all pigs were slaughtered.

Blood was sampled from the anterior vena cava of each pig monthly for 4 months and bimonthly for 12 months for determination of total plasma cholesterol (7) and triglycerides (8). At slaughter, aorta from aortic arch to the bifurcation of the ileac arteries was removed, opened longitudinally, and stained with Sudan IV (9) for identifying lipid deposits. Stained areas were measured planimetrically and the affected area was expressed as a percentage of total aorta surface area. Backfat depth (mean of three measurements taken 5 cm lateral to the vertebral column) and cross-sectional area of longissimus muscle and fat at the 10–11th rib interface were recorded for each pig.

Data were subjected to least-squares analysis of variance (10) with genetic line, age, and diet as main effects for blood and body weight traits and genetic line and diet for aorta and carcass traits; all interactions were tested.

Results. Body weights, backfat thickness, and cross-sectional areas of longissimus muscle and of the associated subcutaneous fat layer are shown in Table II for pigs slaughtered at 6 months and at 18 months of age. Body weight of pigs slaughtered at 6 months was unaffected by breed or by diet, but backfat thickness (mean of three measurements over first rib, last rib, and last lumbar vertebrae) and subcutaneous fat area at the 10–11th rib interface was greater ($P < 0.01$) in obese than lean pigs, as expected, and in pigs fed the high fat diet than in those fed the low fat diet ($P < 0.01$). Longissimus muscle cross-sectional area was greater ($P < 0.01$) in lean than in obese pigs but was unaffected by diet. The same trends persisted in pigs slaughtered at 18 months except that the effect of diet on backfat thickness and subcutaneous fat area at the 10–11th rib interface no longer existed, presumably due to the restricted feed intake imposed on all pigs after 6 months of age.

Plasma cholesterol and triglyceride concentrations and percentages of aorta surface area occupied by Sudan IV stainable tissue are shown in Table III for pigs slaughtered at 6 months of age and in Table IV for those slaughtered at 18 months of age. Data on plasma cholesterol and triglycerides for the period from 12 months to 18 months of age, during which one-half of each genetic line was fed casein instead of soybean meal as the source of supplemental protein, were combined with respect to protein source for presentation in Table IV. This was done because analysis of variance to test protein source failed to reveal an effect for either trait. Neither plasma cholesterol nor triglycerides exceeded 200 mg/dl even at 18 months of age, indicating that both obese and lean pigs are relatively refractory to high fat–high cholesterol diets. There appeared to be a rise in plasma cholesterol in pigs of both genetic

TABLE II. BACKFAT THICKNESS AND CROSS-SECTIONAL AREA OF LONGISSIMUS MUSCLE AND BACKFAT, AT 10–11th RIB INTERFACE OF GENETICALLY LEAN OR OBESE PIGS FED DIETS WITH OR WITHOUT TALLOW–EGG YOLK SUPPLEMENTATION (LEAST-SQUARES MEANS)

Trait	Genetic line (G):	Obese		Lean		SD	Probability
	Diet (D):	Low fat	High fat	Low fat	High fat		
		6 Months old					
Number of pigs[a]		4	4	4	4		
Slaughter weight, kg		71.4	78.3	73.4	78.6	7.6	NS
Backfat, cm		5.2	6.6	2.3	2.8	0.56	G, D < 0.01
Longissimus muscle lean area, cm^2		14.9	13.5	21.7	23.7	3.1	G < 0.01
Longissimus muscle fat area, cm^2		89.7	105.8	26.9	37.5	7.4	G, D < 0.01
		18 Months old					
Number of pigs		4	4	4	4		
Slaughter weight, kg		182.4	163.5	178.7	174.5	6.8	D < 0.01
Backfat, cm		8.5	8.3	3.5	4.4	0.59	G < 0.01
Longissimus muscle lean area, cm^2		21.5	24.5	40.2	37.2	3.0	G < 0.01
Longissimus muscle fat area, cm^2		75.3	75.2	43.1	46.1	5.9	G < 0.01

[a] Initial weight at 7 weeks of age, 9.8 kg for obese and 12.3 kg for lean pigs.

lines fed either diet from the start of the experiment (2 months of age) to 6 months of age. Obese pigs had significantly lower plasma cholesterol ($P < 0.01$) and plasma triglyceride ($P < 0.05$) than lean pigs initially, but the reverse was true at 6 months. The high fat–high cholesterol diet produced significantly greater plasma cholesterol concentrations in both lean and obese pigs than did low fat–low cholesterol at 6 months. Despite

TABLE III. PLASMA CHOLESTEROL AND TRIGLYCERIDES AND PERCENTAGE OF AORTA STAINABLE WITH SUDAN IV OF GENETICALLY LEAN OR OBESE PIGS FED FOR 4 MONTHS DIETS WITH OR WITHOUT TALLOW–EGG YOLK SUPPLEMENTATION (LEAST-SQUARES MEANS)

Trait	Genetic line (G):	Obese		Lean		SD	Probability
	Diet (D):	Low fat	High fat	Low fat	High fat		
		7 Weeks old (initial)					
Number of pigs		8	8	8	8		
Plasma cholesterol, mg/dl		56	51	78	74	4	G < 0.01
Plasma triglycerides, mg/dl		45	60	57	66	27	G < 0.05
		6 Months old					
Number of pigs		8	8	8	8		
Plasma cholesterol, mg/dl		143	174	119	150	25	G < 0.01; D < 0.05
Plasma triglycerides, mg/dl		55	74	22	53	28	G < 0.05
Sudan IV stained aorta, % of area		None (4)[a]	None (4)	None (4)	None (4)		

[a] Number of pigs slaughtered in parentheses.

TABLE IV. PLASMA CHOLESTEROL AND TRIGLYCERIDES AND PERCENTAGE OF AORTA STAINABLE WITH SUDAN IV OF GENETICALLY LEAN OR OBESE PIGS FED FOR 16 MONTHS DIETS WITH OR WITHOUT TALLOW–EGG YOLK SUPPLEMENTATION (LEAST-SQUARES MEANS)

Trait	Genetic line (G):	Obese		Lean		SD	Probability
	Diet (D):	Low fat	High fat	Low fat	High fat		
		6 Months to 12 months old[a]					
Number of pigs		4	4	4	4		
Plasma cholesterol, mg/dl		88	116	73	112	8	G, D, G × D < 0.01
Plasma triglycerides, mg/dl		61	72	38	68	29	G < 0.05; D < 0.01
		12 Months to 18 months old[b]					
Number of pigs		4	4	4	4		
Plasma cholesterol, mg/dl		120	128	87	123	17	G, D < 0.01; G × D < 0.10
Plasma triglycerides, mg/dl		45	42	23	30	13	G < 0.01; G × D < 0.07
Sudan IV stained aorta, % of area		7.4	9.3	10.5	17.7	7.3	NS

[a] Before casein replaced soybean meal in the diet of one-half of the pigs.
[b] After casein replaced soybean meal in the diet of one-half of the pigs. Since there was no significant effect of protein source on any trait measured, data from dietary protein groups were combined.

these moderate rises in plasma cholesterol associated with dietary fat–cholesterol there were no aortic lipid deposits at 6 months as assessed by Sudan IV staining.

Concentration of plasma cholesterol from 6 months to 12 months of age tended to be less in both genetic lines and in both diet groups than that observed at 6 months of age, presumably due to the restricted feed intake imposed after 6 months. Obese pigs had higher cholesterol and higher triglycerides than lean pigs and dietary fat–cholesterol supplementation significantly increased both constituents in both genetic lines at 12 months. There was an interaction between genetic line and diet for plasma cholesterol, such that lean pigs showed a greater response than did obese pigs to dietary fat–cholesterol supplementation.

Pigs slaughtered at 18 months of age differed ($P < 0.01$) in plasma cholesterol from 12 to 18 months between genetic lines (obese > lean) and between dietary fat–cholesterol levels (high fat > low fat). The tendency for lean pigs to show a greater rise in plasma cholesterol in response to diet than obese pigs resulted in an interaction ($P < 0.10$) between genetic line and diets. Plasma triglycerides were higher ($P < 0.01$) in obese than in lean pigs from 12 to 18 months of age and a trend toward opposite response of lean pigs to dietary fat–cholesterol supplementation resulted in a genetic line × diet interaction ($P < 0.07$).

All pigs slaughtered at 18 months of age, regardless of genetic line or of diet, showed some Sudan IV stainable aortic involvement. The mean percentage of the total aorta surface area affected was greater for lean pigs than for obese pigs (14.1% vs 8.4%) and greater for pigs fed high fat–high cholesterol than for those fed low fat–low cholesterol diets (13.5% vs 9.0%) but the differences did not reach statistical significance.

Discussion. The results of this experiment with genetically obese and lean castrated male pigs fed diets containing no animal fat or cholesterol or 11% beef tallow and 1% dried egg yolk (367 mg cholesterol/kg diet) indicate that genetically controlled obesity per se is not necessarily associated with increased plasma cholesterol or triglycerides or with a greater tendency, compared with genetically lean pigs, toward hypercholesterolemia or hypertriglyceridemia when dietary fat and cholesterol are increased. The failure

of obese and lean pigs in this experiment to develop hyperlipidemia when fed a high fat–high cholesterol diet is in contrast to other reports (11–17) in which pigs were of different genetic backgrounds and fed diets higher in cholesterol than in the present experiment. There is evidence (1) of genetic difference in resistance to atherogenesis in response to diet in swine. The failure of a diet containing 11% tallow–1% dried egg yolk to increase plasma cholesterol in the present experiment to levels generally associated with a high incidence of heart disease in humans is in accord with the relative freedom from lipid accumulation in the aortic surface as assessed by Sudan IV stain. Hill (12) observed a high correlation between serum cholesterol concentration and aortic cholesterol content in pigs with aortic lesions following consumption of a high fat–high cholesterol diet. Therefore, the association between serum cholesterol and ischemic heart disease in humans appears to exist also in swine susceptible to atherosclerosis. The serum lipoprotein profile of swine resembles that of humans more closely than other species except nonhuman primates (18–21). Immunogenetically determined polymorphism of β-lipoproteins has been reported in swine (20). The obese and lean swine used in the present research have not been characterized in this regard.

The unexpected refractoriness of plasma cholesterol and triglycerides and of aortic Sudan IV stained lipids to dietary fat in both lean and obese pigs in the present experiment after 4 months on the diet led to the suggestion that soybean meal was a poor choice as the protein supplement because of observations in several species that plant proteins are less atherogenic than animal proteins (22, 23). Therefore, after 10 months, one-half of the pigs in each genetic group and each dietary fat–cholesterol level was changed to casein rather than soybean meal as the protein supplement. Plasma cholesterol and triglyceride concentrations following replacement of soybean meal by casein failed to deviate from the values recorded for pigs continued on the diets containing soybean meal. Likewise, aortic surface area with lipid accumulation (Sudan IV stained) was similar for pigs fed soybean meal and casein, regardless of genetic background or level of fat and cholesterol in the diet. However, the relatively small number of animals in each group precludes a definitive conclusion regarding the effect of dietary protein source on lipid metabolism.

Based on the generally accepted relationship between obesity and incidence of ischemic heart disease, one would expect higher levels of plasma lipids in obese than in lean swine and a greater increase in plasma cholesterol and triglycerides in response to dietary fat and cholesterol in obese than in lean pigs. When fed the low fat diet, obese pigs exceeded lean pigs in both cholesterol and triglycerides at 6, 12, and 18 months of age (each age bracket shown in Tables III and IV includes the mean of monthly samples taken from 2 to 6 months, or bimonthly, from 6 to 18 months), while lean pigs responded to high fat diets with a greater rise in both plasma cholesterol and triglycerides than did obese pigs. This phenomenon suggests the possibility of an analogous segment of the human population whose tendency toward obesity is not accompanied by an increased susceptibility to atherosclerosis and who, in fact, may be more refractory than some genetically lean individuals to elevated plasma cholesterol in response to dietary fat and cholesterol. Unfortunately, nutritional experiments with humans seldom have reported the body type of the subjects. The genetically diverse populations of swine available for use as models and the obese and lean lines selected from a common original population as used in the present experiment offer the possibility of providing answers to questions that remain unanswered in profiling human populations for susceptibility to atherosclerosis.

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