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Original Research

Effect of Caloric Restriction on Metabolic Dysfunction of Young Rapacz Familial Hypercholesterolemic Swine (*Sus scrofa*)

Nell A Bekiares,[1] Andrea S Chen,[1] Dhanansayan Shanmuganayagam,[1] Adrienne Dardenne Meyers,[2] Thomas D Crenshaw,[1] Christian G Krueger,[1] and Jess D Reed[1,*]

The Rapacz familial hypercholesterolemic (FH) swine model is well-characterized and used for studies of both spontaneous and inducible atherosclerosis but has not been used for studies of metabolic dysfunction to date. We examined whether parameters of metabolic syndrome including weight and adiposity, serum cholesterol, and glucoregulatory function could be modulated by restriction of caloric intake in the FH swine. Three groups of FH swine (n = 6 per group) were fed without restriction (AL), 80% of AL caloric intake, or 60% of AL caloric intake for 8.8 ± 0.5 mo beginning 2 wk after weaning. Caloric intake influenced the rate and magnitude of body weight gain and change in adiposity, as determined by dual-emission X-ray absorptiometry. At the conclusion of the study, pigs in the AL group reached a total least-square mean body weight of 94.2 kg and fat mass of 31.1%, whereas those fed 80% AL were 71.6 kg and 24.3% fat, and swine fed 60% AL were 46.1 kg and 14.1% fat. Serum cholesterol was greater in AL than 60% AL pigs at the end of the study. At 10 mo of age, intravenous glucose tolerance testing, performed to assess glucoregulatory function, indicated significant differences in serum glucose clearance profiles and insulin sensitivity between the AL- and 60% AL-fed swine. The AL-fed animals showed almost 5-fold lower insulin sensitivity when compared with animals fed 60% AL caloric intake. These results highlight the value of the FH swine model to study metabolic dysfunction due to changes in caloric intake.

Abbreviations: AL, without restriction (ad libitum); DXA, dual-energy X-ray absorptiometry; FH swine, Rapacz familial hypercholesterolemic swine; IVGTT, intravenous glucose tolerance testing

Obesity currently affects nearly 1/3 of the population in the industrialized world[20,22] and is a leading risk factor for the development of metabolic syndrome. Metabolic syndrome, in turn, increases the risk of morbidity and mortality from coronary heart disease, type 2 diabetes mellitus, cancers (specifically endometrial, breast, and colon), hypertension, dyslipidemia, stroke, and other chronic diseases.[19] Substantial evidence links the accumulation of ectopic visceral fat in obese men and women to insulin resistance, glucose tolerance, cardiovascular disease, and other systemic markers of inflammation.[5,25,34] Controlled prospective studies of obesity and subsequent disease progression in humans are difficult and impractical because of ethical concerns and the challenges in controlling genetic, dietary, and environmental factors. Therefore, relevant studies in animals are required to model the symptoms of metabolic syndrome.

To fully understand how the effects of caloric intake relate to the progression of metabolic syndrome in humans, a relevant animal model with plasticity to respond to dietary inputs is needed. The ideal animal model of obesity would mimic the metabolic pathophysiology of the human condition and would develop end-stage disease as it occurs in human patients. Rapacz familial hypercholesterolemic (FH) swine mimic the progression and end points of human cardiovascular disease and have extensively been studied for that purpose for the past 2 decades.[2,3,11,12,23,24,26,30] Since the 1980s, FH swine have been selectively bred at the University of Wisconsin Swine Research and Teaching Center to have high circulating levels of cholesterol, similar to those in humans diagnosed with hypercholesterolemia.[10] The FH condition is caused by a mutation in the LDL receptor gene (*LDLR*) on chromosome 2; the protein product of this gene normally removes LDL from circulation.[32] The mutation in *LDLR* causes high levels of circulating LDL. Although FH swine have been used previously as a model of cardiovascular disease, the current study is the first to model metabolic syndrome by using these animals. Here, we aimed to evaluate the plasticity of the FH model under 3 levels of daily caloric intake and to assess body weight gain and changes in adiposity, serum cholesterol, and glucoregulatory function as they relate to metabolic syndrome.

Materials and Methods

Study design. The animal study was reviewed and approved by the IACUC at the University of Wisconsin-Madison in compliance with the *Guide for the Care and Use for Laboratory Animals*[14] and the Animal Welfare Act.[1]

Newly weaned female FH piglets (n = 18) were selected from the SPF herd at the University of Wisconsin Swine Research and Teaching Center (Madison, WI), which is specifically protected

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[1]Department of Animal Sciences, University of Wisconsin–Madison, Wisconsin, and [2]Cardiovascular Research Foundation, Skirball Center for Innovation, New York, New York.
[*]Corresponding author. Email: jdreed@wisc.edu

Table 1. Experimental diet composition on an as-fed basis

Ingredients, %	AL	80% AL	60% AL
Corn grain (medium ground)	64.30	52.73	33.34
Soybean meal, (47.5% crude protein)	29.96	40.48	58.12
L-lysine HCl	0.10	0.10	0.10
Calcium phosphate (monocalcium)	1.00	1.52	2.38
Calcium carbonate	1.14	1.29	1.55
Max-Fat[a]	2.00	2.00	2.00
Sodium chloride (iodized)	0.50	0.63	0.83
Vitamin-trace mineral premix[b]	1.00	1.25	1.67
Total	100.00	100.00	100.00

[a]Max-fat (Maxco, Green Bay, WI) provides a blend of animal and vegetable fats
[b]The vitamin–trace mineral premix was supplemented at 1.0% of the complete diet and provided the following nutrients per kilogram of diet: vitamin A, 2,800 IU; vitamin D_3, 280 IU; vitamin E, 14 IU; vitamin K, 0·75 IU; niacin, 22 mg; pantothenic acid, 12 mg; riboflavin, 8 mg; vitamin B_{12}, 33 µg; Cu, 1·5 mg; I, 0·3 mg; Fe, 38 mg; Se, 0·2 mg; and Zn, 90 mg. The amounts of vitamin–trace mineral premix were increased in the 80% and 60% AL diets to supply equal nutrient intakes to that of the AL group.

against atrophic rhinitis, *Mycoplasma hyopneumoniae*, swine dysentery, lice, mange, and internal parasites. The swine were stratified according to weight, age, and weaning date and then randomly assigned to 1 of 3 dietary regimens of energy intake (unrestricted [AL], 80% of AL intake, and 60% of AL). All 3 diets were formulated to ensure that essential macronutrient and micronutrient requirements for normal growth and development were met (Table 1). Feed intake was measured daily and averaged weekly; the average daily feed intake of the AL-fed group was used to adjust the feed intake of the other 2 groups on a weekly basis (Table 2). Animals were fed twice daily, beginning at age 51 ± 1.6 d (2 wk after weaning) and provided with unrestricted access to water. Swine were weighed weekly throughout the trial period. The study concluded when the animals were 10.5 ± 0.6 mo of age and had been fed the dietary regimen for 8.8 ± 0.5 mo.

Swine were individually housed indoors in 1.22 m × 1.22 m or 1.22 m × 2.44 m pens (minimum as determined by body weight) that combined metal and concrete flooring and that provided visual, auditory, olfactory, and tactile contact with neighboring pens. The pens did not contain bedding, and supplemental environmental enrichment was not provided. Swine were exposed to a 12:12-h light:dark photocycle, temperature was maintained at 21.1 °C throughout the study, and humidity was not controlled.

Dual-energy X-ray absorptiometry. Total body fat, lean mass, and bone mass was measured by using dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy, Waukesha, WI) as previously described.[27] The appropriate scan mode (Small Animal [5–20 kg], Adult Thick [above 46 kg], or Adult Standard [20–46 kg]) was selected according to body weight.

DXA measurements were performed weekly for the first 2 mo of the study, and once monthly thereafter. Anesthesia was induced by using tiletamine–zolazepam (2 to 3 mg/kg IM; Fort Dodge Animal Health, Fort Dodge, IA) and maintained (Narkovet 2 anesthesia machine, Drager, Telford, PA) for 10 to 20 min by using 5% isoflurane through a nosecone to prevent animal movement during DXA scan measurements. Anesthetic depth was confirmed by absence of an ocular reflex, loss of muscle tone, absence of a pedal reflex, and deep breathing. An ECG and pulse-oximeter monitor (Instrumentarium, Helsinki, Finland) was used to assess heart rate, respiratory rate, percentages of oxygen and isoflurane, and blood pressure in anesthetized swine.

Ectopic visceral fat. At the conclusion of the study, the chest cavity was exposed, the heart photographed, and the chest and heart visually examined for the presence of pericardial and subcutaneous fat.

Blood collection. While swine were anesthetized for DXA scans, blood samples were obtained monthly for cholesterol analysis. Whole blood was collected by venipuncture of the cranial vena cava by using a syringe containing 1.8 mg EDTA per milliliter of blood (Amresco, Solon, OH). Blood was centrifuged at room temperature for 10 to 15 min at 1500 × *g* (International Equipment Company, Needham Heights, MA). Serum was pipetted into 1.5-mL microfuge tubes and frozen at –80 °C. Serum cholesterol was determined by using an enzymatic dry-slide method (Vitros 5,1 FS chemistry analyzer, Ortho Clinical Diagnostics, Rochester, NY) at the University of Wisconsin School of Veterinary Medicine. Results were reported as milligrams of total per deciliter of blood.

Intravenous glucose tolerance testing (IVGTT). IVGTT was performed when swine were 10.5 ± 0.6 mo of age (that is, at the completion of the study). Feed was withheld for 18 to 24 h before the procedure. Anesthesia was induced with tiletamine–zolazepam (2 to 3 mg/kg IM) and maintained by using 5% isoflurane through a nose cone. Animals were placed dorsally and secured on a surgical table for this terminal procedure; the duration of anesthesia did not exceed 6 h. An intravenous ear line was placed for administration of saline, glucose, and insulin. A small midline incision was made in the neck region to expose the carotid artery, and a catheter was placed for the delivery of fluids, blood sampling, and blood pressure monitoring. Blood samples (4 mL) were drawn from the carotid catheter into syringes and poured immediately into additive-containing vacuum phlebotomy tubes (catalog no. 367921, containing sodium fluoride [5 mg] and potassium oxalate [4 mg], Becton Dickinson, Franklin Lakes, NJ] for glucose determination and into additive-free vacuum phlebotomy tubes (catalog no. 366441, Becton Dickinson) for insulin determination (2 mL blood in each tube). Blood was drawn at 4 time points prior to glucose administration (that is, –15, –10, –5, and –1 min). At time 0 min, a glucose bolus (0.3 g/kg body weight, 50% dextrose solution) was administered through the arterial line in less than 3 min. Subsequent blood samples (4 mL total, as described earlier) were drawn at 0, 2, 4, 6, 8, 19, 22, 30, 40, 50, 70, 90, and 120 min after glucose administration. Insulin (0.3 U/kg body weight IV;

Table 2. Average daily feed intake (kg) of swine according to treatment group

Date	AL	80% AL	60% AL
Before restriction (1–7 June 2010)	0.39	0.36	0.44
7–15 June	0.51	0.36	0.29
15–22 June	0.75	0.41	0.30
22–29 June	0.84	0.47	0.33
29 June–7 July	0.97	0.53	0.35
7–13 July	1.22	0.61	0.39
13–21 July	1.20	0.68	0.41
21–29 July	1.23	0.77	0.46
28 July–3 August	1.35	1.07	0.63
4–10 August	1.45	0.97	0.55
11–17 August	1.55	0.96	0.58
18–24 August	1.35	0.98	0.60
25–31 August	1.40	1.00	0.76
1–7 September	1.66	1.08	0.73
8–14 September	1.65	1.12	0.73
15–21 September	1.73	1.25	0.75
22–28 September	1.58	1.30	0.78
29 September–5 October	1.66	1.10	0.75
6-12 October	1.81	1.11	0.77
13-19 October	1.79	1.11	0.78
20-26 October	1.89	1.12	0.79
27 October–2 November	1.89	1.13	0.81
3–9 November	1.92	1.14	0.81
10–16 November	1.84	1.15	0.81
17–23 November	2.01	1.17	0.82
23–30 November	2.02	1.19	0.82
30 November–7 December	1.66	1.22	0.82
7–14 December	1.82	1.26	0.83
14–21 December	1.85	1.29	0.83
21–28 December	1.77	1.33	0.83
28 December 2010–4 January 2011	1.71	1.33	0.83
4–11 January	1.67	1.33	0.83
11–17 January	2.20	1.55	0.97
17–24 January	2.35	1.55	0.97
24 January–1 February	2.13	1.55	0.97
1-8 February	2.01	1.58	1.06
8-15 February	1.79	1.58	1.06
15–20 February	2.36	1.58	1.08
20–28 February	2.00	1.69	1.09
28 February–7 March	0.50	1.61	1.10
7–15 March	0.79	1.61	1.10
14–21 March	1.33	1.63	1.09
Conclusion (21–28 March 2011)	1.28	1.64	1.10

100 U/mL, Novolin Regular Human Insulin, recombinant DNA origin, Novo Nordisk, Bagsvaerd, Finland) was infused 20 min after glucose administration to induce an additional increment in circulating insulin levels. Blood samples were stored on ice for no more than 30 min and then were centrifuged at room temperature for 10 to 15 min at 1500 × *g* (International Equipment Company). The serum from each vacuum phlebotomy tube was separated into 2 microcentrifuge tubes (0.5 to 1 mL each). Serum was stored at –80 °C until analysis.

While under a surgical plane of anesthesia, the swine were euthanized by using supersaturated potassium chloride (10 mL/45 kg IV through the ear cannula, 4 M KCl injectable, Sigma–Aldrich, St Louis, MO) after the final blood draw in the IVGTT protocol. Death was confirmed through the absence of heart beat and respirations according to the ECG monitor, and tissues were harvested and fixed for future examination.

Frozen serum was processed for glucose concentration by using a modified glucose peroxidase assay. Glucose oxidase–peroxidase solution was prepared monthly. Glucose standards were prepared daily in concentrations to 100 mg/dL by using D-+-glucose (99.5%, Sigma-Aldrich). Samples were read on a spectrophometer (Molecular Devices, Sunnyvale, CA) at 505 nm (SoftMax Pro software, Molecular Devices), and glucose levels were reported in milligrams per deciliter.

Table 3. Least-square means (± SEM) of body weight and lean, bone, and fat content of the swine at the conclusion of the study

Treatment	Body weight (kg)	Lean, kg (%)	Bone, kg (%)	Fat, kg (%)
AL	94.2 ± 1.3[a]	62.0 ± 1.3 (65.8)[a]	2.1 ± 0.04 (2.2)[a]	29.3 ± 0.6 (31.1)[a]
80% AL	71.6 ± 1.3[b]	52.7 ± 1.3 (73.6)[a,b]	1.8 ± 0.04 (2.5)[a,b]	17.4 ± 0.6 (24.3)[a,b]
60% AL	46.1 ± 1.3[c]	38.9 ± 1.3 (84.4)[b,c]	1.4 ± 0.04 (3.0)[b,c]	6.5 ± 0.6 (14.1)[b,c]

Different superscripted letters indicate groups that differ significantly ($P \leq 0.05$) .

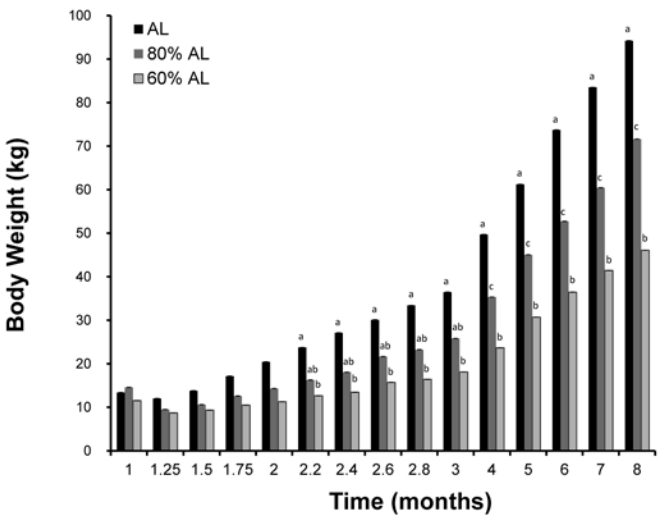

Figure 1. Average body weight of FH swine according to dietary treatment and time. Data are given as least-square means ± SEM (n = 6 per treatment group). At each time point, different letters indicate values that are statistically significant ($P \leq 0.05$). Beginning at 65 d, AL swine weighed significantly more than 60% AL pigs. Weights differed significantly between all groups beginning at 100 d.

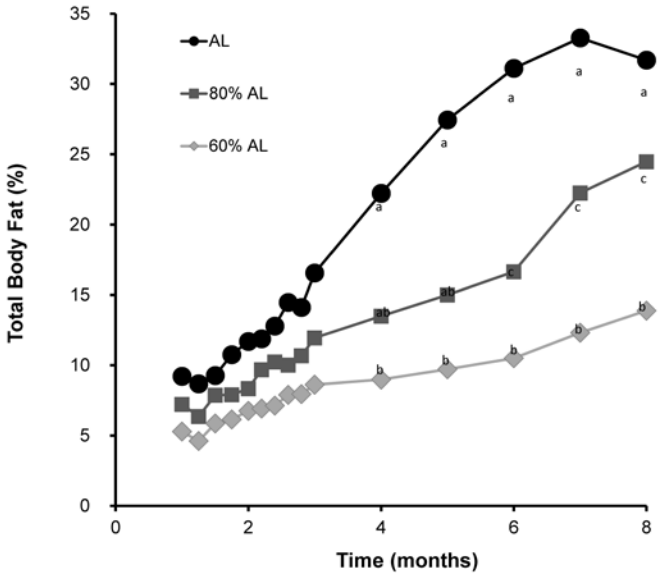

Figure 2. Average percentage total body fat determined from DXA scans of swine according to dietary treatment and time. Data are given as least-square means ± SEM (n = 6 per treatment group). At each time point, different letters indicate values that are statistically significant ($P \leq 0.05$) from that point onward.

Frozen serum was processed for insulin concentration by using a commercially available porcine insulin radioimmunoassay kit (catalog no. PI-12K, Millipore, Billerica, MA). Briefly, assay buffer was placed in each borosilicate tube, followed by the addition of standard or sample. Radioactively labeled insulin and antibodies were added, and reactions were incubated at 4 °C for 20 to 24 h. After incubation, a precipitating reagent was added; reactions were incubated for 1 h and then centrifuged at 3000 × g for 1 h (model no. 6R, Allegra 6 series, Beckman Coulter, Brea, CA). Supernatants were discarded, and the radioactivity in the pellet was counted (Cobra II series, Packard, Ramsey, MN). Results are reported as a percentage of total binding and as converted to µU/mL of porcine insulin.

The glucoregulatory function of the swine in response to IVGTT was determined (Millennium version 6.02, MinMod, Los Angeles, CA)[21] by using the serum glucose and insulin concentration data. This software has been used reliably to model frequently sampled IVGTT data.[13]

Statistical analysis. To test for significant effects of the dietary regimen, a fixed-effects ANOVA model was fit for each longitudinally (repeated measures) and cross-sectionally measured parameter by using the PROC MIXED function of SAS (version 8, SAS Institute, Cary, NC). For longitudinal data sets, diet×time interactions were examined also. Correlations between observations collected on the same animal over time were modeled by using a compound symmetry correlation structure. For each measured dependent variable, the model was fit by using untransformed data, and the residual plots were evaluated to ensure that standard ANOVA assumptions of constant variance and normality were reasonably met; Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises, and Anderson–Darling tests of normality were performed as additional measures. Square-root transformation of the data was performed during significance analysis of the following parameters: total body weight, total fat weight, percentage fat weight, and parameters of glucoregulatory function (basal glucose, basal insulin, glucose clearance, insulin sensitivity, glucose effect, and distributed glucose). Log transformation was applied to the data sets for percentage lean weight and percentage bone weight. Note that, as is common practice, the least-square means reported in the text are of the untransformed data. Type III tests were performed to evaluate significance, and least-square means were calculated for each combination of factors. Any least-square means comparisons made subsequent to the type III tests were adjusted by using the Tukey–Kramer adjustment. The data are reported as least-squares means ± SEM. Statistical significance was accepted at a P value less than 0.05.

Results

All 18 swine completed the study without incident.

Body weight and composition. Weight gain was greater in swine on the AL dietary treatment compared with 80% AL and 60% AL (Table 3, Figure 1) and differed significantly ($P \leq 0.001$) between AL and 60% AL swine after 65 d on study. The weights of animals in all diet groups differed ($P \leq 0.005$) after 100 d on study.

Body mass was significantly ($P < 0.01$) greater in AL swine compared with 60% AL pigs starting at 115 d and greater

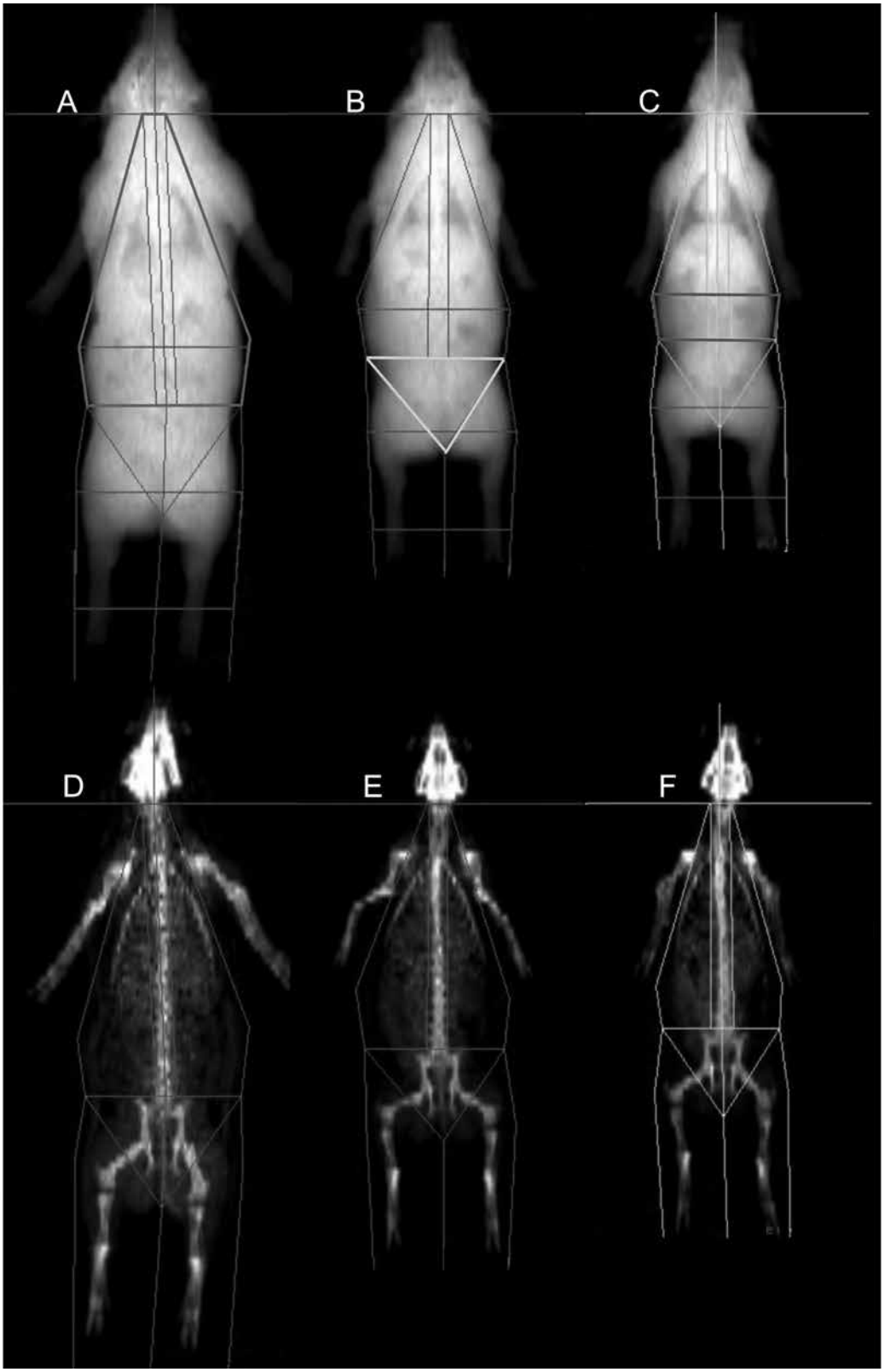

Figure 3. Images of representative pigs from the 3 treatment groups. Whole-body and skeletal DXA scans at 6 mo of representative animals from (A and D) AL, (B and E) 80% AL, and (C and F) 60% AL diet groups. Note that the AL-fed animal is larger in stature and has more adipose tissue.

($P < 0.01$) in the 80% AL group compared with the 60% AL animals starting at 180 d. Percentage fat mass differed ($P \leq 0.01$) from that at baseline for all animals in the AL and 80% AL groups after 115 d and in the 60% AL animals from 155 d onward ($P \leq 0.01$). From 5 mo until the end of the study, percentage total body fat differed ($P \leq 0.01$) between the AL and 60% AL swine. The percentages of lean mass and bone content were significantly ($P \leq 0.01$) higher in AL animals than 60% AL pigs; bone mineral density did not differ between any of the treatments. Swine in the AL group were noticeably larger, with greater

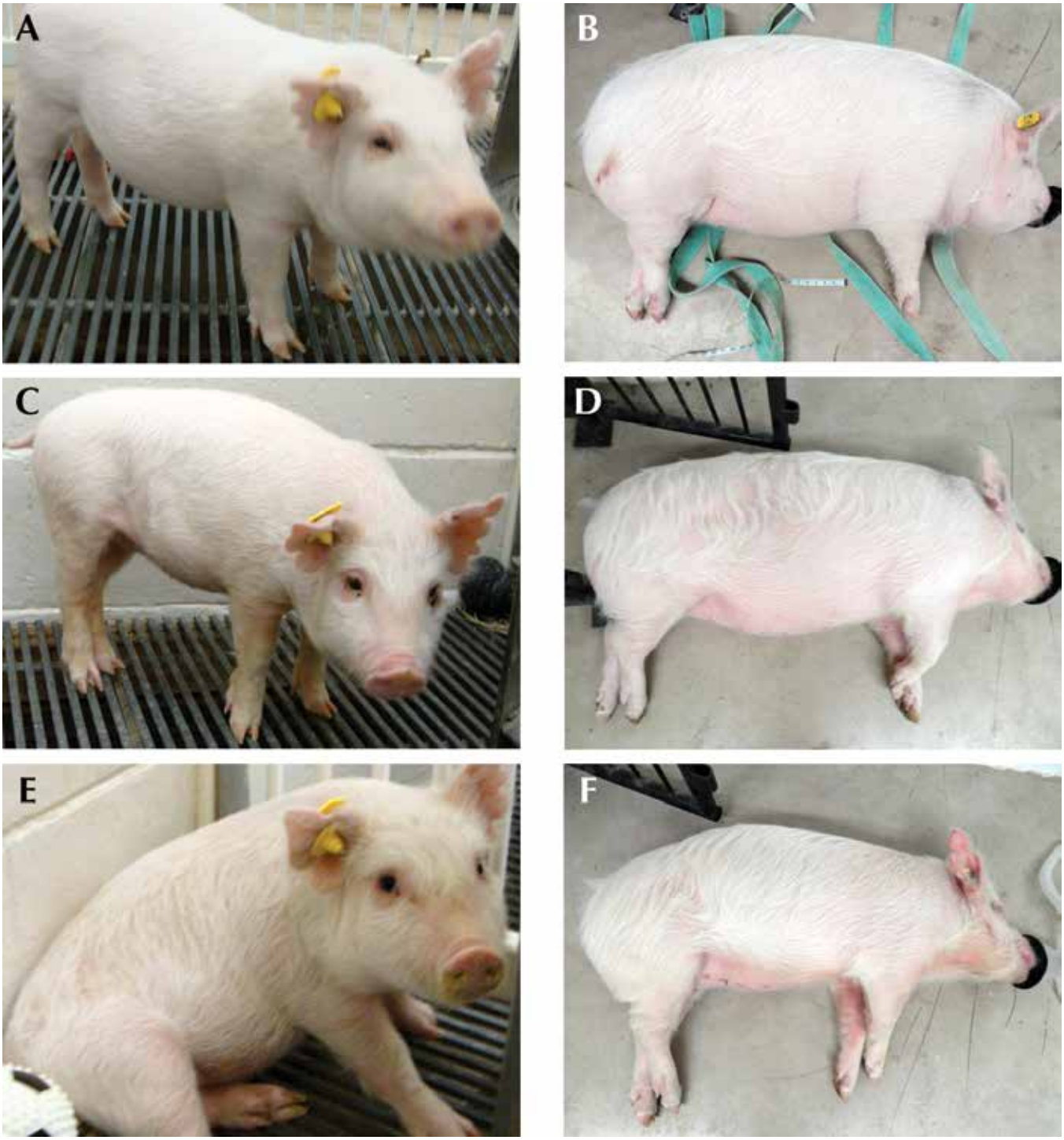

Figure 4. Images of animals from 3 treatment groups at start and end of 8.5 mo of treatment. Representative images of swine in treatment groups: AL (A and B), 80% AL (C and D), and 60% AL (E and F) diet groups.

accumulation of adipose tissue, than their 60% AL counterparts (Figures 2 through 5).

Serum cholesterol. All animals were hypercholesterolemic at the first time point (age, 2.6 mo) and had average serum cholesterol levels of 423 to 486 mg /dL by 9 mo of age. For comparison, total cholesterol levels in adult swine of the same breed but without the *LDLR* mutation are 81 to 103 mg/dL.[12] Average cholesterol values were higher ($P \leq 0.05$) in all AL and 80% AL swine than in the 60% AL group (Figure 6). From 2 to 8 mo of age, all groups had increased ($P \leq 0.05$) serum cholesterol compared with reference values for normal swine.[26]

Glucose tolerance and insulin sensitivity testing. At the completion of the study, IVGTT was conducted to assess glucose clearance and insulin sensitivity (Table 4). Analysis of raw data indicated that all animals displayed at least a 450 mg/dL increase in serum glucose after glucose injection (Figure 7). The 60% AL swine cleared glucose concentrations faster than AL and 80% AL swine. At 2 to 19 min after glucose challenge, the reduction in serum glucose concentration was greater ($P \leq 0.05$) in 60% AL swine when compared with 80% AL swine; this difference was even greater ($P \leq 0.05$) between 60% AL and AL swine. Compared with other groups, the 60% AL swine had

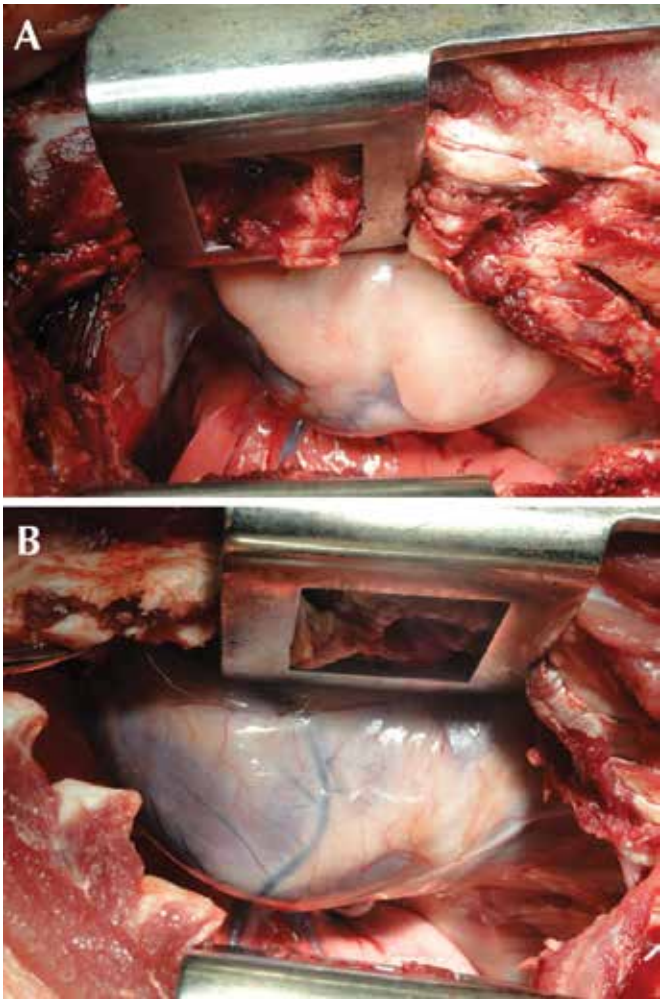

Figure 5. Representative images of heart tissue after 8.5 mo of treatment in (A) AL and (B) 60% AL diet groups. Well-defined pericardial fat is present in (A) AL but not (B) 60% AL swine.

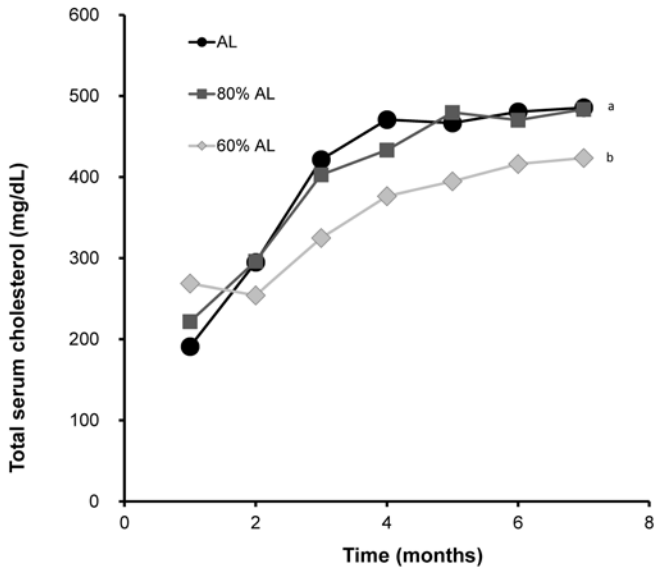

Figure 6. Serum cholesterol levels from baseline. Data are given as least-square means ± SEM (n = 6 per treatment group). At each time point, different letters indicate values that are statistically significant ($P \leq 0.05$).

faster ($P \leq 0.05$) clearance from 2 to 50 min after glucose injection. Glucose clearance was nonsignificantly slower in AL pigs compared with 80% and 60% AL swine.

Insulin sensitivity differed significantly ($P \leq 0.05$) between the AL and 60% AL groups (Figure 8). Serum insulin concentrations were elevated ($P \leq 0.05$) in AL-fed compared with 60% AL pigs at 40 and 50 min after insulin injection. In addition, serum insulin was higher ($P \leq 0.05$) in 80% AL pigs compared with the 60% AL pigs at 4 and 8 min after glucose injection and at 70 and 90 min after insulin injection. Apparent differences in insulin sensitivity between the AL and 80% AL groups were not statistically significant.

Discussion

The strong correlation between obesity and metabolic syndrome[8] makes animal obesity models potential candidates for metabolic syndrome models. As in the Ossabaw swine model, which has been used for obesity studies,[18,29,31,33] we demonstrated that caloric intake significantly influences the rate and magnitude of body weight gain in FH swine.

We used repeated whole-body DXA scans were used to measure the bone mineral density, fat content, and lean content of the 3 diet-treatment groups. These values then were used to calculate the percentage of fat relative to lean and bone mass.[17] Over the course of the study, caloric intake significantly influenced the percentage of body fat. These data demonstrate that AL swine reached a total body fat mass of 31.1%, whereas 80% AL pigs peaked at 24.3% fat, and 60% AL animals were 14.1% fat at the same age. Of importance, bone mass density was similar between groups, indicating the absence of stunting in skeletal growth due to differences in caloric intake. Within this short study, we found differences in animal body composition at young ages.

Definitions of obesity are often based on BMI data.[35] Because height is not a relevant variable for swine, we compared our data with body fat standards for adult human females as determined by DXA.[9] Mature women are similar in mass to female FH swine at the conclusion of the study (approximately 46 to 95 kg). By these definitions, the AL swine would be considered overfat, the 80% AL swine would be healthy to borderline overfat, and the 60% AL group would be underfat to borderline healthy.

Not only the presence of excess fat but also its location[16] are considered in the diagnosis of metabolic dysfunction. Ectopic visceral fat and belly fat have specifically been implicated[25] as symptomatic of metabolic disorder. We noted pericardial fat in the AL swine (Figure 5 A), but the 60% AL swine (Figure 5 B) had little or no fat surrounding the heart. These findings are in contrast to a previous study,[25] which did not find corresponding amounts of abdominal fat or pericardial fat in the obese Ossabaw swine model. This difference implies that FH swine may be a better model of metabolic dysfunction than the Ossabaw breed. More rigorous studies, including quantitative measures of pericardial and subcutaneous fat, are required to establish firm comparative conclusions. In the current study, waist circumference measurements did not yield conclusive results, perhaps because of poor accuracy and precision when obtaining the measurements. Alternatively, waist measurement may not be a relevant measure for swine.

Although blood pressure was monitored during all anesthetic events, the data were not presented here, because of anesthesia's effect on blood pressure.[15] Future work could investigate ways to repeatedly measure blood pressure in swine without using an anesthetic and without causing anxiety to the animal.

Serum cholesterol was studied as a marker of metabolic dysfunction. All swine were hypercholesterolemic at the start of the trial (2 wk after weaning), with total serum cholesterol

Table 4. Least-square means (± SEM) of IVGTT results at the conclusion of the study

	AL	80% AL	60% AL
Basal glucose, mg/dL	77.3 ± 4.3	86.5 ± 4.3	78.8 ± 4.3
Basal insulin, µU/mL	3.8 ± 1.2	2.9 ± 1.2	2.1 ± 1.2
Glucose clearance, mg/min	3.0 ± 0.4	3.6 ± 0.4	3.6 ± 0.4
Insulin sensitivity, (L/µU)/min	0.4 ± 0.5[a]	1.1 ± 0.5[a,b]	1.8 ± 0.5[b]
Glucose effect, /min	1.5 ± 0.3	1.7 ± 0.3	2.1 ± 0.3
Distributed glucose, mg/dL	350.9 ± 17.7	346.8 ± 17.7	315.5 ± 17.7

Different superscripted letters indicate groups that differ significantly ($P \leq 0.05$); no letter indicates no significant difference between groups.

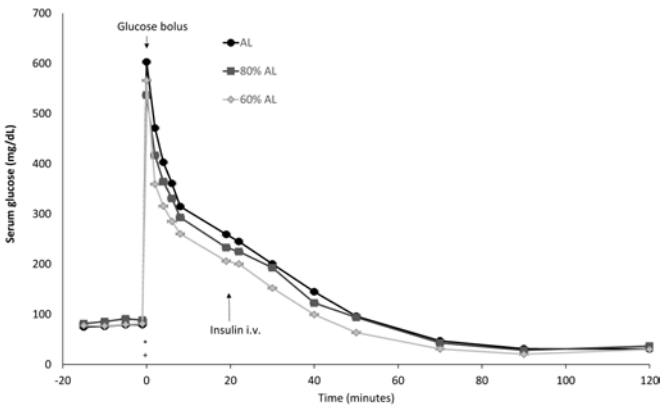

Figure 7. Average serum glucose concentration during IVGTT according to dietary treatment and time. Data are given as least-square means ± SEM (n = 6 per treatment group). Intravenous glucose (0.3 mg/kg BW) was administered at 0 min and insulin (0.3 U/kg BW) was injected intravenously at 20 min after the glucose injection. *, Significant ($P \leq 0.05$) difference between AL and 60% AL swine at every time point from 2 to 50 min after glucose injection; +, significant ($P \leq 0.05$) difference between 80% AL and 60% AL at 2 through 30 min.

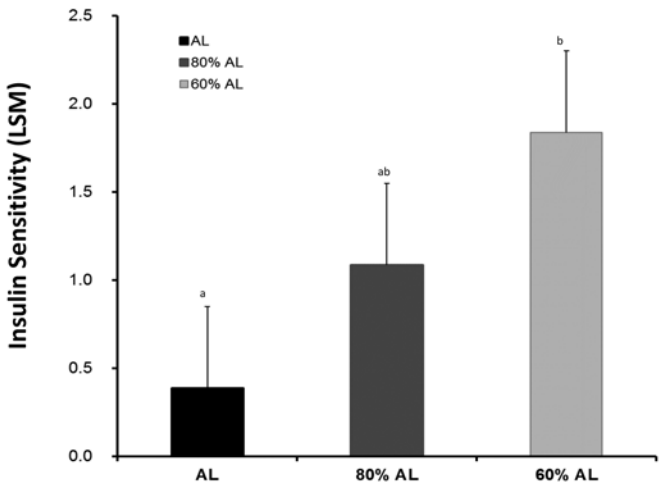

Figure 8. Insulin sensitivity determined through IVGTT. Data are given as least-square means ± SEM (n = 6 per treatment group). Insulin sensitivity differed significantly (a, $P \leq 0.05$) between AL and 60% AL.

concentrations above 240 mg/dL, as is expected for FH swine. The significantly higher serum cholesterol concentrations in 100% AL and 80% AL swine compared with 60% AL swine throughout the duration of the study may indicate a threshold in serum cholesterol responses to caloric intake. Our data suggest that greater than 20% caloric restriction is required to reduce serum cholesterol concentrations in FH swine. All animals had serum cholesterol concentrations between 423 and 486 mg/dL by 9 mo of age, indicating that although caloric restriction affected serum cholesterol levels, all animals remained hypercholesterolemic. Manipulation of caloric intake alone, with corresponding lower body fat, is insufficient to overcome the genetic predisposition (homozygous for mutation of the LDL receptor)[26] of the FH swine to become hypercholesterolemic.

As additional markers of metabolic dysfunction, glucose clearance and insulin sensitivity can be measured noninvasively through blood testing. In various conditions, such as obesity and type 2 diabetes mellitus, the cellular response to insulin becomes impaired and insulin resistance develops. To counteract increased resistance to insulin, the pancreas releases more insulin, creating systemic hyperinsulinemia. This situation impairs the metabolism of glucose and leads to glucose intolerance. When swine were 10.5 ± 0.6 mo of age, IVGTT indicated significant differences in serum glucose clearance profiles between the 80% AL and 60% AL groups, with the 80% AL swine demonstrating impaired clearance. However, the AL and 80% AL swine did not significantly differ in glucose clearance. Much like the cholesterol responses, the impaired glucose clearance responses across caloric restriction groups may be an indication of a threshold of fat mass or caloric intake at which metabolic abnormalities begin to arise. In response to the glucose and insulin challenges, AL swine showed 5-fold lower insulin sensitivity when compared with 60% AL. However, we expected to see a larger increase in insulin secreted during glucose challenge in AL animals. After our studies were completed, we learned that inhaled anesthesia, such as isoflurane, diminishes insulin responses after glucose administration.[7,28] Volatile anesthetics such as isoflurane have a direct inhibitory effect on insulin secretion from the pancreas.[6] Therefore, we speculate that the slight differences between diet groups in regard to insulin and glucose effects associated with IVGTT might have been greater in the absence of anesthesia. Given that most anesthetics have a glucoregulatory effect and because stress hormones can artifactually influence insulin secretion, further studies should be conducted to determine a more appropriate anesthesia method.

Dietary phytoestrogens can influence various symptoms of metabolic syndrome, including body weight, dyslipidemia, and glucoregulatory function.[4] Because soy is rich in phytoestrogens, it could be a compounding factor in our study. Although each diet had a different proportion of soybean meal included, the dietary restriction of the 80% AL and 60% AL treatments caused the actual mass of soy consumed to remain similar across all treatments.

In future studies, additional measures should be added to further support FH swine as a metabolic syndrome model with plasticity to respond to diet. These measures could include assessing adiponectin levels, LDL levels, triglycerides, and nonesterified fatty acids as well as quantifiable measures of pericardial and subcutaneous fat, and blood pressure.

With this study, we evaluated a known model of hypercholesterolemia and cardiovascular disease for its potential as a model of metabolic disease. We hypothesized that a dramatic change in body composition and adiposity in a relatively short time frame might alter glucose metabolism and insulin secretion responses. Our aim was to demonstrate that these changes could model the progression of metabolic dysfunction. In just over 8 mo, we demonstrated that body composition and weight in FH swine changed in response to caloric intake alone. We thus were able to establish pigs that were obese by human definition and that demonstrated symptoms of metabolic dysfunction. The obese condition is extremely important in the application of diabetes and metabolic research that focuses on long-term effects on human health. The current study highlights the importance of early intervention in weight control with the associated metabolic dysfunction and provides a model to study reversibility of these disease states later in life.

Most importantly, we demonstrated that FH swine, if sustained at 100% of unrestricted caloric intake, will develop differences from its leaner counterparts, much like the observed health status between lean and obese humans. This model should be studied in longer and more extensive trials to investigate additional implications of metabolic disease. The magnitude of the changes that occurred after a simple drop in caloric intake—and without the need for additional genetic modification—makes FH swine a valuable model for metabolic dysfunction research.

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