Comparative Medicine
Copyright 2006
by the American Association for Laboratory Animal Science

Vol 56, No 1
February 2006
Pages 35-45

Components of Metabolic Syndrome and Coronary Artery Disease in Female Ossabaw Swine Fed Excess Atherogenic Diet

Melissa C Dyson,[1] Mouhamad Alloosh,[4] James P Vuchetich,[4] Eric A Mokelke,[4] and Michael Sturek[1-4,*]

Ossabaw swine have a 'thrifty genotype' (propensity to obesity) that enables them to survive seasonal food shortages in their native environment. Consumption of excess kcal causes animals of the thrifty genotype to manifest components of the metabolic syndrome, including central (intra-abdominal) obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension. We determined whether female Ossabaw swine manifest multiple components of the metabolic syndrome by comparing lean pigs fed a normal maintenance diet (7% kcal from fat; lean, n = 9) or excess chow with 45% kcal from fat and 2% cholesterol (obese, n = 8). After 9 wk, body composition, glucose tolerance, plasma lipids, and intravascular ultrasonography and histopathology of coronary arteries were assessed. Computed tomography (CT) assessed subcutaneous and intra-abdominal fat deposition and was compared with traditional methods, including anatomical measurements, backfat ultrasonography, and proximate chemical composition analysis. Compared with lean animals, obese swine showed 2-fold greater product of the plasma insulin × glucose concentrations, 4.1-fold greater total cholesterol, 1.6-fold greater postprandial triglycerides, 4.6-fold greater low- to high-density lipoprotein cholesterol ratio, hypertension, and neointimal hyperplasia of coronary arteries. The 1.5-fold greater body weight in obese swine was largely accounted for by the 3-fold greater carcass fat mass. High correlation (0.79 to 0.95) of CT, anatomical measurements, and ultrasonography with direct chemical measures of subcutaneous, retroperitoneal, and visceral fat indicates high validity of all indirect methods. We conclude that relatively brief feeding of excess atherogenic diet produces striking features of metabolic syndrome and coronary artery disease in female Ossabaw swine.

Abbreviations: CT, computed tomography; FPLC, fast protein liquid chromatography; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment insulin resistance; IVGTT, intravenous glucose tolerance test; IVUS, intravenous ultrasonography; LDL, low-density lipoprotein

The metabolic syndrome is a cluster of risk factors that include central (intra-abdominal) obesity, insulin resistance, impaired glucose tolerance, dyslipidemia (low high-density lipoprotein [HDL] cholesterol, increased triglycerides), and hypertension, which increase the risk of developing coronary heart disease, stroke, and type 2 diabetes.[18,21,24,53] Although the definition is controversial,[36] generally the presence of 3 of these characteristics constitutes the metabolic syndrome.[24] The metabolic syndrome affects ≤27% of adults in the United States and continues to increase as obesity and lack of physical activity become commonplace in modern lifestyles.[21] Manifestation of the metabolic syndrome is predictable based on the 'thrifty genotype' hypothesis, which proposes that in the hunter–gatherer stages of human development, the ability to store excess fat enabled survival during periods of famine.[59] The classic example of this thrifty genotype is the Pima Indians in the southwestern United States. Historically these people were lean and active, but exposure to inactivity and Western diets have produced obesity in the majority of adults and the highest incidence of type 2 diabetes in the world.[48]

Received: 15 Jul 2005. Revision requested: 1 Dec 2005. Accepted: 3 Dec 2005.
Department of [1]Medical Pharmacology & Physiology, [2]Internal Medicine, and the [3]Center for Diabetes & Cardiovascular Health School of Medicine, University of Missouri, Columbia, Missouri; [4]Department of Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana.
[*]Corresponding author. Email: msturek@iupui.edu

Swine that were isolated on a barrier island off the coast of Georgia for approximately 500 y and have a thrifty genotype to endure seasonal cycles of feasting and famine provide a large animal model for humans with the metabolic syndrome.[52] Ossabaw Island swine allowed to consume excess food in captivity have the highest levels of total body lipid of any mammal,[7] decreased muscle mass and adult body size compared with those of domestic swine,[10,47] insulin resistance and impaired glucose tolerance,[73,74] hypertriglyceridemia, and hypercholesterolemia compared with that of lean Ossabaw and domestic swine.[19] These initial studies on the physiology of Ossabaw swine, conducted 20 to 30 y ago, were before recognition of the metabolic syndrome as an important health problem. We recently have conducted preliminary studies that confirm many of the complex metabolic syndrome characteristics of male Ossabaw swine,[16,17] thus providing a basis for studies on coronary artery disease, which is the most serious long-term complication of the metabolic syndrome.

One of the most compelling reasons for the use of a large animal model, such as swine, is that coronary atherosclerotic lesions in diabetic swine are virtually indistinguishable from lesions in humans.[22] This mimicry of coronary atherosclerosis in humans suggests that studies of cellular and molecular mechanisms in swine[28,57,68,72] could have the most relevance to human clinical medicine. Perhaps most important, the swine model is the most widely accepted for coronary interventions, such as stent

placement.[34] Although coronary disease is the leading killer of nondiabetic men, it has unusually devastating consequences in diabetic women. Specifically, a significant, 13% decrease in coronary heart disease mortality was achieved in diabetic men over a course of <10 y, but diabetic women experienced a 23% increase.[25] The increasing prevalence of the metabolic syndrome in women is equally astonishing, as shown by a 24% increase in women compared with only a 2% increase for men over a 10-y period.[21] Because the morbidity and mortality of coronary artery disease in women is so profound and the increase in metabolic syndrome in women in the U.S. is continuing virtually unabated, the need for development of animal models that mimic these diseases in women is compelling.

In this study we evaluated the effect of short-term and excess feeding of a diet high in trans fatty acids and cholesterol on female Ossabaw swine, with the overall purpose of optimizing the model for the study of coronary artery disease. Our primary aim was to produce the constellation of features of the metabolic syndrome in the naturally occurring Ossabaw swine model, rather than targeting one putative cause of the metabolic syndrome, as done in rodent models.[49] Because assessment of body composition is central to characterization of the metabolic syndrome, our second aim was to compare computed tomography (CT),[5] indirect anatomical measurements, and backfat ultrasonography with postmortem chemical analysis as the 'gold standard' for body composition evaluation.[8,32,67]

Materials and Methods

All experimental procedures involving animals were approved by the University of Missouri Animal Care and Use Committee and complied fully with the *Guide for the Care and Use of Laboratory Animals*[58] and the American Veterinary Medical Association Panel on Euthanasia.[4] For all surgical procedures and euthanasia, anesthesia was induced with atropine (0.05 mg/kg), tiletamine–zolazepam (5.0 mg/kg), and xylazine (2.2 mg/kg) given intramuscularly; anesthesia subsequently was maintained with isoflurane gas (≤4%). Pigs were euthanized by pneumothorax and cardiectomy while anesthetized.

Ossabaw swine, husbandry, and diet treatment. Female Ossabaw miniature swine (*Sus scrofa*) were bred at the University of Missouri (Columbia, MO). The swine herd has historically tested negative for *Brucella* spp, pseudorabies, vesicular stomatitis virus serovars Indiana and New Jersey, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus, porcine parvovirus, swine influenza virus serotypes H1N1 and H3N2, antibody levels to transmissible gastroenteritis virus, and *Leptospira interrogans* serovars (*canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, *pomona*, and *bratislava*). Pigs were housed in groups of 6 (providing 9 ft^2 per pig, as per reference 58) on a 12:12-h light:dark cycle.

Swine (age, 5 to 6 mo at the beginning of the study) were fed either a lean chow (lean, n = 9) or excess high fat–high cholesterol chow (obese, n = 8) for 9 wk. These n values pertain to all figures and tables, unless otherwise indicated. The lean diet was a maintenance feed for adolescent and adult swine that contained 21.6% of total kcal from protein, 70.9% from carbohydrates, and 7.4% from fat (S-11, Milbank Feeds, Chillicothe, MO). The main components were ground corn, soybean, and alfalfa supplemented with vitamins and minerals. This feed composition was consistent for the total duration of these studies, because the same lot was used. Animals in the lean group ate 1600 to 1900 kcal of feed per day. The high fat–high cholesterol feed was composed of chow supplemented with (percentage by weight) 2.0% cholesterol, 17.1% hydrogenated soybean oil, 2.3% corn oil, and 0.7% sodium cholate. This mixture yielded a composition of 17.0% of total kcal from protein, 37.7% from carbohydrates, and 45.3% from fat, similar to our previous studies,[6,15,77] except that coconut oil was replaced by hydrogenated soybean oil containing 56% trans fatty acids (#170, Columbus Foods, Chicago, IL). Pigs in the obese group ate approximately 3200 to 3800 kcal of feed over the approximately 6-h feeding period during which the pigs had free access to feed each day. Food consumption is stated as a reasonably narrow range, not an exact amount, because pigs were group-housed. As in virtually all nutrition studies, other components proportionally decrease when high fat and cholesterol are added to the diet. Despite this relative reduction on a per-weight basis, the approximately 2-fold greater amount of feed consumed provided 32 vitamins and minerals, and all essential amino acids remained within acceptable ranges for adequate adult swine nutrition. All animals were given free access to water.

Female Yucatan miniature swine (*Sus scrofa*) were bred at the Sinclair Research Center (Columbia, MO). The Sinclair Research Center is a licensed breeder with the U.S. Department of Agriculture (43-R-2499) and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The colony is free of brucellosis and pseudorabies. The breeding colony is routinely vaccinated for parvovirus, leptovirus, *Bordetella pertussis*, swine influenza virus, tetanus, and *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* and has quarterly treatment for parasite prevention by alternation between ivermectin and levamisole. Yucatan swine (n = 10) were compared with age- and weight-matched Ossabaw animals (n = 5) in a brief, 4-wk experiment in which pigs were fed atherogenic diet similar to that described earlier above, except that the fat was coconut oil containing 99% cis fatty acids (#550, Columbus Foods, Chicago, IL), the base chow was Purina Minipig chow (#5082, Purina Mills, St. Louis, MO), and the kcal provided supported normal, not excess, weight gain of Yucatans.[6,46,56] Body weights and subcutaneous fat measures (by ultrasonography, as described later) were obtained to compare the efficiency of adipogenesis ('thriftiness') of the breeds. Unlike those for lean and obese Ossabaw, no other data were collected on the Yucatan pigs during this 4-wk experiment, and the Yucatans then were used in another study.

Blood pressure measurements. From the beginning of the 9-wk study, pigs were acclimated to a low-stress sling[60,61] for conducting intravenous glucose tolerance tests (see below) and blood pressure measurements. Blood pressures were monitored weekly by using a tail cuff sphygmomanometer.[60]

Intravenous glucose tolerance test (IVGTT). Pigs were anesthetized with isoflurane, and right jugular veins were catheterized percutaneously.[11] Pigs were allowed to recover from anesthesia for at least 2 h before the IVGTT was done, to avoid isoflurane-induced decrease in insulin action.[60] Results from IVGTT 2 h and >24 h after isoflurane were not different, thus indicating no effect of brief isoflurane anesthesia. Pigs were placed in a low-stress restraint sling and blood samples were obtained at baseline (–5 and 0 min); animals then were given an intravenous bolus of 0.5 g glucose/kg body weight, and further samples were obtained at 5, 10, 20, 30, 40, 50, and 60 min after injection.[60] Blood glucose was measured on a YSI 2300 Stat Plus analyzer (YSI, Yellowsprings, OH). Plasma insulin levels were obtained by assays done at Linco Research Laboratories (St. Louis, MO) at 0, 5, 10, 20,

40 and 60 min after injection of glucose. The area under the curve was calculated with the baseline at 0 by using SigmaPlot (version 5.0, Jandel Scientific, Corte Madera, CA). The slope of the blood glucose decay was obtained from the log of sample values at 5 through 60 min. A modified homeostatic model assessment insulin resistance method (HOMA-IR; product of the plasma insulin × glucose concentrations) was used to evaluate insulin sensitivity during the IVGTT.[50,66,71]

Plasma lipids. Plasma samples were obtained the day after the IVGTT from conscious pigs by using the jugular catheter. Fasting samples were taken in the morning, and then all animals were fed 12.7 g/kg of high fat–high cholesterol feed, which was consumed in 1 h.[78] Postprandial blood samples were taken 7 h after feeding, which previously was shown to be the peak of the postprandial triglyceride response.[78] Fasting samples were analyzed for triglycerides and total cholesterol and were fractionated into HDL and low-density lipoprotein (LDL) components; postprandial samples were analyzed for triglyceride levels only. As previously described,[14,15] plasma was directly assayed for total cholesterol and triglyceride levels by using a standard enzymatic kit (Thermo Trace, Melbourne, Australia). Cholesterol in lipoprotein fractions was determined after precipitation of HDL by using minor modifications of standard methods.[75] Specifically, apolipoprotein B-containing lipoproteins were precipitated with heparin–$MnCl_2$ and then the same total cholesterol kit and method was used to measure HDL in the supernatant. The LDL level was calculated from the Friedewald equation: LDL = total cholesterol – HDL – (triglycerides/5). We validated the Friedewald equation for LDL cholesterol measurements in pigs by correlating the HDL cholesterol measures obtained by the precipitation method with direct measures of cholesterol in the HDL and LDL fractions that were isolated by gel filtration using fast protein liquid chromatography (FPLC). The precipitation method and FPLC (reference method) were compared using 37 samples having total cholesterol concentrations ranging from 40 to 859 mg/dl and triglyceride concentrations ranging from 7 to 376 mg/dl. Excellent correlation (*R* values) of 0.90 and 0.99 was found for HDL and LDL determined by the Friedewald equation, respectively, between the precipitation and FPLC methods.[12]

Body composition. After 9 wk, animals were evaluated with ultrasonography, CT, anatomical measurements, and proximate chemical analysis (after euthanasia). Ultrasound images were obtained by R. Disselhorst (Agricultural Extension, University of Missouri). While pigs were conscious and in the low-stress sling, images were obtained at locations over the dorsal midline at the 5th, 10th, and last rib for offline analysis of subcutaneous fat thickness. B-mode ultrasonography was performed using an Aloka 500-V ultrasound unit (Corometerics Medical Systems, Wallingford, CT). Two-dimensional images were obtained using a 12-cm 3.5-MHz probe. Offline analysis was performed using the ANIMORPH software program (Woods Hole Educational Associates, Woods Hole, MA) . These measures were averaged to yield a backfat thickness value.

CT scans were performed on a single-slice spiral CT machine (Picker PQ 6000, Picker International, Highland Heights, Ohio) at the University of Missouri College of Veterinary Medicine by using established methods.[5,8,23,32] Before transport to radiology, animals were anesthetized as described earlier. Animals were placed in ventral recumbency on the scanner table, and the abdomen was scanned from cranial to the right kidney to the wings of the ileum. Image slices were 20-mm thick and taken every 0.5 cm. CT images were analyzed using Image Pro Plus 4.0 (Media Cybernetics, Des Moines, IA). We analyzed 11 images in total: 1 at the midpoint at the first lumbar vertebrae (L1) and 5 images each cranial and caudal to this point. Fat in 3 compartments was measured: 1) subcutaneous fat present between the external body wall and internal abdominal muscular layer (transverse abdominal muscle and internal intercostal muscles); 2) retroperitoneal fat present between the internal abdominal muscle layer and external visceral boundary; and 3) visceral fat around organs and in the omentum, excluding air within the viscera.

Anatomical measurements used the apex of the sternum as an anatomical landmark and starting point for all subsequent circumference measurements from anesthetized pigs. The apex of the sternum was defined as the highest point of the breastbone when the pig was dorsally recumbent. Measurements were similar whether pigs were ventrally recumbent (as for CT measures) or dorsally recumbent, but measurements were obtained more conveniently when pigs were dorsally recumbent, because in vivo cardiovascular measures followed (see later description). From the apex of the sternum, the neck location was defined as 20 cm cranial along the central axis of the pig; the mid-abdomen location was defined as 30 cm caudal along the central axis of the pig. The widest girth circumference measurement was defined as the largest circumference measurement around the abdomen of the pig. A sketch illustrating the anatomical sites (Figure 1) is provided in reference 77.

Proximate chemical composition analysis was conducted after in vivo coronary artery procedures and euthanasia (described later). The body was separated into viscera, retroperitoneal tissue (lining the body cavity), and carcass (representing subcutaneous fat deposition). Each compartment was weighed and then ground with a large industrial meat grinder (25 HP, 155 rpm, and a ratio of 11.39 from an electric motor [Lincoln Motors, Cleveland, OH] with a motorreducer [Falk, Milwaukee, WI]). The resultant coarse mixture was further ground to a fine powder by using an industrial grade mixer–processor (two cutting blades, 12 HP, 3500 rpm, with a top stirrer; Hobart, Troy, OH). Samples were frozen and then submitted to the University of Missouri College of Agriculture Experiment Station Chemical Laboratories for quantitative proximate analysis according to standard Association of Analytical Chemists (AOAC) procedures.[30] Crude fat was determined from 2.0-g samples by using the gravimetric method (AOAC 954.02) with acid hydrolysis preceding the ether extraction. Crude protein was determined from 1.0-g samples by using the copper-catalyst Kjeldahl procedure (AOAC 984.13). Crude ash was determined from 2.0-g samples (AOAC 942.05). The moisture content was determined from 2.0-g freeze-dried samples by vacuum drying in an oven at 95 to 100 °C (AOAC 934.01).

Intravascular ultrasonography (IVUS) and histopathology of coronary arteries. After abdominal CT scans, anesthetized animals were moved to a cardiac catheterization laboratory and maintained on isoflurane gas anesthesia for IVUS as previously described.[56] For arterial access, an 8-French sheath was inserted into the femoral artery after surgical exposure. An 8-French Amplatz guiding catheter was inserted through the sheath and introduced into the aortic arch. The tip of the guiding catheter was placed near the ostium of the left main coronary artery. Several angiographic images were obtained to verify placement of a guidewire (Cordis, Miami, FL) and 3.2-French 30-MHz IVUS imaging catheter (Ultracross 3.2, Boston Scientific, Sunnyvale, CA) into the distal portion of the circumflex artery. An automated pullback

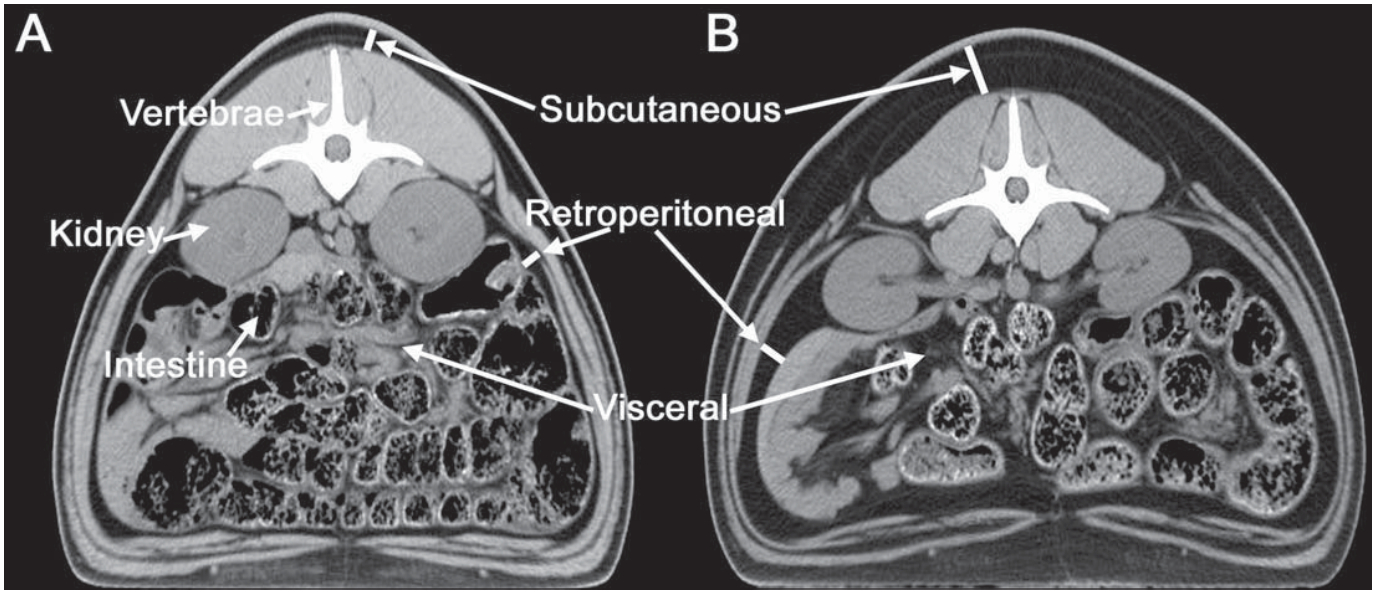

Figure 1. Computed tomography (CT) scan of adipose distribution. CT scans were at the midpoint of the kidneys, shown adjacent to the vertebrae, which is shown by the brightest intensity (most radiopaque). Air in the intestines is shown by the dark intensity (most radiolucent) in the images. The lean pig (panel A) has much less adipose tissue (subcutaneous, retroperitoneal, and visceral) than the obese animal (panel B).

device moved the imaging transducer longitudinally through the artery at 0.5 mm/sec to obtain continuous images ('pullback') of typically a 30- to 70-mm length of the circumflex and left anterior descending arteries.

The presence of atheroma was determined by first identifying the luminal area by the fine scintillations from red blood cells and larger scintillations or turbulence from injection of saline. Atheroma was defined as any fibrous or soft plaque immediately adjacent to the lumen, typically less echogenic than the adventitia, and separated from the adventitia by an echolucent middle layer.[29,45,56] This characteristic atheroma was easily resolved as distinct from the nonlayered appearance of arteries from control pigs. Atheroma was quantified as percent degrees atheroma, which was a modification of our previous methods.[45,56] Atheroma in 1 end-diastolic image for each 1-mm segment of the IVUS pullback was quantified as the total number of 'hours' of atheroma coverage per image, corresponding to the portion of the outer circle of a clock face (representing the interface of the vessel wall and the lumen) covered by atheroma. As little as 0.2 mm of atheroma (intimal thickening) could be resolved. This hour value of atheroma was multiplied by 30 (1 h represents 30° of a circle), divided by 360 (total degrees in circle), and multiplied by 100% to give a percent degrees atheroma value according to the equation:

$$\%\ \text{degrees of atheroma (in one segment)} = (\text{hour value} \times 30) / 360 \times 100\%$$

The percent degrees of atheroma in the artery was the average of all segments (30 to 70) in the pullback. After euthanasia, the coronary arteries were collected, fixed at physiologic pressure (100 mm Hg) in 10% phosphate-buffered formalin solution, paraffin-embedded, and sectioned for histologic analysis, similar to previous immunohistochemical analyses.[69] Histologic sections were made at the bifurcation of the circumflex and left anterior descending arteries,[56] treated with Verhoeff–van Gieson elastin stain, and evaluated. The intima-to-media ratio was calculated as a measure of neointimal thickening.

Statistical analyses. IVGTT data were analyzed with 2-way analysis of variance with repeated measures and Holm–Sidak post hoc analysis (SigmaStat, Jandel Scientific, Corte Madera, CA). Two-tailed unpaired Student *t* tests were used to compare means. Pearson correlation and linear regression analyses (Excel, Microsoft, Redmond, WA) were used for comparing the association of 2 variables. The data that were normally distributed were evaluated with *t* tests. When data failed a Shapiro–Wilk Normality test (heart rate, blood pressure, % atheroma, and coronary artery histology), we performed a Mann–Whitney U test (Analyse-it add-in software for Microsoft Excel, Analyse-it Software, Leeds, England) to determine significant differences. The threshold for statistical significance was $P = 0.05$. Several data sets lack entries due to unforeseen equipment malfunction (catheter failure and loss or destruction of images by the CT machine). These deficits were unrelated to treatment.

Results

Body composition. Starting body weight of lean and obese groups was not different (23.1 ± 2.5 kg versus 24.7 ± 1.2 kg), whereas final mean body weight of obese animals was 1.5-fold greater than that of lean animals, thus indicating the efficacy of the diet treatment on body mass (Table 1). The profound effect of excess high fat–high cholesterol feed on all subcutaneous (carcass), retroperitoneal, and visceral fat compartments can be seen in Figure 1A and B. Quantitative analysis showed obese pigs had greater fat stores in all compartments by all measures, including circumference, backfat ultrasonography, CT, and direct chemical analysis (Table 1). Because proximate chemical composition analysis is the most accurate, 'gold standard' method for assessing body composition[8,32,67] and because body weight did not differ between the 2 groups at the beginning of the study, we point out several comparisons of body composition on the basis of measures of percentage of fat and absolute fat and lean mass in Table 1. First, the percentage of fat in carcass, retroperitoneal, and visceral compartments was significantly increased ($P <$

Table 1. Body composition: circumference, ultrasound, computed tomography, and analytical chemistry analysis measures

	Lean pigs	Obese pigs
Body weight (kg)	29.4 ± 0.9	44.5 ± 1.5*
Circumference (cm)		
Neck	53.7 ± 1.5	56.2 ± 1.2
Midabdomen	72.5 ± 1.7	90.6 ± 1.4*
Widest girth	72.8 ± 1.5	90.8 ± 1.7*
Ultrasound (mm/kg body weight)		
Backfat	0.29 ± 0.02	0.56 ± 0.04*
Computed tomography of fat (cm^2/kg)		
Subcutaneous	5.79 ± 0.55	9.94 ± 0.74*
Retroperitoneal	2.25 ± 0.32	3.31 ± 0.29*
Visceral	0.77 ± 0.04	2.07 ± 0.13*
Analytical chemistry analysis of fat (% of sample)		
Carcass	18.76 ± 0.72	37.50 ± 1.09*
Retroperitoneal	70.56 ± 2.34	81.44 ± 1.50*
Visceral	13.9 ± 1.00	21.04 ± 0.95*
Fat mass (kg)		
Carcass	4.58 ± 0.22	13.79 ± 0.81*
Retroperitoneal	0.20 ± 0.03	1.01 ± 0.15*
Visceral	0.44 ± 0.04	0.98 ± 0.08*
Lean mass (kg)		
Carcass	5.73 ± 0.24	6.57 ± 0.21*
Retroperitoneal	0.013 ± 0.001	0.028 ± 0.004*
Visceral	0.45 ± 0.02	0.57 ± 0.01

*, Statistically significant ($P < 0.05$) versus value for animals on lean diet.

0.05) 2.0-, 1.2-, and 1.5-fold, respectively, in obese compared with lean groups. Second, the absolute mass of fat (weight × fractional composition) was increased 3.0-, 5.0-, and 2.2-fold in carcass, retroperitoneal, and visceral compartments, respectively, of obese animals. Third, absolute lean mass (protein plus ash) increased only 1.1-fold in the carcass compartment (with minor changes in viscera and retroperitoneal), thus indicating minimal effect of excess high fat–high cholesterol feed on lean mass. Overall, pigs did not gain intra-abdominal (retroperitoneal and visceral) fat to a greater magnitude than carcass (subcutaneous) fat.

Further verification of the greater efficiency of adipogenesis ('thriftiness') of Ossabaw (n = 5) compared with Yucatan (n = 10) female pigs was shown in a 4-wk experiment in which animals were fed the normal amount (not excess) of atherogenic diet and backfat was measured with ultrasonography. Body weights did not differ at the end of this short-term study, but the backfat-to-body weight ratio of Ossabaws (0.037 ± 0.004) was 5-fold greater than that of Yucatans (0.0073 ± 0.0006). This result also indicates a predominant increase in body fat compared with lean mass in Ossabaw pigs.

Anatomical circumference, ultrasound, and CT measurements were obtained to assess the validity of noninvasive measures compared with direct chemical measures to estimate body fat compartments in female Ossabaw miniature swine. Linear regression analyses (Table 2) were performed to assess the predictive power of the noninvasive measures. Almost all of the noninvasive measures were significantly ($P < 0.05$) correlated with the amount of fat in the carcass sample (Table 2). However, the strength of the association as indicated by the R value shows that body weight, midabdomen and widest girth circumferences, and CT have the best predictive power ($R = 0.92$, 0.94, 0.95, and 0.87, respectively). Neck circumference, consistent with the nonsignificant increase in the obese pigs, had the lowest and nonsignificant predictive value in the estimation of carcass fat. Because neck circumference and backfat ultrasonography cannot image retroperitoneal and visceral fat compartments, we considered the correlations less relevant and therefore did not include these correlations with chemical composition analysis in Table 2. Overall they were not strong predictors of retroperitoneal or visceral fat (not shown). Similar to the results of regression analyses with the carcass fat, all of the noninvasive measurements were significantly ($P < 0.05$) correlated with the percentage of retroperitoneal fat. For visceral fat, the noninvasive measures with the strongest predictive value were again the midabdominal and widest girth circumference measurements ($R = 0.91$), followed by CT ($R = 0.85$).

Predictive equations. On the basis of the strength of the R value, the single measure that best predicts the percentage of carcass fat is widest girth circumference ($R = 0.95$) and the percentages of retroperitoneal fat and visceral fat is midabdominal girth circumference ($R = 0.87$ and 0.91, respectively). We propose that the following equations derived from the regression analyses can be used for predicting the percentages of carcass, retroperitoneal, and visceral fat by using a single noninvasive measure:

$$\% \text{ carcass fat} = 1 \times (\text{widest girth circumference in cm}) - 53.25$$

$$\% \text{ retroperitoneal fat} = 0.71 \times (\text{midabdominal circumference in cm}) + 19.82$$

$$\% \text{ visceral fat} = 0.44 \times (\text{midabdominal circumference in cm}) - 18.$$

Glucose tolerance and insulin resistance. Obese and lean pigs had similar glucose tolerance (Figure 2A). An exception might be the higher peak plasma glucose at the 5-min sample time in obese compared with lean animals (Figure 2A). However, administration of glucose per kg body weight, instead of per kg lean mass, tends to yield a higher exposure of glucose to metabolically active

Table 2. Linear regression analyses for noninvasive measures versus invasive chemical composition analysis of percentage carcass fat, retroperitoneal fat, and visceral fat

Noninvasive measure	Chemical composition analysis of % fat					
	Carcass		Retroperitoneal		Visceral	
	$P < 0.05$?	R	$P < 0.05$?	R	$P < 0.05$?	R
Body weight (kg)	yes	0.92	yes	0.73	yes	0.83
Circumference (cm)						
Neck	no	0.41	not done	not done	not done	not done
Midabdomen	yes	0.94	yes	0.87	yes	0.91
Widest girth	yes	0.95	yes	0.86	yes	0.91
Ultrasonography (mm/kg body weight)						
Back	yes	0.84	not done	not done	not done	not done
Computed tomography (cm^2/kg)						
Subcutaneous	yes	0.87	not done	not done	not done	not done
Retroperitoneal	not done	not done	yes	0.80	not done	not done
Viscera	not done	not done	not done	not done	yes	0.85

R, correlation coefficient.

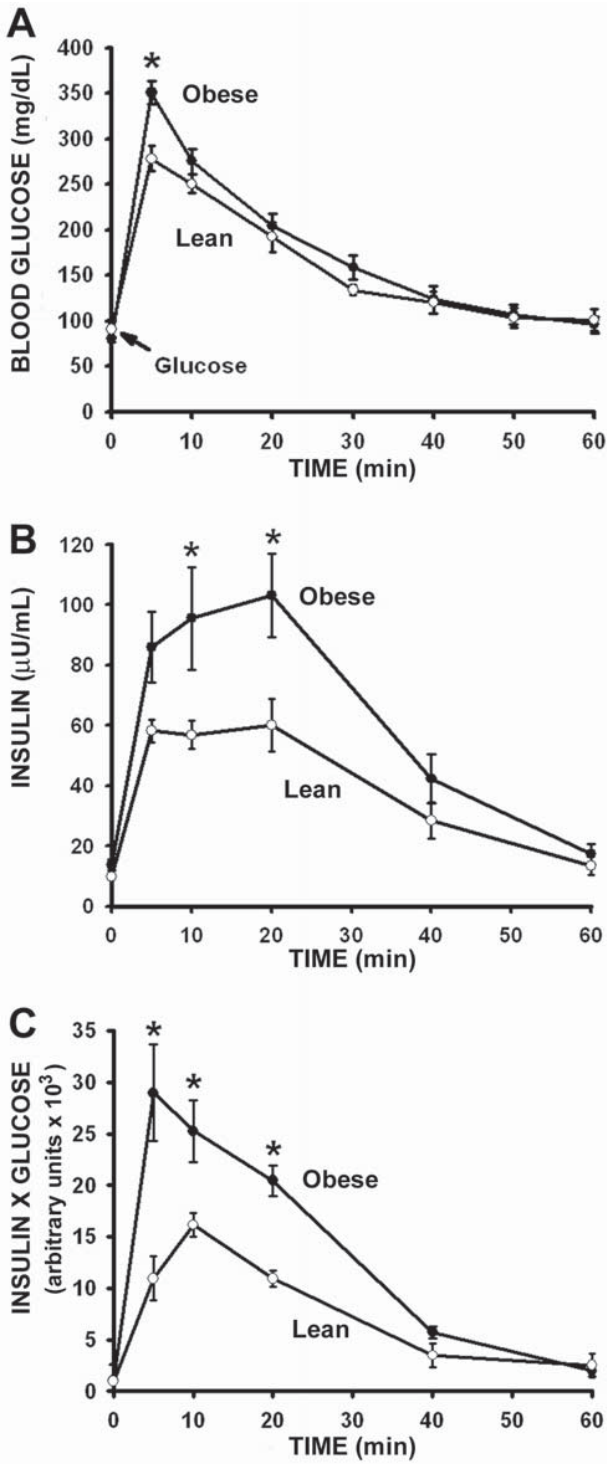

Figure 2. Intravenous glucose tolerance tests. (A) Blood glucose response to intravenous glucose challenge. Baseline, fasting blood glucose was obtained before infusion of glucose (0.5 g/kg) at time 0. Obese pigs (▨, n = 7) showed 26% greater peak response compared with that of lean pigs (■, n = 7; *, $P < 0.05$) only 5 min after glucose infusion. (B) Plasma insulin response. Obese pigs (▨, n = 7) showed 70% greater response compared with that of lean pigs (■, n = 7; *, $P < 0.05$) at 10 and 20 min after infusion. (C) Product of blood glucose × plasma insulin for determination of insulin sensitivity. Values were greater for obese pigs (▨, n = 7) than lean pigs (■, n = 7; *, $P < 0.05$).

tissue (primarily muscle) in obese compared with lean pigs. The equations provided for predicting lean body mass noninvasively will permit the administration of glucose per kg lean mass in future studies. Glucose returned to baseline by 60 min postinjection in both groups, and there were no significant differences in area under the glucose curve or slope of the descending portion of the glucose curve between obese and lean pigs (data not shown). Obese pigs were able to normalize glucose levels within the same time span and at the same rate (indicated by the slope of glucose curve) as lean animals. Obese pigs demonstrated insulin resistance compared with lean animals, as obese animals had higher plasma insulin levels at 10 and 20 min than did lean animals, and obese animals had a greater area under the insulin curve (3811; μUnits insulin × time in minutes) than lean animals (2579). Figure 2B shows that elevated insulin levels during an IVGTT in the obese swine reached statistical significance ($P < 0.05$) at the 10- and 20-min time points. The modified HOMA-IR values from the IVGTT showed that the insulin-×-glucose level was higher in obese pigs at 5, 10, and 20 min (Figure 2C). These data show that compared with lean animals, obese pigs released significantly more insulin to normalize blood glucose levels and thus they are relatively insulin-resistant.

Blood pressure. Compared with lean animals, pigs on the high fat–high cholesterol diet developed mild hypertension midway through the study. In the obese swine, systolic blood pressure was 137 ± 5 mm Hg and diastolic blood pressure was 92 ± 9 mm Hg, compared with 113 ± 8 and 70 ± 4 mm Hg, respectively, in the lean animals. In addition, mean arterial pressure in obese swine (107 ± 7 mm Hg) was significantly higher ($P < 0.05$) compared with that in lean swine (79 ± 3 mm Hg).

Plasma lipids. Pigs on the high fat–high cholesterol diet developed an atherogenic (dyslipidemic) lipid profile. Obese animals had significantly increased fasting total cholesterol, LDL, and LDL:HDL ratio but no change in HDL. Fasting and postprandial triglycerides were greater in obese compared with lean animals (Table 3).

Coronary artery disease. Obese pigs developed early neointimal hyperplasia of coronary arteries. Intravascular ultrasound analysis of both the left anterior descending and circumflex arteries showed increased percentage degrees of atheroma in obese animals compared with lean animals (Figure 3B). Histopathologic analysis of coronary arteries showed increased neointimal area as a percentage of the medial area in obese compared with lean pigs, although this increase did not reach statistical significance ($P < 0.10$; Figure 3D). Consistent with adiposity being a strong risk factor for coronary artery disease,[53] percentage fat in all adipose compartments strongly predicted atheroma quantified by intravascular ultrasonography as shown by significant correlation coefficients (R, $P < 0.05$) of 0.85, 0.65, and 0.70 for carcass, retroperitoneal, and visceral compartments, respectively.

Discussion

The disproportionate 24% increase in prevalence of the metabolic syndrome in women compared with the 2% increase in men[21] and the 23% increase in coronary heart disease mortality in diabetic women compared with the 13% decrease in diabetic men[25] underscore a important health problem and the compelling need for development of animal models for understanding these diseases in women. In addressing our overall purpose of optimizing the female Ossabaw miniature swine model for the study of the metabolic syndrome and coronary artery disease, we

Table 3. Plasma lipids

	Lean pigs	Obese pigs
Cholesterol (mg/dl)		
Total	156.6 ± 13.4	479.0 ±46.7*
LDL	104.2 ± 13.2	430.8 ±48.0*
HDL	44.9 ± 3.8	39.5 ±2.4
LDL:HDL	2.5 ± 0.3	11.4 ±1.8*
Triglycerides (mg/dl)		
Fasting	37.6 ± 1.6	50.5 ±6.8*
Postprandial	80.2 ± 7.4	128.4 ±13.5*

All lipid measures were taken from samples after fasting, except for postprandial triglycerides. Data are presented as mean ± standard error.
LDL, low-density lipoprotein; HDL, high-density lipoprotein.
*, $P < 0.05$ versus lean animals.

have made several noteworthy contributions. By excess feeding of a high fat–high cholesterol diet to female Ossabaw swine for a relatively brief (9-wk) period, we induced 1) substantial obesity, 2) primary insulin resistance, 3) hypertriglyceridemia and increased LDL:HDL ratio, and 4) mild hypertension and coronary artery disease. We also used indirect methods for measurement of percentage of body fat. We found that female Ossabaw swine have 5 of the 6 characteristics of the metabolic syndrome.[24]

Our 1st contribution, induction of substantial obesity, is documented clearly with proximate chemical composition analysis, which is the 'gold standard' of body composition measures.[8,32,67] Although the 'thrifty genotype' propensity to obesity was noted in Ossabaw swine more than 3 decades ago,[47] the comparison with our studies of Yucatan miniature swine is remarkable.[6,77] In the present study, the relative increase in fat in obese Ossabaw pigs was 2-fold greater than the increase in body weight, whereas the relative increase in percentage fat in obese Yucatan pigs was almost identical to the increase in body weight.[77] In addition, although there was not a greater increase in intra-abdominal versus subcutaneous fat, there was a striking, preferential increase in fat mass versus lean mass in Ossabaw pigs (Table 1, Figure 1). Female Göttingen swine on a high fat–high calorie diet increased fat percentage 1.5-fold over lean controls in 5 wk,[33] whereas female Ossabaw swine increased carcass fat percentage 2-fold over lean controls in 9 wk. Considering the different diets, body composition measures, and other factors, the findings are in reasonable agreement but cannot be rigorously compared. Our 4-wk experiment comparing subcutaneous fat thickness clearly indicates a greater efficiency of adipogenesis ('thriftiness') of Ossabaw compared with Yucatan female swine, but we have no such comparison with Göttingen swine. The amount of fat deposition does not necessarily dictate functional properties of the depots, but documentation of these stores is an essential first step in understanding the complex 'adipokines' that underlie the metabolic syndrome and cardiovascular disease.[44,51]

A 2nd contribution from our study is the indication of primary insulin resistance in obese pigs, as evident by hyperinsulinemia (1.7-fold greater than that in lean pigs) at glucose levels similar to control pigs during an IVGTT (Figure 2B). It is striking that insulin resistance was elicited by obesity or excess high fat–high cholesterol feeding in just 9 wk, as this outcome was not achieved in several attempts using male Yucatan pigs over 20- to 40-wk feeding periods.[6,17,60,77] The Yucatan studies included an atherogenic diet group that consumed 1.5-fold greater kcal and increased body weight 1.33-fold greater than that of lean controls after 20 wk of treatment;[6,77] however, insulin resistance was not observed in those mildly obese Yucatan pigs. We used the simpler IVGTT method to screen Ossabaw female pigs as a potential animal model, but we emphasize that 'gold standard' measures such as the hyperinsulinemic–euglycemic clamp[60] should be used to draw broadly definitive conclusions. Phillips and colleagues induced primary insulin resistance in Yucatans, but doing so required almost 10 y of selective breeding[61-63] and, unfortunately, that genetic line no longer exists. Collectively, these findings suggest that Yucatan pigs currently available are a leaner breed of pig, that is, not of the 'thrifty genotype'.

The explanation for our success in achieving primary insulin resistance more rapidly in the present Ossabaw study could be due to several other factors in addition to the genetics of Ossabaws, specifically, greater obesity, female gender, or use of trans fatty acids in the diet. Greater obesity may be linked closely with female gender and also may explain why the study of the Göttingen pig yielded significant obesity and insulin resistance in just 5 wk of excess high fat–high cholesterol feeding;[33] thus, our comparison of male Yucatan pigs versus female Ossabaw swine is done cautiously. Trans fatty acids are the result of the hydrogenation process of unsaturated vegetable oils, such as soybean oil. In the Yucatan studies, coconut oil that was naturally 99% saturated with cis fatty acids was used.[6,17,60,77] Some studies in humans have shown that a diet high in trans fatty acids may be linked to a greater risk of type 2 diabetes in women.[65]

The negligible impairment of glucose tolerance in this other studies may point to an important difference between pigs and humans. Overall, swine have a greater tolerance for glucose challenge, given both orally and intravenously, than humans. The Göttingen minipig's beta cell mass relative to body weight is almost twice as high as that in humans. This difference could indicate a greater insulin secretory reserve compared with humans.[41] However, previous studies have found impaired glucose tolerance in male Ossabaw swine compared with Yucatan and large domestic swine.[17,73] Although our study did not induce impaired glucose tolerance, this fact could simply mean that longer diet studies will be needed in these large animals. Indeed, this need for more time would be entirely consistent with the pathogenesis of type 2 diabetes in humans, as Kahn's influential review[35] clearly indicates that humans may have insulin resistance and compensatory hyperinsulinemia that maintains normal glucose tolerance for >10 y before impaired glucose tolerance occurs.

The trans fatty acid-rich diet in our study also may partially explain our 3rd contribution—demonstration of hypertriglyceridemia and increased LDL:HDL (Table 3). The increased fasting triglyceride concentration is consistent with that found in grossly obese male Ossabaw swine fed a normal chow diet.[19] It is very important to note that increased fasting triglycerides have rarely been found in other swine after consumption of a high fat–high cholesterol diet,[33] unless the swine also are made grossly diabetic by destruction of the insulin-producing cells with diabetogenic toxins.[14,15,22,55,78] Given that the lean and obese Ossabaw groups both consumed the high fat–high cholesterol meal for the postprandial lipemia test, the increased postprandial triglycerides in the obese pigs are also consistent with impaired lipid metabolism in the metabolic syndrome. In human studies, trans fatty acids increase LDL cholesterol and lower HDL cholesterol, alter cardiovascular function, and are correlated with increasing the risk of heart disease.[2,13,54] The astoundingly large LDL:HDL ratio of 11.4 in our study far exceeds any previously reported for swine and is probably due to the lack of increase in HDL that typically occurs after high fat–high cholesterol feeding.[14,15,22,31,33,42,55,78] It

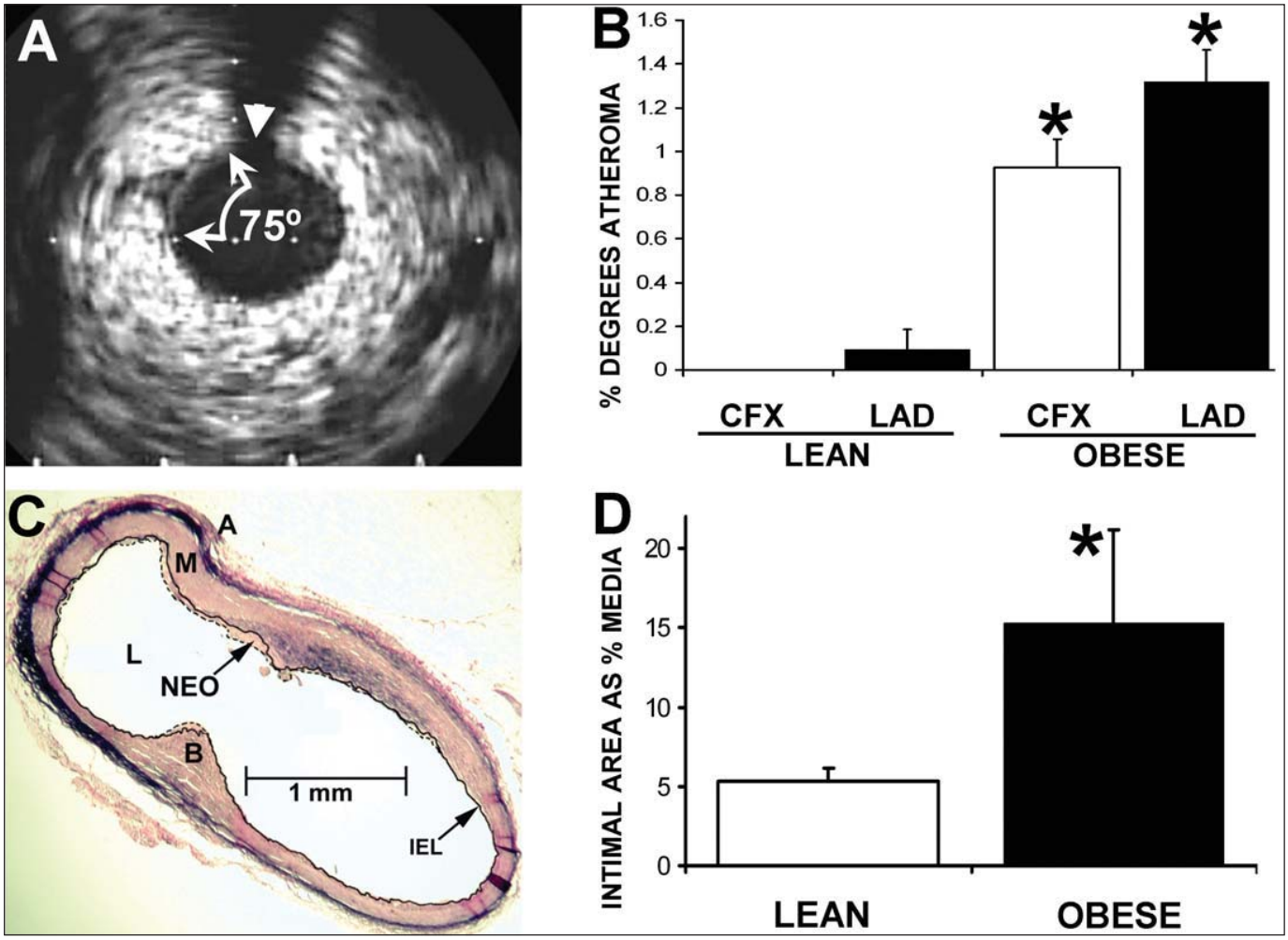

Figure 3. Coronary atherosclerosis. (A) Intravascular ultrasound (IVUS) image. Regions of neointima in the 2-dimensional image are shown by the thickened '3-layer' appearance as degrees of a circle, with 75° shown in this artery. The IVUS catheter is in the center of the image; dots emanating from the center of the IVUS catheter are spaced at 1 mm. The arrowhead shows dropout of the ultrasound image because of guidewire artifact. (B) Percentage degrees of atheroma for the obese group (n = 7) was greater (*, $P < 0.05$) in the circumflex (CFX; open bar) and left anterior descending artery (LAD; solid bar) compared with respective arteries in the lean group (n = 6). (C) Histologic cross-section with Verhoeff–van Gieson stain of the left coronary artery at the bifurcation into the circumflex and left anterior descending artery. A, adventitia; M, media; IEL, internal elastic lamina (delineated by solid line); NEO, neointima (area between solid and dotted lines); L, lumen; B, bifurcation. (D) Neointimal area quantified as percentage of the media showed a trend toward being greater (*, $0.05 < P < 0.10$) in the obese (n = 7) compared with lean group (n = 8).

also should be noted that the LDL:HDL ratio is probably due to a combination of gender and trans fatty acid effects, because a preliminary report on trans fatty acid feeding in male Yucatan and Ossabaw pigs shows more modest LDL:HDL ratios of approximately 4.[27]

We have shown that the LDL:HDL ratio is highly predictive of coronary artery disease,[14] thus implying a highly atherogenic profile in these female Ossabaw swine. Indeed, because cardiovascular disease is the leading cause of mortality in diabetics, the mild hypertension and early coronary atherosclerosis noted in just 9 wk of diet treatment (Figure 3) is our 4th noteworthy contribution, because it provides a basis for studies on therapeutic interventions. Despite the insights provided by rodent models of the metabolic syndrome,[49,76] rodents are not suitable for coronary interventions, such as stent placement.[34] These aforementioned effects induced by obesity and atherogenic diet certainly would be more apparent in a longer, chronic study using Ossabaw swine. Further, we acknowledge that it was not possible to evaluate the separate effects of obesity and atherogenic diet on components of the metabolic syndrome and cardiovascular disease in the present study. There is a compelling need for future studies on this topic.

Our 5th contribution was a main purpose of the study—assessing the validity of indirect measures of body fat compartments in female Ossabaw swine. Increases in intra-abdominal fat stores are a key component in the constellation of factors associated with the metabolic syndrome. Fat is a metabolically active tissue that has endocrine and paracrine functions, and bioactive products of adipose tissue, called adipokines, influence body composition, energy metabolism, lipid metabolism, cardiovascular function, inflammation, and coagulation. These factors include, but are not limited to, leptin, cortisol, tumor necrosis factor α, interleukin 6, 11β-hydroxysteroid dehydrogenase 1, and adiponectin.[20,44,51] Hence, there is great need for accurate measures of adipose distribution in animal models. In particular, a noninvasive technique

that accurately predicts body fat percentage in swine models for biomedical research would enable serial measurements throughout a study, thus minimizing the number of animals used.

The results from this study validate the use of noninvasive body weight, body circumference, ultrasound, and CT measures for the estimation of subcutaneous (carcass), retroperitoneal, and visceral fat percentage in adult (age, 7 to 8 mo) female Ossabaw swine. This finding is very important because although the validity of noninvasive measures has clearly been established for commercial swine,[64] predictions of body fat from noninvasive measures are not the same for miniature feral swine.[67]

It is interesting that the noninvasive measures were more predictive of adipose compartments in Ossabaws than Yucatans.[77] This difference most likely is due to the profoundly greater, almost exclusive, increase in body weight due to fat mass, rather than lean mass, in Ossabaws. This pattern is different from that of Yucatans, which shows comparable increases in lean and fat mass on a high fat–high cholesterol diet. The use of circumference measurements has several distinct advantages over ultrasound and CT techniques, including the minimal cost in purchasing a tape measure, the ability to readily repeat measurements, and the ability to perform measurements in living, conscious animals. However, there are 2 main advantages of the CT scan. First, the ability to conduct extensive post-hoc digital image analysis of the different compartments is virtually unlimited. Second, the use of CT scan to identify structures, in combination with positron emission tomography or magnetic resonance imaging measures of local metabolism, is powerful.

Other important large animal models of metabolic syndrome include Göttingen swine, fat-fed dogs, macaques and baboons, and llamas and alpacas. Göttingen swine have been shown to develop obesity, insulin resistance, and dyslipidemia when fed high-fat diets.[31,33,39,40,42,43] Socially reared bonnet macaques fed commercial monkey chow were shown to have increased abdominal diameter, insulin resistance, and elevated serum lipids.[37] These findings were seen in animals beginning at 3 to 4 y of age and continued to increase in incidence throughout groups of increasing age. Prenatally androgenized male rhesus monkeys were shown to exhibit insulin resistance and impaired insulin secretion.[9] Wild baboons with access to human garbage were found to have high insulin levels, obesity, insulin resistance, and hyperlipidemia. These clinical signs were not seen in wild baboons with low leptin levels.[3] The fat-fed dog model has been shown to develop both subcutaneous and visceral adiposity and insulin resistance.[26,38,70] During glucose tolerance tests, llamas and alpacas were found to have naturally higher blood glucose concentration than do humans and other mammals and insulin resistance compared with humans.[1] Swine are often the large animal model of choice for studies of diabetes and cardiovascular disease, because of their anatomical and physiologic similarities to human beings, compared with other large animal species including dogs or ruminants. Nonhuman primates have many similarities to humans in certain aspects of anatomy and physiology, but there are distinct disadvantages to using them as animal models, including small body size, potential for aggressive temperament and injury to staff, zoonotic disease risk, and negative public perception of their use in research.

This study is the 1st to show that female Ossabaw swine fed excess atherogenic diet develop several features of the metabolic syndrome and resulting coronary artery disease. Although Ossabaw swine seem ideally suited to the study of these diseases, we again emphasize that several other large animal models should be noted. It is predicted that canine models will have minimal coronary atherosclerosis due to their lower triglycerides, total cholesterol, and LDL:HDL ratio. Therefore, further comparison of large animal models should provide keen insights into cardiovascular complications in the metabolic syndrome.

Acknowledgments

M Dyson was a candidate for the Masters of Science degree in Veterinary Pathobiology and was supported by a National Institutes of Health Comparative Medicine Training Grant (no. T32 RR07004). We thank members of our animal support staff—Robert Boullion, Jerilyn Giles, and James Wenzel—for exemplary care and maintenance of Ossabaw swine; James Vuchetich for anesthesia; Meifang Wang for technical assistance; the School of Veterinary Medicine, University of Missouri, for access to the Research Animal Angiography Laboratory; R Disselhorst of the Agricultural Extension for backfat ultrasound measures; JK Waters and TP Mawhinney and staff of the Agriculture Experiment Station for proximate analyses; MN Zafar for initial intravascular ultrasound analysis; and CD Hardin for careful review of the manuscript. This work was supported by National Institutes of Health grants RR13223 and HL62552 and by American Diabetes Association grants to M Sturek. Contact M Sturek regarding the potential availability of Ossabaw swine.

References

1. **Araya AV, Atwater I, Navia MA, Jeffs S.** 2000. Evaluation of insulin resistance in two kinds of South American camelids: llamas and alpacas. Comp Med **50:**490–494.
2. **Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC.** 1994. Trans-fatty acids intake and risk of myocardial infarction. Circulation **89:**94–101.
3. **Banks WA, Altmann J, Sapolsky RM, Phillips-Conroy JE, Morley JE.** 2003. Serum leptin levels as a marker for a syndrome X-like condition in wild baboons. J Clin Endocrinol Metab **88:**1234–1240.
4. **Beaver BV, Reed W, Leary S, McKiernan B, Bain F, Schultz R, Bennett BT, Pascoe P, Shull E, Cork LC, Francis-Floyd R, Amass KD, Johnson R, Schmidt RH, Underwood W, Thornton GW, Kohn B.** 2001. 2000 Report of the AVMA panel on euthanasia. J Am Vet Med Assoc **218:**669–696.
5. **Borkan G, Gerzof S, Robbins A, Hults D, Silbert C, Silha J**. 1982. Assessment of abdominal fat content by computed tomography. Am J Clin Nutr **36:**172–177.
6. **Boullion RD, Mokelke EA, Wamhoff BR, Otis CR, Wenzel J, Dixon JL, Sturek M.** 2003. Porcine model of diabetic dyslipidemia: insulin and feed algorithms for mimicking diabetes in humans. Comp Med **53:**42–52.
7. **Brisbin IL, Jr.** 1990. A consideration of feral swine (*Sus scrofa*) as a component of conservation concerns and research priorities for the Suidae. Frädrich-Jubiläumsband **18:**283–293.
8. **Brodie DA.** 1988. Techniques of measurement of body composition. Sports Med **5:**11–40.
9. **Bruns CM, Baum ST, Coleman RJ, Eisner JR, Kemnitz JW, Weindruch R, Abbott DH.** 2004. Insulin resistance and impaired insulin secretion in prenatally androgenized male rhesus monkeys. J Clin Endocrinol Metab **89:**6218–6223.
10. **Buhlinger CA, Wangsness PJ, Martin RJ, Ziegler JH.** 1978. Body composition, *in vitro* lipid metabolism and skeletal muscle characteristics in fast-growing, lean and in slow-growing,obese pigs at equal age and weight. Growth **42:**225–236.
11. **Carroll JA, Daniel JA, Keisler DH, Matteri RL.** 1999. Non-surgical catheterization of jugular vein in young pigs. Lab Anim **33:**129–133.
12. **Cole TG, Nowatzke WL, Sturek M, Dixon JL.** 2004. Clinical assays for human lipoproteins are suitable for analysis of HDL-C and LDL-C in diabetic swine plasma. Clin Chem **50:**A189.

13. **de Roos NM, Bots ML, Katan MB.** 2001. Replacement of dietary saturated fatty acids by trans fatty scids lowers serum HDL cholestrol and impairs endothelial function in healthy men and women. Arterioscler Thromb Vasc Biol **21:**1233–1237.
14. **Dixon JL, Shen S, Vuchetich JP, Wysocka E, Sun G, Sturek M.** 2002. Increased atherosclerosis in diabetic dyslipidemic swine: protection by atorvastatin involves decreased VLDL triglycerides but minimal effects on the lipoprotein profile. J Lipid Res **43:**1618–1629.
15. **Dixon JL, Stoops JD, Parker JL, Laughlin MH, Weisman GA, Sturek M.** 1999. Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. Arterioscler Thromb Vasc Biol **19:**2981–2992.
16. **Dyson M, Mokelke EA, Vuchetich J, Sturek M.** 2005. Use of computed tomography to evaluate intra-abdominal fat stores in a swine model of the metabolic syndrome. FASEB J **19:**A191.
17. **Dyson MC, Alloosh M, Boullion RD, Mokelke EA, Sturek M.** 2004. Glucose intolerance and insulin resistance in Ossabaw compared to Yucatan swine. FASEB J **18:**A1224.
18. **Eckel RH, Grundy SM, Zimmet PZ.** 2005. The metabolic syndrome. Lancet **365:**1415–1428.
19. **Etherton TD, Kris-Etherton PM.** 1980. Characterization of plasma lipoproteins in swine with different propensities for obesity. Lipids **15:**823–829.
20. **Fasshauer M, Paschke R.** 2003. Regulation of adipocytokines and insulin resistance. Diabetologia **46:**1594–1603.
21. **Ford ES, Giles WH, Mokdad AH.** 2004. Increasing prevalence of the metabolic syndrome amoung U.S. adults. Diabetes Care **27:**2444–2449.
22. **Gerrity RG, Natarajan R, Nadler JL, Kimsey T.** 2001. Diabetes-induced accelerated atherosclerosis in swine. Diabetes **50:**1654–1665.
23. **Grauer W, Moss A, Cann C, Goldberg H.** 1984. Quantification of body fat distribution in the abdomen using computed tomography. Am J Clin Nutr **39:**631–637.
24. **Grundy SM, Brewer HB, Cleeman JI, Smith SC, Jr, Lenfant C.** 2004. Definition of the metabolic syndrome. Circulation **109:**433–438.
25. **Gu K, Cowie CC, Harris MI.** 1999. Diabetes and decline in heart disease mortality in U.S. adults. J Am Med Assoc **281:**1291–1297.
26. **Hall JE, Brands MW, Zappe DH, Dixon WN, Mizelle HL, Reinhart GA, Hildebrandt DA.** 1995. Hemodynamic and renal responses to chronic hyperinsulinemia in obese, insulin-resistant dogs. Hypertension **25:**994–1002.
27. **Henderson KK, Rector RS, Liu Y, Thomas T, Laughin MH, Sturek M.** 2004. Trans fat increases total/HDL cholesterol ratio in a porcine model of cardiovascular disease. FASEB J **18:**A563.
28. **Hill BJF, Dixon JL, Sturek M.** 2001. Effect of atorvastatin on intracellular calcium uptake in coronary smooth muscle cells from diabetic pigs fed an atherogenic diet. Atherosclerosis **159:**117–124.
29. **Hodgson JM, Reddy KG, Suneja R, Nair RN, Lesnefsky EJ, Sheehan HW.** 1993. Intracoronary ultrasound imaging: correlation of plaque morphology with angiography, clinical syndrome and procedural results in patients undergoing coronary angioplasty. J Am Coll Cardiol **21:**35–44.
30. **Horwitz W, editor.** 2005. Official methods of analysis of AOAC International. Gaithersburg (MD): AOAC International. p 1–2200.
31. **Jacobsson L.** 1989. Comparison of experimental hypercholesterolemia and atherosclerosis in male and female mini-pigs of the Gottingen strain. Artery **16:**105–117.
32. **Jebb SA, Elia M.** 1993. Techniques for the measurement of body composition: a practical guide. Int J Obes **17:**611–621.
33. **Johansen T, Hansen HS, Richelsen B, Malmlof R.** 2001. The obese Gottingen minipig as a model of the metabolic syndrome: dietary effects on obesity, insulin sensitivity, and growth hormone profile. Comp Med **51:**150–155.
34. **Johnson GJ, Griggs TR, Badimon L.** 1999. The utility of animal models in the preclinical study of interventions to prevent human coronary artery restenosis: analysis and recommendations. Thromb Haemost **81:**835–843.
35. **Kahn CR.** 1994. Insulin action, diabetogenes, and the cause of type II diabetes. Diabetes **43:**1066–1084.
36. **Kahn R, Buse J, Ferrannini E, Stern M.** 2005. The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia **48:**1684–1699.
37. **Kaufman D, Smith EL, Gohil BC, Banerji M, Coplan JD, Kral JG, Rosenblum LA.** 2005. Early appearance of the metabolic syndrome in socially reared bonnet macaques. J Clin Endocrinol Metab **90:**404–408.
38. **Kim S, Ellmerer M, Van Citters G, Bergman R.** 2003. Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate fat diet. Diabetes **52:**2453–2460.
39. **Larsen MO, Elander M, Sturis J, Wilken M, Carr RD, Rolin B, Porksen N.** 2002. The conscious Gottingen minipig as a model for studying rapid pulsatile insulin secretion in vivo. Diabetologia **45:**1389–1396.
40. **Larsen MO, Juhl CB, Porksen N, Gotfredsen CF, Carr RD, Ribel U, Wilken M, Rolin B.** 2005. Beta-cell function and islet morphology in normal, obese, and obese beta-cell mass-reduced Gottingen minipigs. Am J Physiol Endocrinol Metab **288:**E412–E421.
41. **Larsen MO, Rolin B.** 2004. Use of the Gottingen minipig as a model of diabetes with special focus on type 1 diabetes research. ILAR J **45:**303–313.
42. **Larsen MO, Rolin B, Wilken M, Carr RD, Svendsen O.** 2002. High-fat high-energy feeding impairs fasting glucose and increases fasting insulin levels in the Gottingen minipig: results from a pilot study. Ann N Y Acad Sci **967:**414–423.
43. **Larsen MO, Wilken M, Gotfredsen CF, Carr RD, Svendsen O, Rolin B.** 2002. Mild streptozotocin diabetes in the Göttingen minipig. A novel model of moderate insulin deficiency and diabetes. Am J Physiol Endocrinol Metab **282:**E1342–E1351.
44. **Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S.** 2005. Adipokines: molecular links between obesity and atherosclerosis. Am J Physiol Heart Circ Physiol **288:**H2031–H2041.
45. **Lee DL, Wamhoff BR, Katwa LC, Reddy HK, Voelker DJ, Dixon JL, Sturek M.** 2003. Increased endothelin-induced Ca^{2+} signaling, tyrosine phosphorylation, and coronary artery disease in diabetic dyslipidemic swine are prevented by atorvastatin. J Pharmacol Exp Ther **306:**132–140.
46. **Liu Y, Rector RS, Thomas TR, Taylor JA, Holiman DA, Henderson KK, Welshons WV, Sturek M.** 2004. Lipoproteins during the estrous cycle in swine. Metabolism **53:**140–141.
47. **Martin RJ, Gobble JL, Hartsock TH, Graves HB, Ziegler JH.** 1973. Characterization of an obese syndrome in the pig. Proc Soc Exp Biol Med **143:**198–203.
48. **Marx J.** 2002. Unraveling the causes of diabetes. Science **296:**686–689.
49. **Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS.** 2001. A transgenic model of visceral obesity and the metabolic syndrome. Science **294:**2166–2170.
50. **Mather KJ, Hunt AE, Steinburg HO, Paradisi G, Hook G, Katz A, Quon MJ, Baron AD.** 2001. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. J Clin Endocrinol Metab **86:**5457–5464.
51. **Matsuzawa Y, Nakamura T, Shimomura I, Kotani K.** 1995. Viseral fat accumulation and cardiovascular disease. Obes Res **5(3 Suppl):**645S–647S.
52. **Mayer JJ, Brisbin IL, Jr.** 1991. Wild pigs of the United States: their history, morphology, and current status. Athens (GA): University of Georgia Press. 313 p.
53. **McGill HC, Jr, McMahan A, Herderick EE, Zieske AW, Malcom GT, Tracy RE, Strong JP, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group**. 2002. Obesity accelerates the progression of coronary atherosclerosis in young men. Circulation **105:**2712–2718.
54. **Mensink RP, Zock PL, Kester AD, Katan MB.** 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholestrol and on serum lipids and apolipoprotein: a meta-analysis of 60 controlled trials. Am J Clin Nutr **77:**1146–1155.

55. **Mokelke EA, Dietz NJ, Eckman DM, Nelson MT, Sturek M.** 2005. Diabetic dyslipidemia and exercise affect coronary tone and differential regulation of conduit and microvessel K^+ current. Am J Physiol Heart Circ Physiol **288:**H1233–H1241.
56. **Mokelke EA, Hu Q, Song M, Toro L, Reddy HK, Sturek M.** 2003. Altered functional coupling of coronary K^+ channels in diabetic dyslipidemic pigs is prevented by exercise. J Appl Physiol **95:**1179–1193.
57. **Natarajan R, Gerrity RG, Gu JL, Lanting L, Thomas L, Nadler JL.** 2002. Role of 12-lipoxygenase and oxidant stress in hyperglycemia-induced acceleration of atherosclerosis in a diabetic pig model. Diabetologia **45:**125–133.
58. **National Research Council.** 1996. Guide for the care and use of laboratory animals. Washington(DC): National Academy Press. 119 p.
59. **Neel JV.** 1962. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet **14:**353–362.
60. **Otis CR, Wamhoff BR, Sturek M.** 2003. Hyperglycemia-induced insulin resistance in diabetic dyslipidemic Yucatan swine. Comp Med **53:**53–64.
61. **Panepinto LM, Phillips RW, Westmoreland NW, Cleek JL.** 1982. Influence of genetics and diet on the development of diabetes in Yucatan miniature swine. J Nutr **112:**2307–2313.
62. **Phillips RW, Panepinto LM, Spangler R, Westmoreland N.** 1982. Yucatan miniature swine as a model for the study of human diabetes-mellitus. Diabetes **31:**30–36.
63. **Phillips RW, Westmoreland N, Panepinto L, Case GL.** 1982. Dietary effects on metabolism of Yucatan miniature swine selected for low and high glucose utilization. J Nutr **112:**104–111.
64. **Realini CE, Williams RE, Pringle TD, Bertrand JK.** 2001. Gluteus medius and rump fat depths as additional live animal ultrasound measurements for predicting retail product and trimmable fat in beef carcasses. J Anim Sci **79:**1378–1385.
65. **Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC.** 2001. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr **73:**1019–1026.
66. **Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP.** 2005. Identification of individuals with insulin resistance using routine clinical measurements. Diabetes **54:**333–339.
67. **Stribling HL, Jr, Brisbin L, Sweeney JR, Stribling LA.** 1984. Body fat reserves and their prediction in two populations of feral swine. J Wildl Manag **48:**635–639.
68. **Suzuki LA, Poot M, Gerrity RG, Bornfeldt KE.** 2001. Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. Diabetes **50:**851–860.
69. **Turk JR, Carroll JA, Laughlin MH, Thomas TR, Casati J, Bowles DK, Sturek M.** 2003. C-reactive protein correlates with macrophage accumulation in coronary arteries of hypercholesterolemic pigs. J Appl Physiol **95:**1301–1304.
70. **Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald G, Georges M, Andersson L.** 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature **425:**832–836.
71. **Wallace TM, Levy JC, Matthews DR.** 2004. Use and abuse of HOMA modeling. Diabetes Care **27:**1487–1495.
72. **Wamhoff BR, Dixon JL, Sturek M.** 2002. Atorvastatin treatment prevents alterations in coronary smooth muscle nuclear Ca^{2+} signaling associated with diabetic dyslipidemia. J Vasc Res **39:**208–220.
73. **Wangsness PJ, Martin RJ, Gahagan JH.** 1977. Insulin and growth hormone in lean and obese pigs. Am J Physiol **233:**E104–E108.
74. **Wangsness PJ, Martin RJ, Gatchel BB.** 1980. Insulin-induced growth hormone response in fast-growing, lean and in slow-growing, obese pigs. Growth **44:**318–326.
75. **Warnick G, Benderson J, Albers JJ.** 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of HDL cholesterol. Clin Chem **28:**1379–1388.
76. **Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernstrom M, Rezaei K, Lee SJ, Koch LG, Britton SL.** 2005. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science **307:**418–421.
77. **Witczak CA, Mokelke EA, Boullion RD, Wenzel J, Keisler DH, Sturek M.** 2005. Noninvasive measures of body fat percentage in male Yucatan swine. Comp Med **55:**445–451.
78. **Witczak CA, Sturek M.** 2004. Exercise prevents diabetes-induced impairment in superficial buffer barrier in porcine coronary smooth muscle. J Appl Physiol **96:**1069-1079.