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Swine Disease Models for Optimal Vascular Engineering

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Keywords

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Abstract

Swine disease models are essential for mimicry of human metabolic and vascular pathophysiology, thereby enabling high-fidelity translation to human medicine. The worldwide epidemic of obesity, metabolic disease, and diabetes has prompted the focus on these diseases in this review. We highlight the remarkable similarity between Ossabaw miniature swine and humans with metabolic syndrome and atherosclerosis. Although the evidence is strongest for swine models of coronary artery disease, findings are generally applicable to any vascular bed. We discuss the major strengths and weaknesses of swine models. The development of vascular imaging is an example of optimal vascular engineering in swine. Although challenges regarding infrastructure and training of engineers in the use of swine models exist, opportunities are ripe for gene editing, studies of molecular mechanisms, and use of swine in coronary artery imaging and testing of devices that can move quickly to human clinical studies.

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1. INTRODUCTION

The worldwide epidemic of obesity, metabolic syndrome (MetS; also known as prediabetes and insulin resistance syndrome), and type 2 diabetes (T2D) has prompted the focus on these diseases in this review. The burden is tremendous, because obesity is the major driver of progression to T2D and, by the year 2030, half of the adults in the United States will be obese (1). MetS and diabetes increase the risk for coronary artery disease (2, 3). Despite advances in care, coronary artery disease continues to be the primary cause of mortality worldwide and accounts for one-seventh of deaths in the United States (4). A better understanding of the complexity of the underlying mechanisms of coronary disease is essential to develop therapies to prevent, treat, and reverse the disease.

Animal models are pivotal to our understanding of human vascular biology. The past ~15–20 years have seen a transition to overdependence on mouse models, which make up 95% of all animal studies (5). Furthermore, 98% of animal research funded by the National Institutes of Health employs rodents, mainly mice, and the small number of funded grants using large animals decreased by 30% from 2002 to 2006 (5). Unfortunately, there have been numerous failures to translate findings in mice to human clinical medicine. This review considers major strengths and weaknesses of miniature swine models of MetS and T2D, provides examples of swine model utility for coronary artery disease research and translation to the clinic, and considers future directions for research.

2. TRANSLATION OF BIOMEDICAL ENGINEERING RESEARCH FROM BENCH TO BEDSIDE

True translation of biomedical research from bench to bedside, or from basic science to clinical relevance in an academic medical center, requires an appreciation of and intensive research on biological models at multiple levels of complexity (**Figure 1**). This first translational level of bench to bedside, or T1, must occur before T2, which is widespread clinical practice in primary care, health education, and so forth. Work must progress from the medicinal chemist doing molecular modeling to cell culture and organ culture; to simple animal models such as *Caenorhabditis elegans*; to mouse, rat, rabbit, dog, sheep, pig, and nonhuman primate; and finally to humans (i.e., to the bedside). Translational research is broadly defined as the application of discoveries from research,

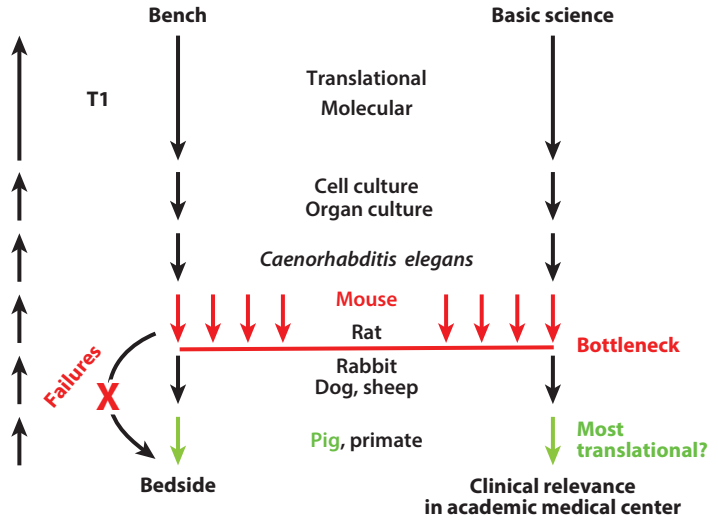

Figure 1

Pathway for bench-to-bedside translation from animal models to human clinical medicine. The downward arrows represent the flow of research from the molecular level to multiple levels of complexity and integrative animal models that most closely mimic humans. The upward arrows at left represent the influence of clinical medicine on the formulation of new hypotheses at the basic bench level. Abbreviation: T1, translational level 1.

including lab bench work, animal studies, and so forth, to the development of clinical studies in humans. Our perspective is that this broad definition implies degrees of translational research, with swine research being much more translational than, for example, the medicinal chemist's modeling of binding sites on molecules that target the diseased vasculature. The numerous failures in translation of promising findings in mouse models to human clinical medicine have created a substantial bottleneck of knowledge (**Figure 1**). This bottleneck has occurred largely because of technology for gene editing in mice, which caused an explosion of research. Perhaps the most striking example of the bottleneck and the failure of translation is that among genes changing significantly in humans in response to inflammation, mapping between mouse and human orthologs is nearly random (6).

Clearly, research at all levels and the use of multiple animal models can contribute to a translational pipeline, but the use of models that might mimic human diseases, such as swine, is lacking overall. We argue that clinical medicine must drive the formulation of new hypotheses and provide relevance to studies at the basic bench level (**Figure 1**). In other words, research needs to go from bench to bedside and back to the bench. For example, the use of a 10-fold increase in glucose above normal fasting levels (e.g., 900 mg/dL) for extended periods in monolayer cell culture to mimic uncontrolled diabetes is simply not compatible with life, not to mention that it has minimal relevance to diabetic vascular complications. Such glucose levels over chronic periods have been virtually nonexistent for almost a century, since before the discovery of insulin. Clinical research data must also drive gene editing in swine models (more preferably, all animal models). Whole-genome sequencing of human patients who have profound metabolic and/or vascular disease has identified the key, naturally occurring mutations that should have the highest priority for editing in animals. A classic example in humans is the profound alterations of plasma low-density lipoprotein (LDL) and atherosclerosis in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) mutation (7). The failures to translate and the bottleneck might be minimized if relevant pig models more closely mimicking humans are thoroughly studied and appreciated.

Most importantly, swine models present a nearly ideal opportunity to achieve integration and translation to human clinical medicine—in essence, to bridge the gap between simpler models (e.g., rodents) and humans. The need for a large-animal (i.e., swine) model of the metabolic syndrome, instead of rodent models, has been poignantly asserted. For example, in his article titled "Resistin: Yet Another Adipokine Tells Us That Men Are Not Mice," Arner (8) points out that many studies on resistin in small animals do not translate well to humans. Despite the significant advances in mouse models of diabetes and vascular disease that have been facilitated by the Animal Models of Diabetic Complications Consortium (9), the use of animal models in translational research requires large-animal models such as swine to be complete. In another example of failure to translate, all 125 therapeutics for β cell replacement that were successful in mouse models of type 1 diabetes failed in human clinical studies over a 5-year period through 2011 (10). All of these treatments would have required safety testing in large animals (e.g., dog, pig), but not efficacy, before human clinical studies. The desire to move a safe compound or device into humans to accelerate potential treatment of human disease is laudable, but it must be weighed against the cost and time of failed human clinical trials. An example of failure to translate vascular disease therapy is angiotensin receptor blocker inhibition of restenosis. This strategy met with ample success in rat models (11) but with failure in both pig (12) and human (13) coronary artery restenosis. In summary, despite the overwhelming success of therapy in mice, swine disease models seem more likely to correctly predict failure, and hopefully success, of therapy in humans.

Figure 2 illustrates the natural development of MetS and T2D in humans (14). Normal glucose tolerance and insulin sensitivity progress to insulin resistance and impaired glucose tolerance, which are among the criteria for MetS. At the time of impaired fasting glucose, the diagnosis of

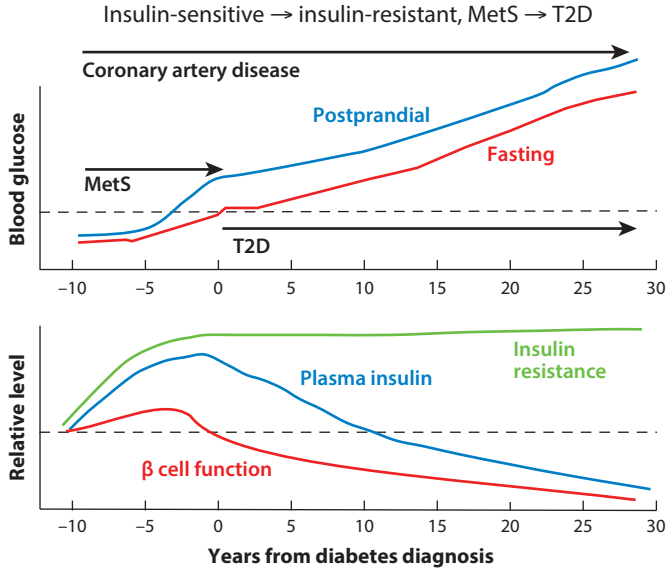

Figure 2

Time course of pathogenesis of MetS, T2D, and coronary artery disease in humans. The dashed line in the upper blood glucose graph represents the threshold level for clinical diagnosis of T2D. The dashed line in the lower graph represents normal levels for the parameters. The rightward arrows represent the continuum of the pathogenesis and the duration of MetS, T2D, and coronary artery disease. Abbreviations: MetS, metabolic syndrome; T2D, type 2 diabetes.

diabetes, specifically T2D, is made (15). MetS is characterized by compensatory hyperinsulinemia, but upon significant loss of pancreatic β cell function fasting hyperglycemia occurs and worsens until the person becomes insulin dependent after several decades. **Figure 2** shows glucose levels expressed in relative units over the years since diabetes diagnosis. Relatively normal glycemia is maintained in the MetS phase, even when the patient has a postprandial glucose influx challenge (or an oral or intravenous glucose tolerance test in the clinic). The MetS period can persist for 10 or more years before a diabetes diagnosis is made by pancreatic β cell expansion and compensatory hyperinsulinemia to compensate for the target organ's steadily increasing insulin resistance. Crucially, coronary artery disease is developing throughout the duration.

Natural models of disease that arise due to adaptation of animals to unique selection pressures can give insights into similarly complex, multifactorial diseases in humans. A prominent hypothesis, supported by wide genome scans for susceptibility genes, is that MetS and resulting cardiovascular disease are complex, polygenic diseases (16). Indeed, a single gene among those interacting in the pathogenesis of obesity and T2D has only a modest impact (1). According to the thrifty genotype hypothesis, in the hunter-gatherer stage of human development the ability to store excess fat enabled survival during periods of famine (17). Pima Indians in the southwestern United States represent the classical example of the thrifty genotype. Historically, these people were lean and active, but exposure to inactivity and Western diets has led to obesity in the majority of adults and the highest incidence of T2D in the world (16). The thrifty genes that allowed rapid accumulation of fat depots were a beneficial adaptation in earlier human cultures and in some animal species, but they are detrimental in the modern era of plentiful food sources and minimal physical activity. Furthermore, extreme inactivity and poor diet, which are now commonplace in modern lifestyles, amplify the manifestation of the thrifty genotype in people from most ethnic backgrounds, further increasing obesity-associated MetS abnormalities (1) and coronary artery disease (2) in the United States (18). Key components of the pathologies of MetS are central (intra-abdominal) obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension. Although the definition and precise clinical utility of MetS have recently been controversial, the presence of three of these characteristics generally prompts a diagnosis of MetS (19).

3. SWINE MODELS OF METABOLIC SYNDROME AND DIABETES

3.1. Advantages of the Use of Swine Models

As we move forward in translating basic research, the primary rationale for using a swine model (or any animal model) is the animal model's overall striking similarities to humans.

3.1.1. Behavior, especially sedentary. Pigs strongly prefer to remain sedentary, if permitted. In contrast, dogs pace their cages and rodents spontaneously run several kilometers per night (20). Overall, pigs are intelligent and gregarious, facilitating husbandry.

3.1.2. Lipoprotein profile. Pigs are omnivorous and monogastric, and their metabolism of foodstuffs (21), specifically lipoprotein metabolism (22–24), is similar to that of humans. The pig has a lipoprotein distribution similar to that of humans (25) and, on a low-fat, low-cholesterol diet, carries 50–60% of plasma total cholesterol (TC) in LDL particles (22, 23, 26). In contrast, rabbits and mice carry almost all of their cholesterol in very low density lipoprotein (VLDL) and high-density lipoprotein (HDL) particles (27), and dogs carry five- to sevenfold more cholesterol in HDL than in LDL (28).

3.1.3. Ample tissue. As Roberts et al. (5) stated regarding the use of farm animals in research: "Size matters." Although some researchers consider pigs' large size a challenge for husbandry (29), their human-like size (~40–80 kg for miniature swine) is an advantage for adequate blood sampling volumes (typically >20 mL/sample), instrumentation, and longitudinal measures and tissue biopsies in the same pig (30).

3.1.4. Measurement of cardiovascular parameters. For studies of exercise training, the maximum oxygen consumption (V_{O2max})/kg body weight of pigs is similar to that of humans (31, 32), as are the cardiovascular adaptations to exercise (33–36). Notably, measurement of cardiovascular parameters (including coronary) in conscious, exercising swine cannot be achieved in smaller mammals (30, 37, 38).

3.1.5. Integrative, comprehensive study. Translational medicine is facilitated by the many experimental methods that can be used in swine (30).

3.1.6. Shared resource for team science. Despite the common opinion that swine models are expensive, they are cost-effective when used as a shared resource, a feature that is fundamental to and promotes team science.

3.1.7. Cardiovascular system. The most important similarity between pigs and humans in the context of this review is the cardiovascular system, specifically the heart and coronary circulation and the susceptibility to coronary artery disease. Prominent points of similarity include:

1. Gross anatomy, including the paucity of innate collateral arteries (39, 40).
2. The pharmacology of coronary artery reactivity (41).
3. Heart rate and, thus, metabolic demand on the heart and cyclic changes in coronary blood flow (42). For example, resting bradycardia after chronic exercise training can be 50–60 bpm (34, 43). In contrast, rodents (e.g., mice, rats) have resting heart rates of 300–500 bpm (20), yielding metabolic demands that are dramatically different from those of humans.
4. Atherosclerotic lesions (44–52). In contrast, vascular disease occurring in mice results primarily in development of fatty streaks, not the full progression to calcification, necrotic core, expansion of vasa vasorum, and so forth observed in human atherosclerosis (9, 53).
5. Size of the heart. This enabled trials in pigs (36) of percutaneous catheter interventions for revascularization to be conducted with the same devices used in humans (54).

3.1.8. Kidney. Renal morphology and function in pigs are similar to those in humans (55, 56). The size of the pig kidney is also similar to the human kidney, thus enabling interventions with the same methods used in humans, such as lithotripsy (56) or optical fiber catheters that allow rapid determination of the glomerular filtration rate. The catheters were tested for safety and efficacy in pigs (57) and quickly advanced to human clinical trials (58). The kidney is relevant to our discussion of coronary artery disease in MetS because of the major influence of plasma electrolytes on vascular calcification.

3.1.9. Genome sequencing. The Swine Genome Sequencing Consortium has generated a draft of the pig genome sequence (see **http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/**) (59), and gene-editing methods (e.g., TALEN, CRISPR) have been vastly improved (53, 60). These methods have allowed the creation of gene-edited miniature swine, including Yucatan (51) and Ossabaw (50, 61, 62). Since the pig genome is closer to that of primates than to those of rats

and mice, it will help fill the gap in knowledge. Gene editing in pigs can be done using the relevant human mutations.

3.2. Disadvantages of the Use of Swine Models

Several disadvantages of the use of swine models also deserve attention. Below, we describe these limitations and comment on strategies to overcome them.

3.2.1. Expertise and resources. Because even miniature swine are relatively large (30–80 kg at sexual maturity and adulthood, versus ~30 g for mice), investigators may not have the expertise and physical resources to manage swine. Indeed, a chapter on animal models in the influential book *Joslin's Diabetes Mellitus* (29) devotes only a couple of brief paragraphs to porcine models of diabetes, which the authors dismissed as unfeasible. It is essential to draw on interdisciplinary talents in order to utilize swine models effectively, particularly researchers skilled in veterinary and comparative medicine, metabolic phenotyping, and coronary physiology in swine. Infrastructure and formal training of engineers in the use of swine models are minimal. There are 12 nonhuman primate research resources supported by the National Institutes of Health Office of Research Infrastructure Programs but only 1 swine center.

3.2.2. Systemic drug delivery. Even if an investigative team can handle swine, their large size (>30 kg) precludes testing of some experimental drugs that are available only in low quantities. Nevertheless, for studies of the vasculature and specific organs, local delivery devices and use of drug-eluting vascular stents can partially overcome this limitation.

3.2.3. Limited antibodies. Selective reagents, including antibodies, have limited availability. Typically, antibodies to human proteins cross-react very well with swine proteins, but many reagents have been designed for rodents.

3.2.4. No naturally occurring profound diabetes. Spontaneous diabetes, either type 1 or type 2, has not been reported in any swine model. Thus, the fasting glucose threshold of 126 mg/dL required for clinical diagnosis of diabetes in humans has not been attained in swine. As in other animal models, healthy swine have a set point for normal plasma glucose that differs from that in humans. Although the swine set point, typically ~60–70 mg/dL, is very close to human levels, swine typically have even less variability, only a few milligrams per deciliter (e.g., 63, 64). Even if the diagnosis of diabetes in swine is set at two or three standard deviations above the mean or statistically greater than a control group, few studies meet that criterion (30, 36, 47, 48, 63, 65–76). This limitation can be partially overcome, as profound hyperglycemia can be reliably elicited with the selective pancreatic β cell toxins streptozotocin (44, 47, 49) and alloxan (35, 77–79). Although these toxins are highly selective, potential renal toxicity has been nullified with intravenous saline before and after dosing with the toxins (35). An outstanding question is whether natural pathogenesis causes additional molecular signaling that can contribute to vascular complications.

3.2.5. Genome database. Not every pig breed has had its genome sequenced. The ~3-billion-base-pair swine genome and coding of ~24,000 proteins are similar in complexity to those of humans, presenting a major challenge.

3.2.6. Gene editing in swine is difficult. Despite major advances in gene-editing techniques, the gestation of nearly 4 months for pigs contributes to cost and length of time required for studies.

Table 1 Metabolic syndrome, type 2 diabetes, and coronary artery disease characteristics in Yucatan, Ossabaw, and Göttingen miniature swine[a]

Characteristic	Yucatan	Ossabaw	Göttingen
Obesity	No, mild (35, 82, 87) Yes (65, 88)	Yes; Ossabaw∼Göttingen> Yucatan (30, 36, 63, 66, 67, 69–73, 89–94)	Yes; Ossabaw∼Göttingen> Yucatan (47, 48, 74–76, 83, 84, 95–97)
Insulin resistance	No (64, 82, 88) Yes (65)	Yes (30, 36, 56, 63, 66, 67, 70–72, 82, 92)	Yes (47, 48, 74–76, 84, 95, 96, 98)
Glucose intolerance	No (82) Yes (65)	Yes (30, 36, 56, 63, 66–72, 82, 91, 92)	Yes (47, 48, 74–76, 84, 97)
Dyslipidemia (↑LDL/HDL or ↑LDL/TC)	Yes (23, 26, 65, 99–101)	Yes (30, 36, 63, 66–73, 94)	Yes (45, 47, 48, 73–76, 95–97, 102)
Dyslipidemia (↑TG)	No (23, 26, 34, 43, 87, 99–101) Yes (65)	Yes (30, 36, 63, 66–73)	Yes (47, 48, 74–76)
Hypertension	No (64, 82, 101) Yes (65)	Yes (30, 36, 56, 63, 64, 67–73, 91, 93, 97, 103–107)	No data
Type 2 diabetes (fasting hyperglycemia)	No (34, 35, 64, 82, 88) Yes (65)	Yes, variable (36, 63, 66, 68, 69, 108)	Yes, variable (45, 48, 75, 76)
Coronary artery disease	Yes	Yes; >Yucatan	Yes
Macrovascular vasoreactivity			
↑Constriction	Yes (43)	Yes (109)	No data
↓Dilation	Yes (82)	Yes (68, 69, 104)	No data
Atherosclerosis	Yes (23, 43, 51, 52, 82, 99)	Yes (23, 36, 50, 67, 72, 77, 82, 94, 110–115)	Yes (45–49)
Calcification	No (30, 43, 82, 99, 101, 116, 117) Yes (51, 52)	Yes (30, 50, 77, 82, 111, 112, 118, 119)	No data
Microvascular vasoreactivity			
↑Constriction	No data	Yes (104)	No data
↓Dilation	No (82)	Yes (82, 103, 108, 120)	No data

[a]Table is adapted from References 30, 82, and 121.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; ∼, similar to; >, greater than.

In contrast, phenomenal research has been conducted using transgenic mouse models (e.g., 80) to study the mechanisms underlying MetS, diabetes, and vascular disease (9).

3.3. Comparison of Major Swine Models

Table 1 compares swine breeds in terms of the key components of naturally occurring MetS and T2D in humans, as depicted in **Figure 2**. Consistent with the goal of observing natural pathogenesis of MetS, T2D, and coronary artery disease, all of the reports listed in the table involve environmentally induced (i.e., diet-induced) changes. More specifically, the diet used to elicit MetS characteristics is high calorie (>50% increase), high fat (>40% kcal), high fructose or sucrose (>20% kcal), and high cholesterol (>1% of diet by weight). For brevity, we refer to this diet as the MetS diet (another common term is Western diet). The table notes some exceptions in diets. The focus is on Yucatan, Ossabaw, and Göttingen miniature swine, largely because they are the

major miniature swine breeds used for biomedical research in MetS and coronary vascular disease. Although Sinclair pigs are used extensively for commercial toxicology and some MetS research, there is a paucity of published data on this breed (22, 81). It is difficult to quantitatively compare MetS and coronary vascular characteristics because of differences in age, gender, diet composition, kilocalorie intake, and duration of treatment in almost all studies. Furthermore, any quantitative comparison of the data will be tenuous, because no single controlled study has compared all three breeds. A partial exception is a study by Neeb et al. (82), who compared only Yucatan and Ossabaw pigs by using precisely matched conditions. Also, the measures of MetS are not the same in all studies. For example, different studies might use the intravenous glucose tolerance test, fasting plasma glucose values, or glucose clamp assessments (36, 64, 83, 84). For these reasons, **Table 1** lists either the presence or absence of these characteristics and/or qualitative descriptors. We provide a more detailed discussion of specific studies, as well as additional features and interpretations regarding **Table 1**, in Sections 3.3.1–3.3.8, below.

Many other swine breeds could be considered for such studies, but there has been much less research on them. The Bama miniature pig from China showed profound obesity after 23 months of feeding a hypercaloric, high-fat, and high-sucrose diet (85). The pigs met the criteria for MetS, namely insulin resistance, glucose intolerance, and dyslipidemia. No measures of coronary artery disease were reported. The Bama breed has been under development for about 20 years, but we are not aware of its availability outside China. Although outstanding research has shown that a line of crossbred domestic pigs with familial hypercholesterolemia will develop MetS (86), use of standard-sized domestic swine for this purpose is not practical because they weigh more than 250 kg and are 2 years old by the time MetS occurs. Typical domestic swine are sexually mature at 5–6 months but might weigh 100 kg at 4 months of age (81). The size and modest MetS traits of domestic swine make it difficult to use them in chronic metabolic disease studies. However, juvenile domestic pigs are acceptable for initial, short-term studies of device deployment due to their human-like size.

3.3.1. Obesity. Overall, a typical increase in body weight by more than 60% above lean, age-matched controls is required to elicit glucose tolerance and insulin resistance. Unfortunately, no studies have used the exact same parameters to directly compare the three major miniature swine breeds. According to **Table 1**, the breed with the fewest measures of MetS is the Yucatan, although there has been a tremendous amount of research on these swine in other areas. Despite intensive efforts to induce insulin resistance and glucose intolerance in Yucatan swine on MetS diets, most current studies have not been able to reliably reproduce the findings of Phillips et al. (122) from at least eight publications nearly 35 years ago. This failure to replicate could be explained, in part, by the fact that the Phillips group bred Yucatan pigs selectively for insulin resistance for several generations, and when the research was discontinued that line was lost. We emphasize that currently available lines of Yucatan swine do not naturally develop the insulin resistance or robust obesity associated with MetS diet consumption (35, 64, 82, 88).

A study on ad libitum feeding of Yucatan pigs a MetS diet for 4 months showed a 40% increase in body weight (88), which is the largest we have found in the literature. Computed tomography (CT) showed a clear increase in adiposity. The changes in adiposity and body weight are not as compelling as for Ossabaw and Göttingen pigs, and the lack of absolute food consumption values for the ad libitum groups makes the interpretation of propensity to obesity unclear. The more genetically lean Yucatan pig responds in an anabolic manner to exercise training, even when dyslipidemic and rendered diabetic with alloxan (123). Greater body weight gain in exercised pigs versus sedentary diabetic dyslipidemic controls occurred with matching food consumption (35, 123). Taking into consideration the vast amount of research on Yucatan swine, the field may

benefit from additional studies of glucose tolerance and insulin resistance in Yucatan pigs fed a MetS diet with a substantially increased caloric load for more than 6 months. Such studies would provide a rigorous test of whether Yucatan pigs are relatively more metabolically healthy than other miniature swine breeds. Neeb et al. (82) kilocalorie-matched the MetS diet and lean control diet groups, so the main comparison was to Ossabaw miniature swine. Clearly, a MetS diet that is kilocalorie-matched to normal chow typically induces few MetS characteristics (64), except increased plasma cholesterol (43, 64, 82, 120). The only robust MetS phenotype for Yucatan swine was found in Yucatan microswine, which manifest, exceptionally, all six MetS characteristics (65).

3.3.2. Insulin resistance. Ossabaw and Göttingen pig groups reliably develop insulin resistance when they are placed on a MetS diet and their body weight is increased, but not when their body weight is maintained at healthy, lean levels (120). Unfortunately, few studies have been conducted in Yucatan pigs using glucose tolerance or direct insulin sensitivity tests or the homeostatic model assessment–insulin resistance (HOMA-IR) measure. As shown by the Yucatan micropig study (65), if body weight gain is sufficient, Yucatan miniature pigs might manifest insulin resistance. The finding that group data on pigs demonstrate insulin resistance is important and represents a very interesting statistical aspect of MetS in swine. Sham et al. (92) demonstrated the heterogeneity of insulin resistance in obese Ossabaws. Insulin resistance of an individual pig consuming a MetS diet was defined by the HOMA-IR measure as more than two standard deviations above the mean of the group consuming a lean diet. Although the MetS group had statistically greater HOMA-IR than the lean group, as noted in numerous studies cited in **Table 1**, only ~70% of the pigs in the MetS diet group developed insulin resistance. Indeed, Sham et al. (92) noted that the heterogeneity of insulin resistance in Ossabaw pigs is completely consistent with human epidemiological data and indicates the translational relevance of the swine model.

3.3.3. Glucose intolerance (or impaired glucose tolerance). Intravenous glucose tolerance tests conducted routinely in Ossabaw and Göttingen pigs strongly support the finding of glucose intolerance. Tests in Yucatan pigs are largely confined to those in eight or more papers summarized nearly 35 years ago (122). A 12-week MetS feeding study of Sinclair miniature pigs showed mildly higher (27%) postprandial glucose than in healthy diet controls (22). Excellent oral glucose tolerance tests have been conducted in Göttingen pigs, but they often have poor reproducibility and the outcome is uncontrolled for differences in absorption of glucose from the gastrointestinal tract (75). Since gastric emptying takes longer in pigs than in other species (124), these tests have not been widely used. The translational relevance of the Ossabaw model is demonstrated by the improved glucose tolerance resulting from metformin treatment (71).

3.3.4. Dyslipidemia (increased LDL/HDL or increased LDL/TC). Increased plasma LDL and TC in Yucatan, Ossabaw, and Göttingen pigs is a reliable finding when the diet includes more than 1% cholesterol. Thus, the colloquial term high-fat diet is erroneous, because an increased fat percentage with minimally increased cholesterol and no cholate in the diet yields only modest increases in plasma lipids (88). Increased fat and/or fructose in the complete absence of cholesterol yields no increase in plasma TC and LDL cholesterol (63, 93). Virtually all studies also find increases in plasma HDL cholesterol; thus, ratios of LDL/HDL and LDL/TC are crucial to predict atherogenicity.

3.3.5. Dyslipidemia (increased triglycerides). A large proportion of studies has shown that Ossabaw and Göttingen pigs fed a MetS diet have increased plasma triglycerides, although the level is not robust. In contrast, Yucatan pigs fed a MetS diet seldom show increases in plasma

triglycerides (65). Additionally, Yucatan and almost all domestic pigs do not show increased triglycerides unless insulin secretion is substantially decreased by alloxan- or streptozotocin-induced diabetes (23, 44, 99).

3.3.6. Hypertension. Hypertension is a hallmark of MetS in humans, and Ossabaw miniature swine express this characteristic. There are no data available on Göttingen pigs, and Yucatan pigs clearly do not have this MetS trait. The translational relevance of the Ossabaw in this respect is illustrated by the complete reversal of hypertension by metformin treatment (71) and catheter-based radio-frequency renal nerve denervation (107). The size of the pig is optimal for catheter-based interventions that can be used in humans.

3.3.7. Type 2 diabetes (fasting hyperglycemia). Fasting hyperglycemia in MetS models is not consistently found in all Ossabaw and Göttingen pig MetS diet studies, but it is sufficient to be consistent with the time course of the pathogenesis in humans (**Figure 2**). Normal blood glucose is maintained in Yucatan pigs fed a MetS diet, with one exception showing hyperglycemia (65). The greater insulin secretory reserve in swine (125) versus humans probably explains the modest fasting hyperglycemia in pigs. Additional studies should compare in more detail β cell mass between the three breeds of pigs (126). The compensatory β cell expansion could potentially classify pigs as metabolically healthy obese.

3.3.8. Coronary artery disease. Vascular disease is a major endpoint and long-term complication of MetS and diabetes. There is a relative paucity of characterization of coronary artery macro- and microvascular vasoreactivity in the Göttingen pig, but significant atherosclerosis has been noted. Yucatan pigs show altered coronary vasoreactivity and atherosclerosis due to dyslipidemia, but not the full spectrum of MetS. Yucatan pigs with the *PCSK9* mutation have quite striking coronary atherosclerosis and calcification (51). Extremely high levels of plasma LDL and total cholesterol drive atherosclerosis independently of any obesity, glucose intolerance, or hypertension, since all of these were normal. The dominant effect of dyslipidemia versus obesity and other MetS factors of insulin resistance, impaired glucose tolerance on coronary atherosclerosis, was observed in the Göttingen pig with morbid obesity (body fat was 50% of body mass) (48). In animals fed the MetS diet, plasma cholesterol peaked at only ~125 mg/dL after 30 weeks but decreased to only ~85 mg/dL, levels similar to those of healthy lean pigs, after 54 weeks. There were no blood pressure measures, but profound resting tachycardia in obese versus lean pigs (97 versus 59 bpm) was observed. Only fatty streaks in coronary arteries were noted; these were most likely due to the modest increase in total plasma cholesterol, which also recovered to levels found in healthy lean pigs. These findings are consistent with a major role of dyslipidemia in coronary atherosclerosis in MetS, as shown by serial intravascular ultrasound measures of coronary artery plaque burden in humans (127). The individual component risk factors, mainly dyslipidemia, and not the diagnosis of MetS were predominant in atherosclerosis progression in humans (127). Compelling evidence for the value of intravascular ultrasound measures in swine studies is the direct relationship among intravascular ultrasound measures of coronary atherosclerotic plaque burden, its progression, and adverse cardiovascular events in humans (128).

The role of glucose was tested with chemically induced diabetes in swine models. Poor glycemic control did not accelerate atherosclerosis in genetically modified *PCSK9* hypercholesterolemic (129) or Göttingen (47) pigs. It is important to interpret these findings carefully, as there is a preponderance of evidence implicating diabetes with increased coronary artery disease in human patients (3, 130). In animal models in particular, the effects of very high levels of plasma cholesterol may mask any proatherosclerotic effect of hyperglycemia (43). Other studies have found that

hyperglycemia can potentiate hypercholesterolemia-induced coronary disease in domestic (44), Yucatan (99), and Ossabaw miniature swine (77). Swine models have also shown that hyperglycemia alone clearly does not increase macrovascular coronary artery disease (43, 44).

Ossabaw pigs with MetS can develop all coronary vascular pathologies, including significant coronary atheroma. These data and the association of obesity with other MetS characteristics, especially hypertension, are consistent with findings in human disease (1, 127). Compared with Yucatan miniature swine, Ossabaw swine have greater diffuse atherosclerosis, calcification, and in-stent stenosis (82), which are hallmarks of T2D and MetS in humans (130). All three major swine models develop coronary atherosclerosis that could be severe enough, after a long period of development, to make it feasible to stent natural atherosclerotic lesions, not balloon-injured healthy arteries (e.g., 54, 131). Indeed, the size and characteristics of coronary artery disease that mimic human disease so well have made the pig the premier model. It is hoped that swine can shed light on the major cause of acute myocardial infarction, namely unstable plaque, via state-of-the-art imaging methods (132–136).

Excellent imaging studies of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake measures of inflammation with positron emission tomography and hybrid magnetic resonance imaging (PET-MR) of aortic morphology have been conducted in Göttingen swine (49). After 30 weeks on a MetS diet, one group was switched to the diet of the lean control group for another 30 weeks. PET-MR resolved a decrease in vascular inflammation of the MetS group to baseline levels in the lean control group, despite decreasing body weight from the obese 97% above lean to only 34% above lean control. Aortic gene expression in the diet normalization group similarly showed a recovery to near-lean-control levels. Aortic atherosclerosis, however, did not significantly decrease over the 30-week diet-normalization period. The important points are that PET-MR can provide measures of inflammation that parallel gene expression and that structural atherosclerotic changes may require much longer to occur. These timelines are critical when designing studies to measure the efficacy of therapeutic interventions.

A subtle detail in all studies on coronary artery disease is the feeding regimen, including composition, timing, and quantity. The need for increased dietary cholesterol is discussed in Section 3.3.4, above. Once-per-day feeding (i.e., gorging behavior) in Yucatan swine elicits much greater coronary atherosclerosis (43) than twice-per-day feeding of the same total amount of food (23, 99). There is every reason to suspect that similar results would be found in other swine breeds, and from an animal husbandry and nutritional control standpoint, once-daily feeding is superior.

3.4. Developmental Stage

The age and sexual maturity of pigs are variables that deserve much more attention in swine MetS and coronary artery disease studies. There are only a few reports of direct comparisons. Ossabaw swine have been used for studies of juvenile obesity, and their coronary transcriptome has been characterized (96) but not compared with that of adults. There is a profound resistance to alloxan-induced diabetes in juvenile (age <5 months) versus young-adult Ossabaw swine that are sexually mature (78). At the other extreme, coronary artery plaques are more advanced and complex in older MetS Ossabaw swine (age 8.8 years) versus young adults (age 2.5 years) (114). The so-called aging milieu potentiates the effects of coronary smooth muscle cell Ca^{2+} dysregulation (114), but these cellular mechanisms are only a hint of the altered signaling pathways in aged swine versus young adults. Moreover, the relatively small size (~30–40 kg) of miniature swine at sexual maturity provides another advantage for husbandry and for studies of sex differences in MetS, T2D, coronary artery disease, and so forth. There is greater insulin resistance in MetS female versus male Göttingen pigs (83).

Related to developmental state are the rate of disease development and the duration of study protocols. For example, the daily quantity of MetS diet directly increases both cholesterol levels and the rate of development of coronary disease. Studies of much longer duration will be necessary to definitively determine whether the pig pancreatic β cell capacity can be challenged enough to render robust T2D. Since the ratio of pancreatic β cells to body mass in swine is twice that in humans, there is considerable insulin secretory reserve in swine (125). For example, more robust MetS and achievement of T2D have been noted after 13 months on a MetS diet (36) versus 2 months (67).

3.5. Metabolically Healthy Obese

The concept of metabolically healthy obesity has received support from human epidemiological data (cited in 92), the heterogeneity of insulin resistance in Ossabaw swine fed a MetS diet (92), controlled studies comparing Lee-Sung and Lanyu miniature swine (137, 138), and studies on most domestic pigs and Yucatan pigs (**Table 1**). Similar to Yucatan pigs, Lanyu pigs fed a MetS diet (without added cholesterol) showed modest increases in body weight and adiposity and no change in plasma lipids, fasting blood glucose, or lipid liver accumulation (137). In contrast, Lee-Sung pigs had five of six features of MetS (137) as well as cardiac lipid accumulation (138). The heterogeneity of response of domestic pigs fed MetS diets may be partially the result of shorter durations of feeding, due to the excessive increase in body frame and lean mass that makes husbandry difficult. Subsets of domestic pigs develop the MetS characteristics of obesity, glucose intolerance, and hypertension (71; reviewed in 139).

3.6. Need for Natural Pathogenesis of Metabolic and Vascular Disease

A highly focused, targeted genetic approach is very powerful because it can elucidate the involvement of a single protein in a disease; however, it can be artificially narrow. Typically, no single gene mutation recapitulates the complexity of the metabolic syndrome (1, 16). Thus, researchers should place a higher priority on more human-like animal models for translational research, rather than relying solely on gene-edited animals and mouse models that have been shown to poorly reproduce human inflammatory diseases (6).

Swine—and, we strongly argue, Ossabaw miniature swine—are essential for biomedical engineering research because they mimic humans across the spectrum from healthy to disease states (14, 15). The pathogenesis of MetS and the underlying mechanisms of subsequent long-term health complications in MetS and T2D are multifactorial; thus, there is a compelling need for animal models of complex, polygenic diseases (1, 16). Therefore, a major reason to study Ossabaw swine is that natural models of disease that arise due to adaptation of animals to unique selection pressures can provide insights into similarly complex, multifactorial diseases in humans. Ossabaw miniature swine may recapitulate the natural pathogenesis of MetS and T2D due to their thrifty genotype, that is, propensity to obesity (17), which has enabled them to survive in the feast-and-famine ecology of Ossabaw Island in Georgia, USA (140), since their introduction to the isolated barrier island nearly 500 years ago (**Figure 3*a***) (30). Indeed, the Ossabaw pig has the highest fat stores of any terrestrial mammal (140). Harvest and study of pigs from Ossabaw Island showed components of the pathologies listed in **Table 1**. An important point is that Ossabaw swine mimic the whole spectrum of progression from relatively insulin sensitive to insulin resistant, MetS to T2D, as in humans.

Figure 3*d* presents a graphical summary of idealized experimental designs and predicted data for optimal MetS swine models and development of coronary artery disease. Pigs progress from a normal, healthy, lean state at birth. Note that pigs younger than ~6 months are considered to

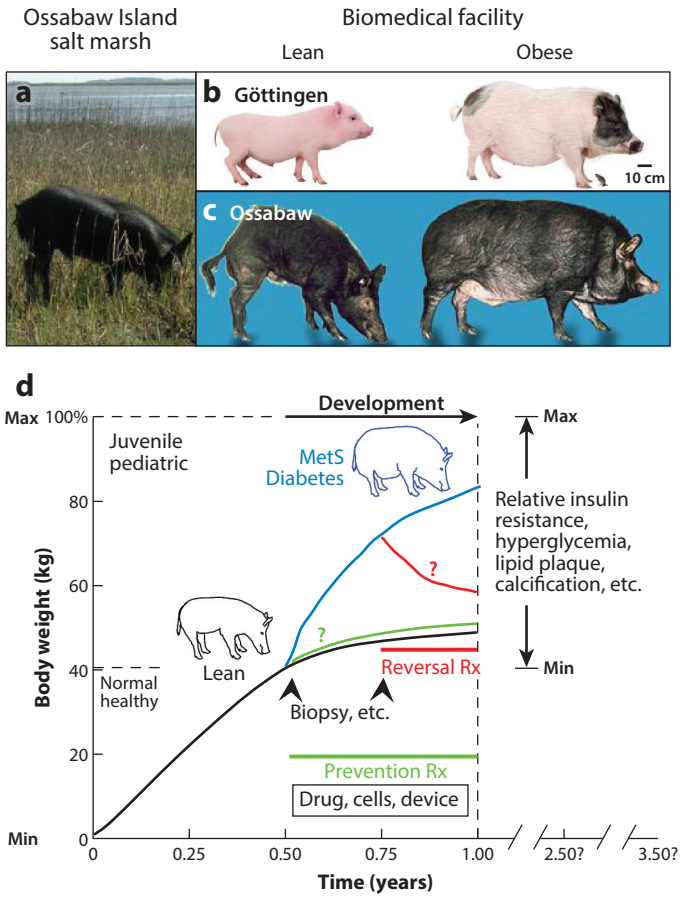

Figure 3

Overall design to achieve the most natural pathogenesis of metabolic syndrome and vascular disease studies in biomedical facilities. (*a*) An Ossabaw pig in its native salt marsh environment on Ossabaw Island, Georgia, USA (30). The pig was in a relatively lean state in the spring after surviving a winter of food scarcity. It will cycle through a period of obesity as it feasts for months before the next winter. Biomedical facilities house pigs under much tighter experimental control. (*b*) Lean and obese Göttingen pigs. The mouse to the left of the scale bar shows the substantial difference in size compared with pigs. (*c*) Lean and obese Ossabaw pigs (70). (*d*) Idealized experimental designs and predicted data for optimal MetS swine models and development of vascular disease in a biomedical facility. Abbreviations: MetS, metabolic syndrome; Rx, treatment. Panel *a* adapted with permission from CRC Press. Panel *c* adapted with permission from Dove Medical Press, Ltd.

be in the pediatric through juvenile states. The body weight gain is relatively representative of the growth curves for Yucatan and Ossabaw, although the smaller Göttingen are approximately 50–70% of their weight. Sexual maturity occurs at age ~6 months. Although these breeds may be genetically predisposed to metabolic disease, they are considered relatively lean. We urge that experiments initiated on pigs under age 6 months take into consideration the pediatric through juvenile stages of development. With rare exceptions (e.g., 44), almost all studies of domestic pigs involve juveniles. Studies of juvenile obesity certainly are an urgent, unmet clinical need, and recognizing that need is crucial (96). All data should be interpreted within the pigs' developmental phase for optimal translation to human clinical medicine. For example, plasma lipid responses to

the MetS diet, capacity for recovery from vascular injury, and atherogenic potential are likely to be different for young and adult pigs. Evidence reinforcing this point comes from a study of geriatric and young adult Ossabaw swine in which the geriatric subjects consumed a MetS diet for 11 months starting at age 8.8 years; compared with their young adult counterparts (age 2.5 years), the older pigs developed an approximately threefold-greater coronary intimal/medial ratio (114). The baseline was the intimal and medial thickening of the healthy, lean aged pig (114).

The ideal age to initiate MetS development in normal, healthy pigs is ~6 months because that is when body weight starts to plateau (**Figure 3**). The relatively steady body weight and health status facilitate baseline physiological measures, tissue biopsies, glucose tolerance tests, assessment of coronary atherosclerosis with intravascular ultrasound, and so forth (30, 110). The 6-month starting age represents a compromise between cost and desire for robust effects of the MetS diet on metabolic and coronary artery disease. The data presented in this review indicate overall that 6 months of the MetS diet (high in kilocalories, fat, fructose, and cholesterol) is sufficient for the development of MetS. Transition to mild T2D is variable. Drug, cellular, and device therapies can be started concomitantly with the MetS diet for prevention studies. A separate control group can be used, or repeat measures in the same pigs can substitute. The trajectory of body weight must be carefully monitored, as it is a surrogate for body composition (35, 67, 87), other MetS characteristics (**Table 1**), and coronary disease. Reversal treatment studies can be used instead of prevention, but stabilization of body weight is recommended if a placebo control group is not used. Ideally, a lean control group should be included in all studies. Ablation of β cells to elicit profound hyperglycemia will further maximize the diabetic milieu and subsequent coronary artery disease and other comorbidities. The diet elicits MetS, which progresses in severity as the pigs become more obese and insulin resistant up to some hypothetical maximum (**Figure 3**). It is not known for every MetS characteristic or for coronary atherosclerosis, calcification, and so forth whether the trajectory to maximum is steep or shallow. Despite the natural pathogenesis of metabolic and vascular disease in some animals, all experimental models aim for accelerated disease progression for the practical reasons that studies must be completed in a timely manner and costs of maintaining the animals kept low. Swine models are especially problematic in this regard, due to the cost of maintaining them over their long life span (approaching ~15 years).

A major consideration is whether the underlying pathogenesis may be different in accelerated experimental models compared with natural progression over the animal's lifetime. On the basis of this consideration, it would be provocative to increase the experimental treatment of miniature swine to 2 or 3 years (**Figure 3*d***). Specifically, the development of more robust T2D and more complex coronary artery atherosclerosis almost certainly requires longer durations of study. Alternatively, screening pigs for robust insulin resistance (92) and coronary disease with serial intravascular ultrasound (110) would facilitate selection of optimally diseased subjects. A compromise that combines some natural models with targeted gene editing may facilitate disease severity. An advantage of natural and inherently complex models is that diet-induced disease models may more faithfully recapitulate the fundamental pathology in vascular calcification, which is therapeutically most translational to patients with MetS and T2D (141).

4. POSITRON EMISSION TOMOGRAPHY–COMPUTED TOMOGRAPHY IMAGING OF CORONARY ARTERY CALCIFICATION

The importance of coronary artery calcification in humans is best known through the increasingly wide use of calcium scores from CT imaging (112). Calcium scores can predict adverse coronary outcomes but do not address the possible role of calcification in unstable plaque rupture leading to acute myocardial infarction (132–136), and they do not provide insights into the mechanisms of early development of calcification (112) and treatments to prevent calcification (112, 142, 143).

A thorough and rigorous study (144) demonstrated the value of ^{18}F–sodium fluoride (^{18}F-NaF) for imaging of vascular calcification (first achieved in 2010; reviewed in 112). Irkle et al. (144) verified that very high picomolar affinity binding occurs via the exchange of fluoride with hydroxyls on hydroxyapatite, the major chemical species of extracellular calcification. The binding sites of hydroxyapatite in early microcalcification are exposed, so the PET signal for ^{18}F-NaF is high. As calcification becomes thicker, more extensive, and visible with CT, the binding sites of hydroxyapatite are relatively well covered. Consequently, the PET signal for ^{18}F-NaF may be lower compared with the high volume of calcification, that is, macrocalcification. This chemistry is nearly ideal for sensitive ^{18}F-NaF PET imaging of early microcalcification, but a major problem is that the signal can be blurred by coronary artery motion in the beating heart. Efforts to solve this problem for imaging molecular calcification have largely utilized human clinical studies (132–136).

The advantages of appropriate MetS characteristics, coronary artery physiology, and pathophysiology of calcification in Ossabaw swine (30, 50, 77, 82, 111, 112, 118, 119), as well as their human-like size, could help solve the ^{18}F-NaF PET imaging problem. Stabley & Towler (141) note that for the translational pathogenesis of calcification, natural induction of MetS with diet is the most relevant approach for animal models. Again, "size matters" for the use of swine models (5). The 0.1-mm scale bar in **Figure 4*b*** (111) is the size of the typical epicardial conduit artery in the mouse, dashing any hope of conducting ^{18}F-NaF PET imaging studies in the mouse. In close analogy to human studies, contrast CT–enhanced imaging in swine enables coregistration of the PET and CT signals. Respiratory motion artifacts can be eliminated because the pig is fully anesthetized and on a ventilator. Intravascular ultrasound imaging quantifies wall coverage by neointima. The ^{18}F-NaF signals shown in **Figure 4*d*** and ***e*** are barely at the level of visual detection but are resolved quantitatively (111). The significant correlation ($r = 0.67$) between coronary artery ^{18}F-NaF uptake and intimal thickening in **Figure 4*e*** indicates molecular calcification in very early stage type I, II, and III atherosclerotic lesions (111). **Figure 4*f*** shows low, diffuse levels of ^{18}F-NaF uptake and focal ^{18}F-NaF visually detected in a region of stable plaque in a human patient (134).

With the use of ^{18}F-NaF PET-CT imaging, swine disease models could facilitate optimal vascular imaging and engineering:

1. After state-of-the-art ^{18}F-NaF PET-CT imaging with coregistration of the PET signal with CT-resolved artery morphology, coronary arteries could be harvested to quantify the coronary plaque ^{18}F-NaF signal ex vivo and compare it with the maximum total background and other quantitative measures of in vivo PET images (135). The ^{18}F-NaF uptake measured in vivo could actually underestimate the true uptake of ^{18}F-NaF.
2. Longitudinal ^{18}F-NaF studies from early neointimal lesions (111) through more advanced, clinically significant lesions would help elucidate the relationship of microcalcification to the mechanisms of coronary atherosclerosis.
3. Early evaluation of therapeutic efficacy in preclinical studies could be improved. Researchers could determine whether imaging with ^{18}F-NaF PET-CT can resolve whether statin therapy prevents coronary artery calcification at the earliest stage (142), concomitant with prevention of aberrant Ca^{2+} signaling at the single-cell level (99).

Statins clearly reduce the volume of lipids in coronary lesions, but they cannot reverse the bonelike, CT-resolvable calcification (143). The result is an increase in coronary artery calcification on a relative volume basis (143). Plaque stability is a certainly a positive adaptation and is thought to be one of the main causes of improved outcomes. The neglected adverse effect is the decreased distensibility of conduit coronary arteries, which adversely affects downstream

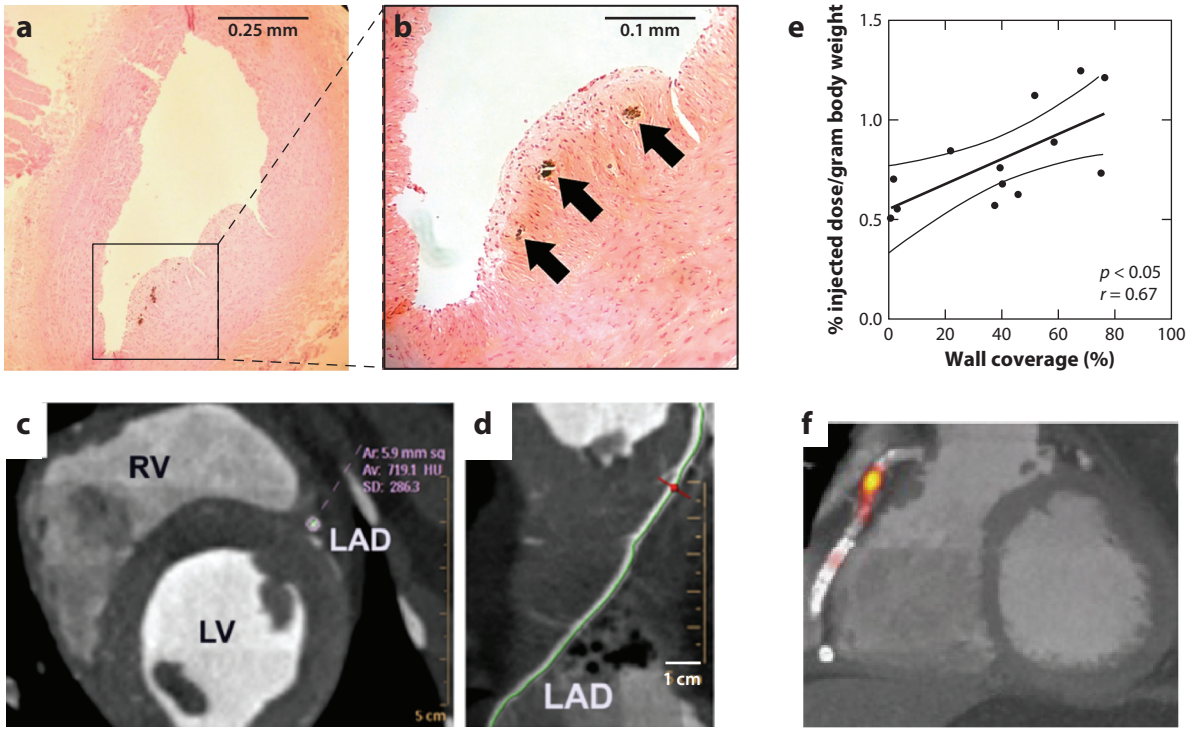

Figure 4

Coronary artery microcalcification detected with ^{18}F-NaF PET-CT. (*a*) Hematoxylin and eosin stain of epicardial conduit coronary artery from an Ossabaw swine. The dark speckles in the box are microcalcifications. (*b*) Higher magnification of similar area in the boxed region of panel *a* from an adjacent section of the artery. Arrows show microcalcifications within a region of neointimal thickening in the earliest stages of atherosclerosis (111). (*c*) Transverse section of the heart during contrast-enhanced CT imaging of the Ossabaw swine to locate conduit arteries (e.g., LAD) and coregister for PET imaging. (*d*) LAD coronary artery from panel *c* shown along its linear axis for calculation of ^{18}F-NaF uptake in the artery. (*e*) Quantification of ^{18}F-NaF uptake in Ossabaw swine coronary arteries as a function of the percentage of wall coverage of the artery with intimal thickening as measured by intravascular ultrasound imaging (111). (*f*) Focal ^{18}F-NaF uptake in an epicardial conduit artery of a human. Heat map color imaging shows a greater intensity of ^{18}F-NaF binding in the proximal section of the artery (*yellow*) at the site of stable plaque (134). Abbreviations: CT, computed tomography; LAD, left anterior descending; LV, left ventricle; RV, right ventricle; PET, positron emission tomography; ^{18}F-NaF, ^{18}F–sodium fluoride. Panels *b* and *e* adapted with permission from the American College of Cardiology. Panel *f* adapted with permission from the American Heart Association.

microvascular function (141). These issues require early therapy and imaging of microcalcification, which can be performed in swine models.

SUMMARY POINTS

1. We have presented an overall design aiming to achieve the most natural pathogenesis of MetS and vascular disease studies in biomedical facilities (**Figure 3**).
2. No single swine model can recapitulate all aspects of human metabolic and vascular disease, but the advantages of these models for translation to humans greatly exceed those of small-animal models.

3. Swine models with the most robust metabolic syndrome traits are the most relevant for humans, but lean breeds and metabolically healthy obese models can provide helpful contrasts.
4. Miniature swine are essential for longer-term studies of disease progression.
5. PET-CT imaging of coronary artery calcification exemplifies the use of MetS swine models for optimal vascular engineering.

FUTURE ISSUES

1. Infrastructure for the maintenance of swine disease models is scarce, especially for long-term MetS and coronary artery disease progression studies.
2. Training of basic scientists in large-animal methods is minimal.
3. We must change our attitudes to move out of our comfort zone with smaller animals (e.g., mice), which offer a robust phenotype but limited translation to human medicine.
4. Sharing of resources will be essential to overcome the expense of chronic swine studies. It is fruitful to consider the actual financial savings and reduced number of complications, adverse events, and so forth that would be achieved in human clinical trials if swine models of vascular disease were used more extensively to predict therapeutic efficacy.
5. Team science can overcome the weaknesses and maximize the strengths of swine disease models.
6. Tools for the use of swine in biomedical engineering research are available. Many of these tools are used in humans.
7. Use of swine in coronary artery imaging and device testing enables these technologies to move quickly to human clinical studies. The low-hanging fruit might be imaging methods such as PET-CT. The technology is largely in place, and the instrumentation needs to be synthesized with the appropriate swine disease model(s).

DISCLOSURE STATEMENT

M.S. and M.A. are cofounders of CorVus Biomedical, LLC. F.W.S. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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