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Investigating the metabolic syndrome: Contributions of swine models

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Abstract

The metabolic syndrome (MetS), a cluster of dyslipidemia, hypertension, and diabetes, and an important contributor to cardiovascular morbidity and mortality, occurs in nearly 35% of adults and 50% of the aging population in the United States. However, the underlying mechanisms by which MetS orchestrates and amplifies cardiovascular events remain elusive. Furthermore, traditional therapeutic strategies addressing lifestyle modifications and individual components of MetS are often unsuccessful in decreasing morbidity due to MetS. The availability of an adequate experimental platform that mimics the complexity of MetS may allow development of novel management techniques. Swine models, including domestic pigs and minipigs, have made important contributions to our understanding of many aspects of MetS. Given their similarity to human anatomy and physiology, those models may have significant predictive power for elucidating the pathophysiology of MetS in a manner applicable to humans. Moreover, experimental maneuvers and drugs can be tested in these pre-clinical models before application in patients with MetS. This review highlights the utility of the pig as an animal model for metabolic disorders, which may play a crucial role in novel drug development to optimize management of MetS.

Keywords

Metabolic syndrome; insulin resistance; adiposity; swine; hypertension

Overview

The prevalence of obesity and metabolic syndrome (MetS) is increasing in the developed world (Eckel *et al.*, 2005). MetS is defined by the constellation of obesity, dyslipidemia, hypertension, insulin resistance (IR), and increased levels of serum proinflammatory markers (Grundy *et al.*, 2004). According to American Heart Association, individuals are diagnosed MetS when they show ≥3 of following components: 1) Central or abdominal obesity (measured by waist circumference); 2) Elevated triglyceride levels; 3) Low high-density lipoprotein (HDL); 4) Hypertension; 5) Elevated fasting glucose. The International Diabetes Federation criteria are similar, but more restrictive for central obesity (Parikh and

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Disclosure

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Mohan, 2012). MetS is an important contributor to cardiovascular morbidity and mortality, which can be observed in nearly 35% of adults in the United States and 50% of the aging population (Aguilar *et al.*, 2015). MetS patients are also at increased risk of developing microalbuminuria (Hoehner *et al.*, 2002) and renal dysfunction (Chen *et al.*, 2004).

Investigation of the pathogenesis of MetS and its adverse outcomes have been facilitated by development of animal models of MetS. Various species have been used as models of MetS, particularly rats and mice, whereas studies in swine models to date account for no more than 10% of the total number of publications in this area identified in a search of the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed; http://www.ncbi.nlm.nih.gov/pubmed?term=(%22Metabolic+Syndrome+X%22%5BMesh%5D)+AND+%22Swine%22%5BMesh%5D; year 1960–2015). However, although rodents are small and thus useful for multi-variable experiments, they show several important differences in metabolism and physiology from humans (Davis *et al.*, 2013, Arner, 2005). For example, normal mouse lipoprotein profiles have primarily atheroprotective HDL, whereas normal human lipoprotein profiles contain primarily atherogenic low-density lipoprotein (LDL)(Kennedy *et al.*, 2010). Adipsin, an adipokine that is increased in human Mets, is found lower in rodents. (Rosen *et al.*, 1989, Napolitano *et al.*, 1994) Unlike swine (Table 1), Rodents may not always develop hypertension (Mark *et al.*, 1999). As they do not readily and simultaneously exhibit all the clinical signs of MetS, (Spurlock and Gabler, 2008) the translation of rodent data for humans have been hindered. In contrast, pigs possess many anatomical and physiological similarities to humans, as well as a high sequence and chromosome structure homology. (Vamathevan *et al.*, 2013, Groenen *et al.*, 2012) Comparisons of 317 known human drug target genes revealed less variation from minipigs (19 genes) than from beagle (41 genes). (Vamathevan *et al.*, 2013) However, pigs do show some anatomical difference from humans. In the kidney, the avascular plane is transverse in swine, rather than longitudinal as in humans, and it has 2 large venous trunks (humans have 3) and no large veins on the dorsal surface of the pelvis. (Bagetti Filho *et al.*, 2008) In the heart, innervation of the atrioventricular node and ventricular conduction tissues also differs from humans. (Crick *et al.*, 1999) Nonetheless, many similarities to humans support the use of the pig model for investigating MetS (Spurlock and Gabler, 2008).

Inducing MetS in swine

MetS has been induced in different strains of pigs by high caloric diets (Table 1), mostly including 15–25% (by weight) fatty acids (mainly lard supplemented with hydrogenated soya bean and coconut oil), 1–2% cholesterol, 40% refined sugars (commonly 20% fructose and 20% sucrose), 17% protein, and 15% other carbohydrates like starches and fibers. In most MetS models, features of this disorder appear after a 3–6 months diet, but in some can emerge as early as after 2–5 weeks of high-fat/high-energy diet. (Christoffersen *et al.*, 2013, Johansen *et al.*, 2001) At 12 weeks after high-fat/high-sucrose diet there are usually apparent manifestations of MetS (Xi *et al.*, 2004), which progressively aggravate over time (Pawar *et al.*, 2015). At 6 months of high-fat/ high-fructose diet, some atherosclerotic plaque formation in the coronary artery may be evident (McKenney *et al.*, 2014, Borbouse *et al.*, 2009) (Table 2).

Minipig breeds frequently used to simulate MetS include Yucatan, (Lee *et al.*, 2010) developed in the Yorkshire area of northern England and imported into the United States in 1890s; Sinclair, originally developed at the Hormel Institute of the University of Minnesota in the 1950s; Göttingen (Johansen *et al.*, 2001), developed in the 1960s at the Institute of Animal Breeding and Genetics of the University of Göttingen, Germany, by crossbreeding the Vietnamese, Hormel, and German improved Landrace swine; the Chinese Guizhou (Xi *et al.*, 2004) and Taiwan Lee-Sung (Li *et al.*, 2015) strains, as well as Ossabaw pigs.

The Ossabaw strain encompasses descendants of minipigs brought from Spain that were isolated on the Ossabaw Islands off the coast of Georgia, (Brisbin *et al.*, 1977), and develop MetS upon high-fat/high-sucrose feeding (Clark *et al.*, 2011, Lee *et al.*, 2009, Zhang *et al.*, 2013) and readily exhibit obesity, insulin resistance, and hypertension, not often seen in other breeds (Litten-Brown *et al.*, 2010). This unique strain has lived in relative genetic isolation for centuries, surviving on abundant food in the fall and famine conditions in the winter, a scenario that may have selected for a “thrifty genotype”. (Speakman, 2008) Hypothetically, in early evolutionary history genes promoting efficient fat deposition might have been advantageous by allowing survival at periods of famine. In modern society, such genes are disadvantageous because famine does not necessarily come, resulting in facilitated evolution of metabolic disorders. Ossabaw pigs have low insulin binding affinity for liver microenzymes and thereby are relatively insensitive to insulin (Meserole and Etherton, 1984) and susceptible to MetS. (Elmadhun *et al.*, 2014a, Zhang *et al.*, 2013) This strain, however, is costly, and does not grow to the full body size of domestic pigs, possibly due to lower plasma levels of growth hormone (Kasser *et al.*, 1981, Wangsness *et al.*, 1977), resulting in more limited sample collection capacity.

Recently, MetS was successfully induced in large domestic pigs such as American Yorkshire (Sus scrofa domesticus) using high saturated-fat/cholesterol/sugar (te Pas *et al.*, 2013) or high-fat/high-fructose diet. (Pawar *et al.*, 2015, Ma *et al.*, 2015) In domestic pigs, a 2% high-cholesterol diet causes only endothelial dysfunction and early changes of atherosclerosis, but not obesity, IR, or hypertension (Eirin *et al.*, 2014, Urbieta-Caceres *et al.*, 2010). Contrarily, a 3–4 months ad-libitum high-fat/high-fructose diet (Table 1) induced significant manifestations of MetS resembling those observed in Ossabaw pigs. A 12–16 weeks of diet elicited a 20% increase in blood pressure, 2-fold increase in IR (Figure 1A), a 4-fold increase in serum cholesterol, and doubling of triglyceride levels. In addition, plasma tumor necrosis factor (TNF)-α levels were also elevated by 3-fold (Li *et al.*, 2012, Pawar *et al.*, 2015, Zhang *et al.*, 2013). These findings suggest that the MetS model in domestic pigs is effective and may offer some advantages in cost and genetic heterogeneity.

Interestingly, female pigs seem to develop MetS models more readily than their counterpart male pigs. While male pigs also become obese and increase fasting blood glucose and insulin levels following a MetS diet (Larsen *et al.*, 2001), female pigs develop a larger abdominal circumference and higher concentrations of plasma insulin, triglyceride, total cholesterol, and leptin. Compared with male pigs, females are more insulin-resistant and might therefore constitute better models for MetS (Christoffersen *et al.*, 2013, Christoffersen *et al.*, 2007).

In addition to diet-induced MetS, a genetically modified MetS model has been induced using a novel technique. Increased activity of proprotein convertase subtilisin/kexin type (PCSK)9 leads to lower liver LDL-receptor levels, a reduction in LDL uptake from the circulation, and thus in hypercholesterolemia and associated atherosclerosis. In human subjects, a PCSK9 gain-of function mutation (D374Y) can lead to LDL cholesterol levels increase (Abifadel *et al.*, 2003). Recently, Yucatan minipigs induced with liver-specific expression of human D374Y-PCSK9 displayed reduced hepatic LDL receptor levels, severe hypercholesterolemia, and spontaneous development of progressive aortic atherosclerotic lesions with human-like histopathological features (Al-Mashhadi *et al.*, 2013). This model may be useful for translational research in atherosclerosis, although it requires a relatively long induction period, often 12 months. Additional studies are also required to determine whether this model exhibits indices of MetS such as IR and hypertension.

MetS pig models have shown activation of distinct injurious features in many vital organs and tissues. Swine MetS has provided insight regarding obesity-induced alterations in the central nervous system, (Karmi *et al.*, 2010) digestive system, (Liang *et al.*, 2015) pancreas, (Fullenkamp *et al.*, 2011) gut microbiome, (Pedersen *et al.*, 2013) heart, (Neeb *et al.*, 2010) kidney, (Li *et al.*, 2011) reproductive, (Newell-Fugate *et al.*, 2014) and musculoskeletal (Karmi *et al.*, 2010) systems, as well as metabolic health in the offspring. (Arentson-Lantz *et al.*, 2014) On the other hand, the higher cost of large compared to small animals may limit the experimental sample size, and assessment of central obesity in swine is limited by missing firm standards for waist circumference or body-mass index. Nevertheless, this model holds promise for exploring clinically-relevant pathways of metabolic disorders.

In recent years the impact of MetS on the heart and kidney has been characterized in swine models.

MetS and the swine heart

A recent study showed that normal weight patients with MetS had a 1.6-fold and obese patients with MetS a 2.5-fold increased risk for death from cardiovascular events as compared with normal weight patients without MetS (Arnlov *et al.*, 2010). Pigs are useful models for the study of cardiovascular disease, owing to a 90% similarity of morphology and physical function of the cardiovascular system to human subjects (Smith and Swindle, 2006, Hughes, 1986).

We have shown in MetS Ossabaw pigs that a 16-week high-fat/high-fructose diet induced a 30% increase in the rate-pressure product, impaired myocardial perfusion, and blunted response to vasoactive challenge by adenosine (Li *et al.*, 2012), and myocardial oxygenation was greatly reduced in both Ossabaw and domestic MetS models (Pawar *et al.*, 2015, Li *et al.*, 2012). MetS diet also accelerated formation of atherosclerotic plaque and aggravated in-stent stenosis in both Yucatan and Ossabaw pigs, but to a greater extent in the latter breed (Neeb *et al.*, 2010). Ossabaw MetS pigs also showed decreased microvascular density in the sub-epicardial myocardium, as shown by micro-computed tomography (Li *et al.*, 2014) (Figure 1B), indicating adverse effects of MetS in both the cardiac macro- and micro-circulation. Altered myocardial vascular function in Ossabaw pigs fed with atherogenic diet

has been linked to decreased myocardial endothelial nitric oxide (NO) synthase functionality and NO bioavailability (Bradley *et al.*, 2015), and in Yucatan pigs to impaired function of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (Mokelke *et al.*, 2005). MetS in Ossabaw pigs induced by high-cholesterol diet also impairs signaling of cardiac angiogenesis (Elmadhun *et al.*, 2014a) thereby restricting the compensatory capacity of the myocardium for recruiting adequate blood and oxygen supplies.

In Ossabaw MetS models, myocardial oxidative stress was found to be increased and antioxidant enzymes reduced (Bradley *et al.*, 2015). Moreover, MetS attenuated myocardial autophagy in response to overwhelming nutrient supplies which were observed at both early (3 months) (Li *et al.*, 2012) and late (9 months)(Sabe *et al.*, 2014) stages of MetS. Therefore, attenuation of autophagy, a housekeeping process to maintain cellular energy homeostasis, may have profound influence on myocardial viability and cardiac adaptability in MetS. Additionally, in Ossabaw pigs with superimposed renovascular hypertension, we found that MetS magnified downregulation of mitochondrial proteins and activity, (Zhang *et al.*, 2015) and synergistically exacerbated the diastolic dysfunction and myocardial fibrosis induced by hypertension (Zhang *et al.*, 2015). Importantly, in Yucatan pigs chronic (7 months) MetS may lead to cardiac IR, along with impaired insulin signaling such as PI3-kinase activation, Akt phosphorylation and abnormal phosphorylation of insulin receptor substrate-1 (Lee *et al.*, 2010). These effects may further inhibit proper cardiac energy utilization and function. These findings suggest multiple potential pathways that mediate the myocardial injury in MetS, yet their roles in directing pharmacological exploration need to be carefully examined.

MetS and the swine kidney

Patients with 1–2 and those with ≥3 traits of MetS are, respectively, 80 and 130% more likely to have microalbuminuria than those without the syndrome (Hoehner *et al.*, 2002). The incidence of chronic kidney disease in patients with MetS increases progressively with the individual number of the MetS components (Chen *et al.*, 2004). These studies suggest a close link between MetS and renal dysfunction, but their mechanistic relationship is incompletely understood, partly because of the need for appropriate experimental platforms. One of the advantages of pigs as experimental models of kidney research, compared with rats and dogs, is that their kidneys are more similar in anatomy and physiology to the human kidney (Yokota *et al.*, 1985, Schook and Tumbleson, 1996). The porcine kidney is multi-pyramidal with an undivided cortex, and has several different medullary structures. Each medullary pyramid forms a separate papilla and fusion results in the formation of some compound papillae. Pig also possesses maximal urine concentration, glomerular filtration rate (GFR), and renal blood flow (RBF) similar to humans (Sachs, 1994). These important properties render the swine a suitable model to study renal disease, including the effects of MetS on the kidney.

Both Mets Ossabaw and domestic pig models almost double their RBF and GFR upon MetS diet feeding (Li *et al.*, 2011, Ma *et al.*, 2015). In Ossabaw pigs, the augmented renal hemodynamic indices are associated with increased renal cortical volume and microvascular density (Li *et al.*, 2012), linking augmented hemodynamics and microcirculatory remodeling

in the development of kidney hypertrophy (Li *et al.*, 2011). Interestingly, neither circulating nor renal inflammatory and oxidative stress markers in the MetS Ossabaw pigs were different from the control group at 10 weeks (Li *et al.*, 2011), but increased renal deposit of triglycerides was positively associated with increased GFR (Li *et al.*, 2011), suggesting a possible role for excessive lipid deposit in kidney injury in early MetS.

Nevertheless, the enhanced renal hemodynamic state does not seem to be reno-protective. Instead, the proximal and Henle's tubular flow in the MetS kidneys was found to be slower than in healthy pig kidneys, and associated with marked proximal tubular vacuolization, suggesting tubular degenerative changes and atrophy (Li *et al.*, 2011). Furthermore, tubulo-interstitial fibrosis becomes evident in Ossabaw pigs after 16 weeks of diet, although RBF and GFR remain elevated (Zhang *et al.*, 2013). By 16 weeks of diets, MetS Ossabaw pigs exhibited increased renal inflammatory macrophages and plasma ox-LDL levels (Zhang *et al.*, 2013), indicating that inflammation may play a role at later stages of renal injury. Both MetS Ossabaw and domestic pig kidneys are also encased in greater amounts of perirenal fat compared with control kidneys, enriched in inflammatory macrophages and cytokines, including TNF-α(Zhang *et al.*, 2013, Ma *et al.*, 2015). At least in the domestic pig MetS model, the peri-renal fat may not only serve as an inflammatory depot but may also impair renal artery endothelial function (Ma *et al.*, 2015). These findings may have implications in the setting of therapeutic management of MetS-induced kidney disease.

MetS and the adipose tissue

Visceral obesity is a major component of MetS. In high-energy diet induced-MetS impairments in glucose and insulin metabolism are associated with an increase in accumulation of body fat (Christoffersen *et al.*, 2013). We have recently shown that in domestic pigs both abdominal fat tissue volume and adipocyte sizes progressively increased over 16 weeks of MetS diet, accompanied by a parallel increase in intra-adipose capillary count and fibrosis (Pawar *et al.*, 2015). The adipokines adiponectin and leptin are reciprocally regulated by obesity (Spurlock *et al.*, 1998, Jacobi *et al.*, 2004, Pawar *et al.*, 2015). Indeed, MetS upregulates in porcine adipose tissue the release of abundant inflammatory adipokines from the adipose tissue, such as TNF-α, interleukin-6, and monocyte chemotactic protein-1 (Pawar *et al.*, 2015), and stimulates macrophage infiltration (Ma *et al.*, 2015, Zhang *et al.*, 2013). Furthermore, MetS upregulates the expression of toll-like receptor in porcine adipose tissue (Gabler *et al.*, 2008), which is closely associated with development of IR (Shi *et al.*, 2006). Importantly, in the domestic MetS model, the inflamed adipose tissue may affect the organ it encapsulates, at least partly by disrupting the vascular endothelial function, which can be restored by TNF-α inhibition (Ma *et al.*, 2015). Similarly, in Ossabaw MetS, perivascular fat impairs coronary endothelial function, (Payne *et al.*, 2010) and is linked to atherosclerosis formation in the coronary artery wall (McKenney *et al.*, 2014). Whether these changes are similar to human events is unclear and further investigations would be useful to provide more insight in this area.

Experimental Treatment in MetS swine model

Lifestyle changes are the essential and fundamental management strategies in MetS. In a swine model, contemporary pigs fed a Paleolithic diet consistent with the hunter-gather lifestyle of our ancestors are leaner, more sensitive to insulin, and have lower circulating concentrations of C-reactive protein than their counterparts fed a cereal diet reflective of modern-day habits (Jonsson *et al.*, 2006), signifying the importance of dietary modification in alleviating the progression of MetS.

Additionally, medications have been shown to modulate cellular turnover and improve tissue viability. The antidiabetic drug metformin confers a survival advantage in patients with cardiovascular disease, and was recently found to selectively downregulate the apoptosis pathway in MetS pigs and upregulate cardioprotective proteins including mitogen-activated kinase proteins p38 and extracellular signal-regulated protein kinases 1 and 2. (Elmadhun *et al.*, 2014b) Atorvastatin elicits a net decrease in apoptosis as well, (Sabe *et al.*, 2015) and also prevents myocardial autophagic dysfunction produced by MetS, which may in part account for its cardioprotective effect. (Sabe *et al.*, 2014) On the other hand, cholesterol levels in domestic pigs are less responsive to statins than humans (Hasler-Rapacz *et al.*, 1996), possibly due to some differences from humans in lipid metabolism. Like rats and dogs, pigs have low plasma activity of cholesteryl-ester transport protein, and manifest a high HDL and low LDL distribution. (Yin *et al.*, 2012) Many traditional models, including rabbit, Zucker diabetic fatty rat, and mice, do not show overall similarity to dyslipidemic humans. Non-human primates exhibit the most similar lipid profile compared to humans with respect to basal plasma total cholesterol, LDL/HDL ratio, and lipoprotein traces, but ethical issues restrict their widespread use as experimental models. Furthermore, inter-species differences in cholesterol handling might also be related to the primary site for fatty acid synthesis, which is the liver in humans, the adipose tissue in pigs, and both the liver and adipose tissue in rodents and rabbits (Nafikov RA, Beitz DC: Carbohydrate and lipid metabolism in domestic animals. (Nafikov and Beitz, 2007)

Treatment with sustained-release-nitrite reduces myocardial oxidative stress while increasing myocardial antioxidant capacity and NO bioavailability, and is associated with marked improvement in vasoreactivity of coronary arteries. (Bradley *et al.*, 2015) In addition, resveratrol polyphenol, which is often found in high concentrations in red wine and considered cardioprotective, improves regional ejection fraction and myocardial perfusion in the high-cholesterol diet induced-MetS heart with or without chronic ischemia (Robich *et al.*, 2012, Sabe *et al.*, 2013), as well as body mass index (Sabe *et al.*, 2014). Such beneficial effects are thought to be mediated through binding to its primary target protein sirtuin-1, a key regulator of energy metabolism (Li, 2013), and activating NO synthase (Robich *et al.*, 2012). In a Chinese Guizhou pig MetS model, a lipoprotein lipase activator (ibrolipim) decreased ectopic lipid deposition, improved IR, and alleviated the beta cell damage (Yin *et al.*, 2004).

Clearly, these studies have markedly advanced the understanding and identification new pathways contributing to MetS and their interaction with targeted pharmacological agents. Nevertheless, drug metabolism in MetS pigs requires cautious assessment. More studies are

therefore needed to gauge optimal dosages of anti-MetS drugs and to decrease the potential of MetS subjects of experiencing life-threatening toxicity or lack of effects.

Conclusion

It is becoming increasingly apparent that both minipigs and larger pig strains are valuable models to mimic human MetS and test new treatment strategies. Additional advantages of the pig are the ability to use standard diagnostic and treatment technologies like in humans, and collect body fluid and tissues in adequate quantity especially in larger swine, thereby accelerating clinical translation. Future studies need to continue exploring the molecular basis of MetS and its comorbidities, as well as pathogenesis of MetS in other vital organs such as liver (steatohepatitis). Studies that focus on validation of the MetS models by controlling the severity of symptoms of MetS and organ-specific complications through developing and optimizing novel therapeutics would be of great significance.

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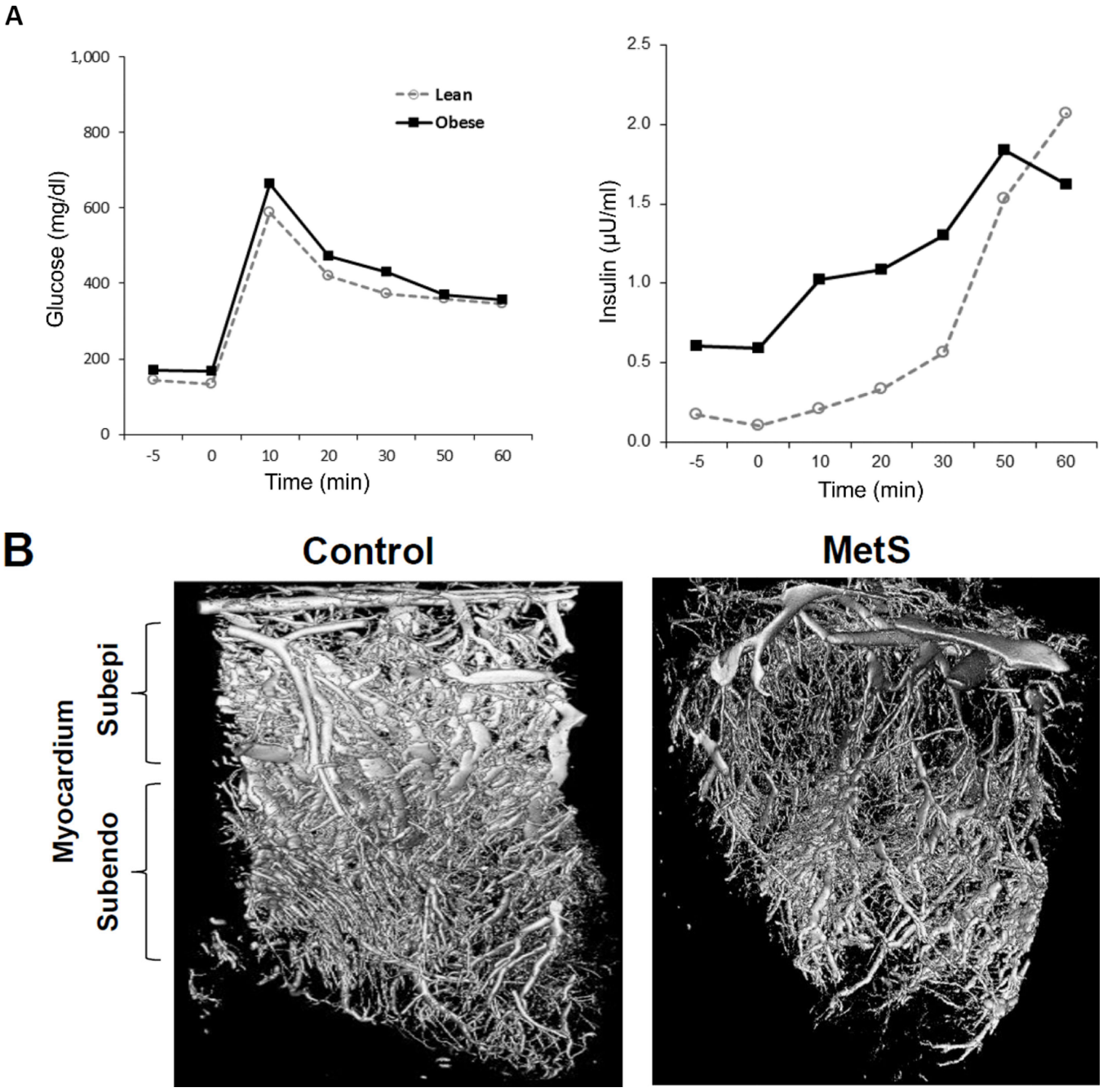

Figure 1.

A. Plasma insulin levels during an intravenous glucose tolerance test in Ossabaw pigs fed with MetS or control diets.

B. Metabolic syndrome in Ossabaw pigs decreased microvascular density in myocardium compared with control using three-dimensional micro-computed tomography.

Table 1

Nutrient compositions of different diets used to induce the metabolic syndrome in pigs

Studies	Fat (%)	Carbohydrate (%)	Fructose (%)	Sucrose (%)	Cholesterol (/Kg)	Sodium cholate (/Kg)
Lee et al., 2010	45	47	11g/Kg			
Elmadhun et al., 2014a					4	1.5
Borbouse et al., 2009	43	20	20		2	0.7
Li et al., 2012	43	40.8	17.8	1.05	2	0.7
Pawar et al., 2015	43	40.8	17.8	1.05	2	0.7
Zhang et al., 2013	43	40.8	17.8	1.05	2	0.7
Robich et al., 2012					4	1.5
Bradley et al., 2015	43		19		2	0.7
Christoffersen et al., 2013	30.7			30.9		
Xi et al., 2004	12.5	69.6		37	10	
Sabe et al., 2014						
Ma et al., 2015	43	40.8	17.8	1.05	2	0.7
te Pas *et al.*, 2013	250/kg		150/kg	100/kg	10/kg	
Johansen *et al.*, 2001	51.3					
Lee *et al.*, 2009	46	43	20	0.9		
Li *et al.*, 2015	45.7	35.3		20		
McKenney *et al.*, 2014	42.9	40.8	19		2.0	0.7

Table 2

Pig characteristics and manifested metabolic syndrome (MetS) components induced by MetS diets.

Studies	Breed	Gender	Age	Diet duration	Reported metabolic syndrome components
Lee et al., 2010	Yucatan	male and female	3 months	6 months	Obesity, hypertension, increased cholesterol, increased TG, insulin resistance
Elmadhun et al., 2014a	Ossabaw	male	6 weeks	9 weeks	Obesity , hypertension, increased cholesterol, impaired glucose intolerance
Borbouse et al., 2009	Ossabaw	male	12 months	3–6 months	Obesity, hyperglycemia, hyperinsulinemia, increased cholesterol, increased LDL/HDL ratio, increased TG
Li et al., 2012	Ossabaw	female	3 months	14 weeks	Obesity, insulin resistance, increased cholesterol, increased LDL/HDL
Pawar et al., 2015	Domestic pigs	female	3 months	16 weeks	Insulin resistance, hypertension
Zhang et al., 2013	Ossabaw	female	3 months	16 weeks	Obesity, insulin resistance, inflammation
Robich et al., 2012	Yorkshire			11 weeks	Obesity, increased cholesterol impaired glucose tolerance
Bradley et al., 2015	Ossabaw	male and female	6–8 month	6 months	Ossabaw, obesity, hypertension, hyperglycemia, increased cholesterol, increased TG, increased LD/cholesterol ration,
Christoffersen et al., 2013	Göttingen	male and female	9 weeks	4 months	Obesity, insulin resistance, impaired glucose tolerance, increased cholesterol, increased TG, visceral adiposity. Changes appeared after 2 weeks.
Xi et al., 2004	Chinese Guizhou	male	3–4 months	6 months	Obesity, hyperglycemia, hyperinsulinemia, increased cholesterol, increased TG,
Sabe et al., 2014	Ossabaw	male		6 months	Obesity, impaired glucose tolerance,
Ma et al., 2015	Domestic pigs	female	3 months	16 weeks	Obesity, hypertension, increased cholesterol, insulin resistance, visceral adiposity
te Pas *et al.*, 2013	YorkshirexLand race		11 weeks	10 weeks	Hyperlipidemia, insulin resistance
Johansen *et al.*, 2001	Göttingen	female	9–10 months	5 weeks	Obesity, increased TG, hyperinsulinemia, impaired glucose tolerance,
Lee *et al.*, 2009	Ossabaw		5–10 months	24 weeks	Obesity, insulin resistance, hyperglycemia, hypertension
Li *et al.*, 2015	Lee-Sung (LS) and Lanyu (LY) Lee-Sung	male and female	5 months	6 months	Hypercholesterolemia, hypertriglyceridemia, obesity, hypertension, hyperglycemia
McKenney *et al.*, 2014	Ossabaw	male	6 months	6 months	Obesity, hypertension, increased LDL/HDL ratio, insulin resistance, increased TG, coronary artery atherosclerosis

TG: triglyceride; LDL: low-density lipoprotein; HDL: high-density lipoprotein.