Chronic exercise attenuates coronary artery disease (CAD) morbidity and mortality in humans, and nearly two-thirds of the benefit is independent of reductions in traditional risk factors (81). The profound implication is that the major protective mechanisms of exercise are direct actions within the coronary vasculature. Further, intensive control of one CAD risk factor in diabetes, i.e., blood glucose, increased mortality in long-term type 2 diabetes patients (1). These findings indicate that knowledge of the cellular and molecular mechanisms of exercise as an adjunct therapy to prevent diabetic CAD could provide insights into pathogenesis and other molecular therapies.

The heterogeneity of macrovascular and microvascular coronary arteries (109) prompts this minireview focus on macrovascular CAD (atherosclerosis) and, more specifically, the role of coronary smooth muscle (CSM) cells. It is also fully recognized that microvascular CAD is a problem in metabolic syndrome and diabetes. Atherosclerotic CAD progresses through stages shown in Fig. 1 (see Refs. 94 and 109 for review). The completely healthy stage of an epicardial conduit coronary artery is characterized by medial CSM cells in their differentiated contractile state with a single layer of endothelium comprising the intima (Fig. 1A). This is almost never seen
in the average person beyond adolescence in Western society. Instead, because of the presence of copious systemic risk factors, including sedentary lifestyle, thin layers of neointima form when lipids and other factors infiltrate the artery (Fig. 1B). The progression continues unabated to form often concentric, thicker neointima with increasing numbers of macrophages that are more lipid laden and transformed to foam cells (Fig. 1C). In addition to the neointima, the vascular media thickens due to CSM proliferation. More severe, complex lesions form in later stages in which CSM proliferation and migration into the neointima accelerate and fibrosis and calcification increase to form a flow-limiting stenosis (Fig. 1D) (76, 94, 109). Eccentric, complex lesions also include areas of soft atheroma and calcification (Fig. 1F). The severity and complexity of atherosclerotic lesions varies according to the magnitude of dyslipidemia and other CAD risk factors and the duration of exposure to the systemic risk factors (48). If the fibrosis and calcification do not form stable lesions, a more lipid-laden core may have just a thin fibrous cap and be susceptible to rupture (16, 76, 127). Such unstable, vulnerable plaque is the major cause of acute myocardial infarction characterized by ST segment elevation in the electrocardiogram and requiring revascularization with percutaneous balloon angioplasty and stenting or coronary bypass surgery. Despite these treatments, survivors of myocardial infarction face >10-fold increased risk of progression to heart failure and death (115).

METABOLIC SYNDROME AND DIABETES AS MAJOR RISK FACTORS FOR CAD

Rapid and robust accumulation of fat depots enabled by “thrifty” genes (22) was a natural, beneficial adaptation in earlier human cultures to adapt to feast and famine ecology, but the “thrifty genotype” is detrimental in our modern era of plentiful food sources and minimal physical activity. Extreme physical inactivity and poor diet that are now commonplace in modern lifestyles amplify manifestation of a thrifty genotype, as up to 27% of adults in the United States have obesity-associated metabolic abnormalities (34) and even young children are affected (104). Key components of the pathologies are propensity to central (intra-abdominal) obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension (38, 53, 104). Although the definition and precise clinical utility have recently been controversial (33), generally the presence of three of these characteristics renders a diagnosis of metabolic syndrome (MetS; “prediabetes”) (38). The diagnosis of type 2 diabetes is rendered by an increase in fasting plasma glucose, which typically occurs after >10 years in the prediabetic/MetS stage.
Hyperglycemia, hyper- and hypoinsulinemia, increased ratio of low-density lipoprotein to high-density lipoprotein (LDL/HDL) cholesterol, triglycerides (TG), and free fatty acids (FFA) are major components of the systemic MetS and diabetic milieu (Fig. 2; Refs. 37, 70, 94). In addition, chronic inflammation is thought to be key to macrovascular CAD (70, 94). A plethora of “outside-in” risk factors from perivascular adipose tissue (adipokines) represent another source of atherogenic factors (92, 98). A central concept is that MetS and diabetes increase CAD by damaging the coronary endothelium, which normally inhibits CSM contraction, migration, and proliferation (Fig. 2A; Ref. 94). Similar factors are thought to alter the contraction and relaxation of CSM in the microvasculature that regulates coronary blood flow (Fig. 2B). Aggregating platelets release a variety of growth factors, including ATP and UTP, and the dysfunctional endothelium allows these and other mitogens and the atherogenic milieu to stimulate CSM via several receptor signaling systems and ion channels as shown in the model in Fig. 2A, and this will be covered in more detail in the next sections. These tonic modulatory functions of the endothelium are mediated by the production and/or release of several vasoactive factors, collectively referred to as endothelium-derived relaxing factors [EDRFs; e.g., nitric oxide (NO), prostacyclin] and endothelium-derived contracting factors [EDCFs; e.g., endothelin (ET)] (for review see Ref. 94). The processes of migration and proliferation of CSM as key steps in atherosclerosis (94) involve dedifferentiation of the cells from their normal contractile phenotype to a synthetic, phenotypically modulated cell (90). Although contraction of CSM in a conduit artery (Fig. 2A, right) that results in coronary vasospasm is potentially life-threatening, it is a lesser problem clinically than atherosclerosis (106). MetS and type 2 diabetes patients are generally found to have increased activity of the renin-angiotensin-aldosterone system (RAAS, Ref. 21; Fig. 2), which further adds to the complexity of the milieu because of the contribution to “obesity hypertension” (Fig. 2; blood pressure; BP) (40). Type 1 diabetes, in contrast, generally involves less extreme dyslipidemia and RAAS activation and greater hyperglycemia compared with MetS and type 2 diabetes (21, 37, 70, 94, 105). Although the “ABCs” of diabetes treatment, i.e., hemoglobin A1C (glucose), blood pressure, and cholesterol management, can attenuate cardiovascular disease (1), adherence is difficult and costly, and intensive glycemic control could not be generally recommended for long-term, established type 2 diabetes due to increased mortality (1).

Despite significant improvements, CAD remains the leading cause of death in our society and is greatly exacerbated in MetS and diabetes (33). Because MetS is increasing in prevalence in our society to levels widely considered epidemic (128), more widespread progression of patients to type 2 diabetes will compound CAD morbidity and mortality, since CAD further increases to fourfold higher in diabetic vs. nondiabetic patients (27). Progression of CAD is greater in MetS and diabetes partly because of the pervasive “diffuse CAD” that is a hallmark of diabetic CAD (15, 82, 83), which was highlighted in a recent pooled analysis (87). Diffuse CAD argues for more systemic therapy afforded by exercise as opposed to localized treatment with coronary angioplasty and stenting.

**CHRONIC EXERCISE-INDUCED ADAPTATIONS**

Exercise is one of the first-line treatments for thus preventing or delaying the onset of type 2 diabetes, and decreases plasma lipids, blood pressure, cardiovascular events, and mortality (19). Given the multiple risk factors for CAD (Fig. 2, above), the integrative effects of exercise seem to be a substantial adjunct to pharmacotherapy, which would require a
“polypill” for such widespread benefit on multiple risk factors. Haskell et al. (42) were one of the first to report profound effects of exercise on coronary arteries in which they showed ultradistance runners had greater coronary artery diameters at rest and after maximal nitroglycerin-induced vasodilatation, suggesting that exercise training in humans elicited structurally larger coronary arteries due to vascular remodeling. Hambrecht and coworkers showed attenuation of plaque formation (99) and improved event-free survival in patients after angioplasty (41). Belardinelli et al. (3) found that exercise training decreased restenosis progression and the natural progression of CAD in arterial segments proximal and distal to the stent, i.e., decreased peri-stent CAD. Unfortunately, few human studies have included sufficient numbers of MetS or diabetic (type 1 or type 2) patients, nor have they studied cellular and molecular mechanisms (3; for review see 30).

Rigorous assessment of CAD. Overall, these significant, but relatively modest, effects on plaque progression and regression in humans do not account fully for the remarkable relief of symptoms of CAD and improved event-free survival. Since vulnerable plaque rupture is the major cause of acute myocardial infarction (above), exercise training-induced plaque stabilization may be much more important than reduction in stenosis (16). Studies that employ high-resolution imaging methods such as intravascular ultrasound, optical coherence tomography, magnetic resonance, near-infrared imaging, and others that enable chemical resolution of collagen and lipid (e.g., 125) will be needed for thorough characterization of the effects of exercise on CAD (127). Further, longitudinal measures of CAD, not just at the end of the study, will provide the most insights into CAD progression and regression (88).

Animal model of CAD, exercise, and the “diabetic milieu.” Invasive studies of cellular and molecular mechanisms of exercise effects on CAD require appropriate animal models. Phenomenal work has been done on transgenic and gene ablation (knockout) mouse models to understand mechanisms of MetS and diabetes, as summarized from work of the Animal Models for Diabetic Cardiovascular Complications (AMDCC) (49). However, transgenic mouse models are simply not adequate for studying CAD, not to mention vascular interventions using stents and catheter devices identical to those used in humans (28, 31, 74, 107, 116, 127), which are essential for translation to the clinic. Further, animal models that develop mature, clinically significant atheroma will provide vast improvement over studies that employ injury of healthy arteries (116). Particularly important for the study of diabetic CAD is use of a model that displays diffuse CAD, which is the hallmark (15, 82, 83, 87). Intravascular ultrasound assessment of CAD in Ossabaw miniature swine with MetS enables interrogation of much of the artery as shown in Fig. 1E. In this example, automated pullback of the intravascular imaging transducer along 60 mm of the coronary shows diffuse stenosis along much of the coronary artery. As predicted, in-stent stenosis is clearly greater along the 8-mm length of the stent compared with other segments of the artery. A more focal CAD occurs in hyperlipidemic Yucatan miniature swine that are not insulin-resistant, as shown by the percent stenosis decreasing to nearly zero in more distant artery segments (84). Progression to clinically detectable coronary calcification is shown in MetS Ossabaw swine in Fig. 1F (84). Although arterial calcium deposition has been shown microscopically in swine models postmortem (36, 125), detection by clinically used intravascular ultrasound in living animals is a significant milestone. Finally, in addition to the study of basic cellular and molecular mechanisms by invasive methods, animal models should ideally include hard clinical endpoints, such as spontaneous myocardial infarction and mortality for translation to the clinic (e.g., 115).

Link et al. (71) nearly 40 years ago reported that exercise training of domestic swine attenuated CAD and Krams et al. (55) showed profound regression of mature atherosclerosis in nonhuman primates. More recent studies by Fleenor and Bowles (31), Long et al. (74), and Edwards et al. (29) showed that exercise training profoundly decreased restenosis and native atherosclerosis progression. These are particularly strong studies because the exercise stimulus prescription was well quantified by intensity, duration, frequency, and total duration of training. Classical training adaptations were elicited, including lower resting heart rate and exercise heart rate at a submaximal workload, increased physical work capacity, and skeletal muscle oxidative enzymes (31, 74). These studies validate by widely accepted exercise training criteria the use of large animal models to study exercise and CAD (20). The next step is to superimpose MetS and diabetes on the background to elicit CAD. Studies to date have all been performed on swine.

The MetS and diabetic milieus have qualitative, quantitative (severity), and duration differences in animal models and it is essential that one be cognizant of these variables for interpretation of exercise study outcomes and CAD mechanisms (6, 29, 36). Table 1 compares Yucatan, Ossabaw, and Göttingen miniature swine, which are collectively the most widely used miniature swine breeds for laboratory animal medicine for MetS, diabetic dyslipidemia, CAD, and exercise training studies. Direct, quantitative comparisons are not ideal because no single study has systematically compared the three breeds when controlling for age, sex, diet composition calories, and duration of treatment. Neeb et al. (84) compared only Yucatan and Ossabaw using precisely matched conditions. The measures of MetS and diabetes are also not the same, e.g., intravenous glucose tolerance test or fasting values or glucose clamp (17, 29, 63, 89), which makes quantitative comparisons between all studies tenuous. See also the review by Varga et al. that encompasses swine and other animal models of MetS (120). Table 1 summarizes the six key components of MetS schematically shown in Fig. 2, progression to type 2 diabetes (item 7), and the incidence of CAD (item 8). 1) The first major characteristic of MetS is obesity. Yucatan pigs could be made only mildly obese by consumption of excess calorie atherogenic diet; in contrast, Ossabaw and Göttingen pigs are superior for studying the pathogenesis of obesity and related disorders. 2) Yucatan miniature swine do not naturally develop obesity-associated insulin resistance. 3) As insulin resistance is a precursor to glucose intolerance, Ossabaw and Göttingen pigs also show marked increases elicited by excess calorie diet. 4) A high fat and cholesterol diet elicits dyslipidemia (hypercholesterolemia) in domestic (36) and all three miniature swine breeds that are almost exclusively used in laboratory animal medicine and exercise studies (17, 24, 25, 52, 52, 77, 78, 130, 132). 5) The increased plasma triglycerides noted only in Ossabaw and Yucatan are probably linked to the decreased insulin action. Several decades of work on domestic and Yucatan breeds have shown no increase in plasma triglycer-
Table 1. **Metabolic syndrome, diabetes, and coronary artery disease characteristics in Yucatan, Ossabaw, and Göttingen miniature swine**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Yucatan</th>
<th>Ossabaw</th>
<th>Göttingen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Obesity</td>
<td>No</td>
<td>Yes</td>
<td>No data</td>
<td>11, 61, 14, 17, 18, 26, 32, 52, 56, 63, 64, 68, 84, 107, 129</td>
</tr>
<tr>
<td>2) Insulin resistance</td>
<td>No; secondary</td>
<td>Yes</td>
<td>Göttingen &gt; Yucatan</td>
<td></td>
</tr>
<tr>
<td>3) Glucose intolerance (or impaired glucose tolerance, [IGT])</td>
<td>No</td>
<td>Yes</td>
<td>No data</td>
<td>61, 65, 89</td>
</tr>
<tr>
<td>4) Dyslipidemia (↑ LDL/HDL or ↑ LDL/TC)</td>
<td>Yes</td>
<td>No data</td>
<td>No data</td>
<td>24, 44, 67, 77-79, 96, 123, 129, 130</td>
</tr>
<tr>
<td>5) Dyslipidemia (↑ triglycerides)</td>
<td>Yes</td>
<td>No data</td>
<td>No data</td>
<td>24, 44, 67, 78, 96, 123, 130</td>
</tr>
<tr>
<td>6) Hypertension</td>
<td>No</td>
<td>No data</td>
<td>No data</td>
<td>77, 89</td>
</tr>
<tr>
<td>7) Type 1 diabetes (fasting hyperglycemia)</td>
<td>Yes</td>
<td>No data</td>
<td>No data</td>
<td>24, 67, 78, 79, 123</td>
</tr>
<tr>
<td>8) Coronary artery disease</td>
<td>Yes; &gt;nondiab.</td>
<td>No data</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>CAD attenuated by exercise</td>
<td>Yes*; vasomotor</td>
<td>No data</td>
<td>No data</td>
<td></td>
</tr>
</tbody>
</table>

**Metabolic syndrome, type 2 diabetes, coronary artery disease**

- 1) Obesity
- 2) Insulin resistance
- 3) Glucose intolerance (or impaired glucose tolerance, [IGT])
- 4) Dyslipidemia (↑ LDL/HDL or ↑ LDL/TC)
- 5) Dyslipidemia (↑ triglycerides)
- 6) Hypertension
- 7) Type 2 diabetes (fasting hyperglycemia)
- 8) Coronary artery disease

**Diabetes (toxin-induced type 1) and dyslipidemia, coronary artery disease**

- 1) Obesity
- 2) Insulin resistance
- 3) Glucose intolerance (or impaired glucose tolerance, [IGT])
- 4) Dyslipidemia (↑ LDL/HDL or ↑ LDL/TC)
- 5) Dyslipidemia (↑ triglycerides)
- 6) Hypertension
- 7) Type 1 diabetes (fasting hyperglycemia)
- 8) Coronary artery disease

All studies cited include atherogenic diet (high fat/cholesterol; often excess calorie). *Characteristics 1–6 define metabolic syndrome (MetS) and metabolic abnormalities, and characteristic 7 defines diabetes specifically. All type 1 diabetes models required pancreatic beta cell-selective toxins alloxan or streptozotocin. “No data” denotes that there were no studies or the study did not include these measures. LDL; low-density lipoprotein; HDL, high-density lipoprotein; TC, total cholesterol. *Conduit artery vasomotor responses were attenuated, not atherosclerosis. Highlighted entries are underlined.
ides, until atherogenic diet was combined with impaired insulin action in these breeds by chemically induced diabetes (e.g., 36, 67; and see below). 6) Genetically leaner Yucatan pigs made mildly obese and hyperlipidemic by consumption of excess caloric atherogenic diet did not become hypertensive (11, 130) and blood pressure measures have not been reported for Göttingen. In summary, Ossabaw miniature swine fed a high-caloric diet display a natural pathogenesis of all MetS characteristics (84). 7) Yucatan and Göttingen swine do not progress to type 2 diabetes (e.g., 63, 89, 93). Ossabaw pigs develop type 2 diabetes as evidenced by a significantly increased fasting plasma glucose of ~30% above healthy lean pigs. This degree of fasting hyperglycemia clearly shows only the earliest diagnosis of type 2 diabetes and is not nearly the magnitude commonly found in type 2 diabetic humans (1) or in rodent models (37, 120); thus, longer duration is probably needed to elicit robust type 2 diabetes in Ossabaw swine. Göttingen pigs, however, will reliably develop mild MetS (17, 52, 63). Although outstanding work shows that a line of cross-bred domestic pigs with familial hypercholesterolemia will develop MetS (5), use of the standard-sized domestic swine is not practical because they weigh >250 kg and are 2 yr of age before type 2 diabetes develops. A 250-kg pig is not amenable to use of conventional treadmill exercise equipment and angiography instrumentation needed for clinically relevant atherosclerosis assessment and stent deployment. 8) All three breeds develop CAD (coronary atherosclerosis) when fed an atherogenic diet. CAD is more widely documented in Yucatan and Ossabaw breeds (e.g., 84), while there are few studies of atherosclerosis in Göttingen (50) and Göttingen-Yucatan cross-bred pigs (47). There are no studies of coronary catheter interventions in lean Göttingen pigs, almost certainly due to the diminutive size of only ~20–30 kg, which would preclude use of standard human clinical devices. Direct, carefully controlled comparison showed that Ossabaw developed more extensive and diffuse CAD and restenosis responses to stenting than Yucatan pigs (84). In Yucatan and Ossabaw pigs exercise training attenuated CAD. Highlights of the MetS and type 2 diabetes section of Table 1 are underlined. The finding that dyslipidemia, in the absence of insulin resistance or hyperglycemia, elicited CAD in Yucatan pigs affirms a major role for plasma lipids. Despite the outstanding metabolic data in the Göttingen, there is a paucity of cardiovascular and CAD data (47, 50) and there have been no exercise training studies.

Inducing type 1 diabetes (plus or minus dyslipidemia) is relatively straightforward in swine by pancreatic beta cell toxins streptozotocin or alloxan, which yield within ~24 h plasma glucose values ranging from 200 to 400 mg/dl, depending on the specific doses of toxin (e.g., 11, 25, 36, 62, 89; Table 1, item 7). The lesser role of glucose in atherosclerosis (36, 78) was the reason for including in Table 1 only studies using the combination of chemically induced diabetes and atherogenic diet to promote dyslipidemia, while excluding studies of purely toxin-induced diabetic (hyperglycemic) and normolipidemic pigs when fed normal low-fat/cholesterol diet. The comparisons in Table 1 exclude Ossabaw swine because they have been used solely for studies of the natural progression of diet-induced obesity, MetS, type 2 diabetes, and CAD; thus there are no data on beta cell toxin-induced models of type 1 diabetes. 1) Similar to the above, the Göttingen shows more robust obesity than Yucatan pigs. 2) Insulin resistance in the Yucatan is secondary to the profound hyperglycemia, not due to primary insulin resistance of peripheral target tissues (mainly skeletal muscle and adipose) (89). In the Göttingen pig insulin resistance is partially due to primary insulin resistance and secondarily due to the streptozotocin destruction of beta cell mass (61, 65). 3) Clearly, when insulin-producing pancreatic beta cells are destroyed, there is profound glucose intolerance. 4) Although there are no data on plasma lipids in the streptozotocin-treated and atherogenic diet-fed Göttingen, it is reasonable to assume that LDL/HDL and triglycerides would be elevated above healthy controls based on data from atherogenic diet feeding alone (50, 52, 64). 5) Increased plasma triglycerides in Yucatan have only been found reliably in chemically induced diabetic pigs, again reinforcing the linkage to decreased insulin action (e.g., 36, 67). 6) It is clear that there is no hypertension in Yucatan swine, despite the extreme diabetic dyslipidemia, thus suggesting other components of the MetS milieu drive hypertension. I could find no data on hypertension in Göttingen pigs. 7) Fasting hyperglycemia in graded severity has been achieved in several studies and, despite chemically induced diabetes being an artificial experimental intervention, not natural pathogenesis, this diabetes model is an excellent means of studying the effect of the very extreme diabetic dyslipidemic milieu on CAD. 8) CAD in diabetic dyslipidemic Yucatan was greater than in normoglycemic (nondiabetic) dyslipidemic pigs when postprandial lipids were not greater than fasting (36, 67), thus validating the swine model by its mimicry of the greater CAD in diabetic humans (15, 27, 82, 83). However, when postprandial lipids (i.e., triglycerides) were much greater than fasting due to a “gorging” meal regimen (130), then diabetes (hyperglycemia) did not further exacerbate the CAD (78). There are no data in Göttingen pigs. It is important to note (asterisk in Table 1) that the only measures of exercise training attenuation of macrovascular and microvascular CAD were decreases in vasomotor impairment, not atherosclerosis (77–79). Exercise training has not been studied in the Göttingen pig. A summary of the diabetic dyslipidemia section of Table 1 is the preponderance of metabolic and CAD data in the Yucatan pig, while there is a paucity of CAD data in the Göttingen and no data whatsoever on Ossabaw swine. A striking observation overall is that exercise training had almost no effect on metabolic parameters (insulin resistance, glucose regulation, lipids, hypertension) in Yucatan and Ossabaw swine, yet there was attenuation of CAD (29, 31, 71, 74, 77–79).

The importance of the diabetic milieu is epitomized by the greater susceptibility of microvasculature to glucose toxicity shown in other animal models (37) and humans in the Diabetes Control and Complications Trial [DCCT (114)]. In contrast, it was clearly shown that dyslipidemia is a much greater factor than hyperglycemia in macrovascular CAD (atherosclerosis) in swine models (36, 78), consistent with the greater CAD in type 2 diabetic humans who are generally more dyslipidemic compared with type 1 diabetics (105). Another example of the potentially major impact of the specific diabetic milieu on CAD is that increased aldosterone is more predominant in MetS and type 2 diabetes compared with type 1 diabetes (21, 94) and aldosterone is a powerful inducer of coronary calcification (51, 76). Further, the RAAS activation in MetS could explain why “obesity hypertension” is found in the Ossabaw swine model (40). Because most of exercise training-induced
cardioprotection is not due to reduction of traditional risk factors (81), the diabetic milieu must be well characterized to draw the conclusion that exercise effects are partly due to direct actions on the vasculature. Studies in experimental animals described in the next section provide support for this.

**CORONARY SMOOTH MUSCLE (CSM) Ca²⁺ REGULATION**

It is clear that cytosolic Ca²⁺ is a primary regulator of smooth muscle contraction and Ca²⁺ signaling is involved in “phenotypic modulation” of CSM (90), characterized by proliferation and migration in several in vitro cell culture models (46, 121, 122). Although the focus here is Ca²⁺ signaling, I recognize that there are numerous other mechanisms contributing to atherosclerosis (e.g., platelet aggregation and release of growth-promoting and vasoactive molecules, lipid accumulation, adhesion molecules, macrophage invasion, immune response, increased extracellular matrix production, etc.). Rapidly proliferating CSM are characterized by decreased smooth muscle myosin and actin contractile proteins (90), decreased contraction (90), increased amount and perinuclear distribution of sarcoplasmic reticulum (SR) (46, 90, 123), altered membrane ionic signaling mechanisms, and increased DNA synthesis (90). Phenotypic modulation of CSM in atherosclerosis also involves dedifferentiation to a more osteogenic phenotype that contributes to vascular calcification (51, 76). Underwood et al. (118) and Bowles and Wamhoff (13) reviewed CSM Ca²⁺ regulation in exercise, but not in CAD. In essence, they considered how exercise converted CSM in healthy arteries (Fig. 1A) to super-healthy and/or primed the CSM to defend against atherogenic factors. I review salient features of those data on healthy CSM that have bearing on CSM Ca²⁺ regulation in MetS and diabetes, which are summarized schematically in Fig. 3.

**Ca²⁺ regulation in healthy CSM.** Studies of CSM from Yucatan and domestic swine have been conducted over the past almost 20 years. I focus on CSM from sedentary animals and ignore the changes in diabetic dyslipidemia and MetS in Fig. 3A, left. In all cases the data summarized in Fig. 3 are from direct functional measures of intracellular free Ca²⁺ with fluorescent indicators (fura-2, fura red, fluo-4) using widefield epifluorescence and confocal microscopy, patch-clamp electrophysiology of ion channels (including simultaneously with imaging), and molecular measures of protein and mRNA expression. Use of acutely isolated CSM cells, instead of cultured cells exposed to “diabetic milieu,” provide more confidence that the findings represent most closely Ca²⁺ regulation in the intact organism. There is almost uniform agreement that localized subsarcornemal Ca²⁺ (Fig. 3A, Caₔ) is regulated at higher levels than bulk cytosolic Ca²⁺ (Fig. 3A, Caₓ) (103). These gradients of Caₔ and Caₓ are largely maintained by a superficial buffer barrier (SBB; Fig. 3A; 109, 119) that involves close proximity of sarcornemal ion channels and transporters with superficial sarcoplasmic reticulum (SR). Ca²⁺ entering CSM via voltage-gated Ca²⁺ channels (VGCC; Fig. 3A) is rapidly sequestered by the SR Ca²⁺ pump (SERCA) superficial SR, thus buffering (attenuating) strongly the rise in Caₓ (108, 109, 118). Ca²⁺ influx through ligand-gated channels, now known widely as transient receptor potential canonical (TRPC; Fig. 3A) channels can also refill the SR or participate in excitation-contraction coupling, excitation-contraction coupling, and other processes. Preferential release of Ca²⁺ from the superficial SR toward the sarcolemma in the regions of the SR Ca²⁺ pump and Na⁺/Ca²⁺ exchange results in Ca²⁺ extrusion from CSM, a process that we first termed “SR Ca²⁺ unloading” (102, 103). The mechanism also includes internal cycling of Ca²⁺ between the SR and another intracellular Ca²⁺ store during SR Ca²⁺ unloading (131). Mitochondria (Fig. 3A) was suspected as the store, but selective inhibitors of mitochondrial Ca²⁺ transport were not used to clearly identify the store. Alternatively, superficial SR Ca²⁺ release and occurrence of localized “Ca²⁺ sparks” can be coupled to stimulation of large-conductance, Ca²⁺-activated K⁺ channels. The hyperpolarization acts as a negative feedback system to close VGCC, thereby decreasing Ca²⁺ influx (Fig. 3A, K_ca; circled minus sign, and dashed line; 77, 78, 86, 103). This functional association of Ca²⁺ sparks and K_ca is another prime example of vascular heterogeneity, as microvascular CSM has strong coupling of Ca²⁺ sparks and K_ca, while healthy conduit CSM has minimal coupling (77, 109). Conduit CSM displays very intricate morphology of the transnuclear SR, which functionally forms a nuclear buffer barrier (NBB; Fig. 3A; 109, 121, 123). The specificity of CSM Ca²⁺ signaling is further reinforced by comparing the effects of receptor agonists. For example, endothelin elicits a substantial release of Ca²⁺ from the SR, which decays to nearly baseline levels (67, 121, 123). The results are CSM proliferation and the most efficacious contraction of any coronary vasoactive agent. In contrast, although the UTP-induced Ca²⁺ transient in CSM of in vitro organ cultured arteries is robust and similar in amplitude as the response to endothelin, the UTP-induced Ca²⁺ transient does not elicit contraction of CSM (46); instead, the Ca²⁺ transient and kinase activation elicit CSM proliferation only (101). This principle of specificity is applicable to the effects of MetS, diabetic dyslipidemia, and exercise training.

**Exercise training effects on CSM Ca²⁺ regulation in diabetic dyslipidemia.** The past decade of work has shown altered regulation of several domains of intracellular free Ca²⁺ in diabetic dyslipidemia. I show the spatial relationships in Fig. 3A and list the many changes in Table 2, **bottom [Diabetes (toxin-induced type 1) and dyslipidemia]** because there are at least 10 Ca regulatory processes, proteins, and membrane domains that influence Ca signaling and the diabetic milieu is different in the studies of diabetic dyslipidemic Yucatan pigs. Cytosolic Ca²⁺ (Caₔ) and nuclear Ca²⁺ (Fig. 3A, Caₓ) are modulated by several Ca²⁺ transporters in diabetic dyslipidemia-induced CAD. The sarcomemal Ca²⁺ pump (CP; Fig. 3A) appears to be the first Ca²⁺ transporter to be impaired in diabetes (130), along with loss of the coupling of SR Ca²⁺ release from ryanodine-sensitive SR Ca²⁺ channels (CR; Fig. 3A) to Ca²⁺ extrusion via the sarcomemal Ca²⁺ pump and Na⁺/Ca²⁺ exchange (NX; Fig. 3A). The coupling is functional and physical, as digital imaging of fluorescent markers of the SR and sarcomemal shows that the SR retracted significantly from the sarcomemal (Fig. 3A, red double arrow; 130). The distance of the SR from sarcomemal was returned to healthy control after exercise training of diabetic dyslipidemic pigs (Fig. 3B, green double arrow; 130). Conduit (macrovascular) CSM may compensate for the defective Ca²⁺ extrusion by the upregulation of K_ca channels in the sarcomemal that are activated by Ca²⁺ sparks and other Ca²⁺ release from the SR (77, 78). These large and transient K_ca currents activated are
Fig. 3. Schematic model of Ca²⁺ regulation abnormalities in macrovascular CSM in diabetic dyslipidemia and MetS and attenuation of abnormalities by exercise training. A: sedentary. CSM cells from a sedentary pig show abnormalities of at least 10 Ca²⁺ regulatory processes, proteins, and membrane domains that influence Ca²⁺ signaling. The red font and structures denote functional and molecular changes (actual protein expression or membrane domain differences). For example, SERCA function and SERCA2b protein (S) are increased in diabetic dyslipidemia. There is a retraction of the SR from the sarcolemma (long red arrow), thereby impairing the superficial buffer barrier (SBB) function. Overall, decreased Ca²⁺ extrusion via the plasmalemmal Ca²⁺ pump and Na⁺/Ca²⁺ exchange is a major defect in Ca²⁺ regulation. SERCA and Kₐᵣ, channel function increase, including a closer coupling of Ca²⁺ release from caffeine-sensitive SR Ca²⁺ release channels and increased subsarcolemmal Ca²⁺ (Caₙ), perhaps partially compensating for other defects. [The IP₃-sensitive and caffeine/ryanodine-sensitive Ca²⁺ release channels (CR) are shown generically as one channel for simplicity, but are not the same molecular entity.] Voltage-gated Ca²⁺ channel current is downregulated. The net effect of these Ca²⁺ regulation abnormalities is increased cytosolic Ca²⁺ (Cac). The transnuclear SR membrane is retracted (dashed lines), thereby impairing the nuclear buffer barrier (NBB), and is associated with increased localized nuclear Ca²⁺ (Can) responses to endothelin in diabetic dyslipidemia. CSM in MetS have impaired Ca²⁺ extrusion and SERCA pump function. TRPC channel functional Ca²⁺ influx (red arrow in channel) and TRPC1 isoform protein expression (red channel structure) are increased, which are very closely associated with increased STIM1 protein expression. There is decreased Kᵥ₉, channel function, despite increased molecular expression. The precise subcellular localization of Ca²⁺ transport molecules and processes is emphasized for the crucial role in CSM function.

B: exercise training. Aerobic exercise training attenuates 8 of the abnormalities in Ca²⁺ transport protein and membrane domains. The green font and structures denote those changed by exercise training. For example, the shorter double green arrow shows the shorter distance of the SR from sarcolemma after exercise training, thus attenuating the defect in SBB. The effects of exercise training on Ca²⁺ regulation are relatively independent of changes in the diabetic dyslipidemia or MetS milieu (shown by the red letters). There have been no studies to determine whether exercise attenuates abnormalities of the NBB, plasmalemmal Ca²⁺ pump and Na⁺/Ca²⁺ exchange, SERCA, Kᵥ₁₀, channels, or Caₙ (shown by “?”). See text and Table 2 for more details. ET, endothelin, receptor; TRPC, transient receptor potential canonical channels; VGCC, voltage-gated Ca²⁺ channels; CP, plasmalemma Ca²⁺ ATPase (PMCA pump); NX, Na⁺/Ca²⁺ exchange; Kᵥ₉, Ca²⁺-activated K channels; Caₙ, subsarcolemmal Ca²⁺; Mito, mitochondria; IP₃, inositol trisphosphate; STIM1, stromal interaction molecule type 1; CR, ryanodine-sensitive Ca²⁺ release channels; Caₙ, cytosolic Ca²⁺; NPC, nuclear pore complex; Caₙ, nuclear Ca²⁺; MetS, metabolic syndrome; S = SERCA = sarcoplasmic-endoplasmic reticulum Ca²⁺ ATPase; SR, sarcoplasmic reticulum; †, hyperpolarization-induced stimulation of ion flux; ‡, hyperpolarization-induced inhibition of channel opening; ↑, increased; ↓, decreased; |, attenuated or returned diabetic dyslipidemia- or MetS-induced changes toward normal; red dashed lines, retracted transnuclear SR.
Table 2. Coronary vascular smooth muscle Ca\textsuperscript{2+} signaling abnormalities in Yucatan and Ossabaw miniature swine and effects of exercise training

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Yucatan</th>
<th>Exercise</th>
<th>Ossabaw</th>
<th>Sedentary</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Plasmalemmal Ca\textsuperscript{2+} pump</td>
<td>No Δ</td>
<td>No data</td>
<td>No data</td>
<td>84, 130, 132</td>
<td></td>
</tr>
<tr>
<td>2) Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange</td>
<td>No Δ</td>
<td>No data</td>
<td>No data</td>
<td>84, 130, 132</td>
<td></td>
</tr>
<tr>
<td>3) SERCA</td>
<td>NoΔ mild CAD;</td>
<td>No data</td>
<td>↑ moderate CAD;</td>
<td>84, 130, 132</td>
<td></td>
</tr>
<tr>
<td>4) Voltage-gated Ca\textsuperscript{2+} channel</td>
<td>↓</td>
<td>No data</td>
<td>↓ severe CAD</td>
<td>84, 130, 132</td>
<td></td>
</tr>
<tr>
<td>5) TRPC channel, store-operated Ca\textsuperscript{2+} entry</td>
<td>Absent</td>
<td>Absent</td>
<td>↑</td>
<td>29, 132</td>
<td></td>
</tr>
<tr>
<td>6) STIM1</td>
<td>No data</td>
<td>No data</td>
<td>↓</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>7) K\textsubscript{Ca} channel</td>
<td>↑</td>
<td>No data</td>
<td>No data</td>
<td>8, 78</td>
<td></td>
</tr>
<tr>
<td>8) SR Ca\textsuperscript{2+} release</td>
<td>No Δ, ↑\textsuperscript{3}</td>
<td>No data</td>
<td>No Δ</td>
<td>29, 67, 78</td>
<td></td>
</tr>
<tr>
<td>9) Superficial buffer barrier (SBB)</td>
<td>↓</td>
<td>No data</td>
<td>No data</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>10) Nuclear buffer barrier (NBB)</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>11) Increased C\textsubscript{a}</td>
<td>↑</td>
<td>No data</td>
<td>↑</td>
<td>8, 130, 132</td>
<td></td>
</tr>
<tr>
<td>12) Increased C\textsubscript{n}</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td></td>
</tr>
</tbody>
</table>

**Diabetes (toxin-induced type 1) and dyslipidemia**

1) Plasmalemmal Ca\textsuperscript{2+} pump | ↓ | I | No data | 130, 132 |
2) Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange | ↓ | I | No data | 80, 130, 132 |
3) SERCA | ↑ | I | No data | 44, 45, 130, 132 |
4) Voltage-gated Ca\textsuperscript{2+} channel | ↓ | I | No data | 132 |
5) TRPC channel, store-operated Ca\textsuperscript{2+} entry | Absent | Absent | ↑ | 23, 29 |
6) STIM1 | No data | No data | No data | No data |
7) K\textsubscript{Ca} channel | ↑ | I | No data | 77, 78 |
8) SR Ca\textsuperscript{2+} release | ↑\textsuperscript{4} | I | No data | 67, 78, 123 |
9) Superficial buffer barrier (SBB) | ↓ | I | No data | 78, 130 |
10) Nuclear buffer barrier (NBB) | ↓ | I | No data | 123 |
11) Increased C\textsubscript{a} | ↑ | I | No data | 130, 132 |
12) Increased C\textsubscript{n} | ↑ | No data | No data | 123 |

\footnotesize{All studies include excess calorie atherogenic diet (high fat and high cholesterol) feeding. MetS, type 2 diabetes, and diabetic dyslipidemia characteristics are in Table 1. Göttingen are not included because no Ca\textsuperscript{2+} signaling studies have been done. “No data” denotes that there were no studies or the study did not include these measures. “Absent” indicates no evidence for store-operated Ca\textsuperscript{2+} entry. TRPC, transient receptor potential canonical channels; K\textsubscript{Ca}, Ca\textsuperscript{2+}-activated K channels; C\textsubscript{a}, subsarcolemmal Ca; STIM1, stromal interaction molecule type 1; C\textsubscript{a}, cytosolic Ca; C\textsubscript{n}, nuclear Ca; SERCA, sarcoplasmic-endoplasmic reticulum Ca\textsuperscript{2+} ATPase; SR, sarcoplasmic reticulum; No Δ, no change; ↑, increased; ↓, decreased; |, attenuated or returned MetS- or diabetic dyslipidemia-induced changes toward normal. \textsuperscript{1}Although Yucatan do not meet criteria to be defined as having MetS, data are included because Yucatans have the major component of dyslipidemia. \textsuperscript{2}Plasmalemmal Ca\textsuperscript{2+} extrusion mechanisms are decreased in “healthy” lean Ossabaw vs. Yucatan (84). \textsuperscript{3}SR Ca\textsuperscript{2+} release was not changed in response to endothelin (67), but coupling of caffeine-induced SR Ca\textsuperscript{2+} release to activation of K\textsubscript{Ca} channels was increased (78). \textsuperscript{4}Bulk SR Ca\textsuperscript{2+} release was increased in response to endothelin (67, 123) and coupling of SR Ca\textsuperscript{2+} release to activation of K\textsubscript{Ca} channels was increased (78).}

referred to as spontaneous transient outward currents (STOCs) (72, 77, 78, 86, 103). It is worth noting the analogous result that aldosterone hypertensive rats had profoundly increased K\textsubscript{Ca} (72). The upregulation of K\textsubscript{Ca} channels was clearly a compensatory response to increased Ca\textsuperscript{2+} influx through VGCC (72), as inhibition of K\textsubscript{Ca} channels caused more membrane depolarization in hypertensive compared with normoten-

The CAD in our studies on diabetic dyslipidemic Yucatan pigs was relatively mild (44, 45, 132) compared with the gross atherosclerosis in the rabbits (2). Our studies in Yucatan and Ossabaw swine CSM show that Ca\textsuperscript{2+} modulation by SERCA function progresses from increased function (compensation) in mild/moderate atherosclerosis to severe dysfunction with more severe disease elicited by coronary stenting in atherosclerotic pigs (84) (Table 2). Collectively, these studies show that Ca\textsuperscript{2+} transporter changes can be beneficial in an attempt to compensate for an adverse change (defect) in another transporter. Adaptations in Ca\textsuperscript{2+} regulatory mechanisms should not be taken uniformly as causing CAD in diabetes.

Perhaps surprising is the downregulation of VGCC in CSM of diabetic dyslipidemic pigs (VGCC; Fig. 3A; 132). In con-

TRPC, transient receptor potential canonical channels; KCa, Ca\textsuperscript{2+}-activated K channels; C\textsubscript{a}, subsarcolemmal Ca; STIM1, stromal interaction molecule type 1; C\textsubscript{a}, cytosolic Ca; C\textsubscript{n}, nuclear Ca; SERCA, sarcoplasmic-endoplasmic reticulum Ca\textsuperscript{2+} ATPase; SR, sarcoplasmic reticulum; No Δ, no change; ↑, increased; ↓, decreased; |, attenuated or returned MetS- or diabetic dyslipidemia-induced changes toward normal. Although Yucatan do not meet criteria to be defined as having MetS, data are included because Yucatans have the major component of dyslipidemia. Plasmalemmal Ca\textsuperscript{2+} extrusion mechanisms are decreased in “healthy” lean Ossabaw vs. Yucatan (84). SR Ca\textsuperscript{2+} release was not changed in response to endothelin (67), but coupling of caffeine-induced SR Ca\textsuperscript{2+} release to activation of KCa channels was increased (78). Bulk SR Ca\textsuperscript{2+} release was increased in response to endothelin (67, 123) and coupling of SR Ca\textsuperscript{2+} release to activation of KCa channels was increased (78).
dyslipidemia is very consistent with the concept of excitation-transcription coupling (124), wherein Ca$^{2+}$ influx and localized Ca$^{2+}$ signaling may alter CSM phenotype. Wamhoff et al. selectively increased or decreased L-type VGCC gene expression and Ca$^{2+}$ influx and found parallel changes in smooth muscle contractile gene and protein expression (122). Exercise training of diabetic dyslipidemic Yucatan pigs prevented the decrease in VGCC current (132), which is consistent with a less proliferative, more differentiated CSM phenotype (Fig. 3B; Table 2).

Cytosolic and localized Ca$_{in}$ were increased in response to the mitogen endothelin (ET; Fig. 3A) in diabetic dyslipidemia and were directly associated with increased coronary atherosclerosis (67, 123). The transnuclear SR was more fragmented, thus potentially providing more structural evidence for a breakdown in the NBB. The lipid-lowering agent atorvastatin prevented the increases in Ca$_{in}$, defects in the transnuclear SR morphology, and coronary atherosclerosis. These data are consistent with a causal role of Ca$_{in}$ in atherosclerosis, especially considering that it is highly plausible because of the known role of Ca$_{in}$ in regulation of gene transcription in other cells (124). This is not definitive evidence, however, because there is no selective inhibitor of Ca$_{in}$, transients. No studies have been performed to determine whether aerobic exercise training will prevent the increases in CSM Ca$_{in}$ or transnuclear SR distribution in diabetic dyslipidemia (Fig. 3B, red font “Diabetes” [Ca$_{in}$], broken red lines; Table 2). However, the attenuation of the endothelin-induced increase in CSM Ca$_{in}$ by exercise training of lean healthy pigs (121) and global effects on all other Ca$^{2+}$ transporters suggest that exercise could attenuate diabetes dyslipidemia-induced increases in CSM Ca$_{in}$.

Finally, to integrate these findings with human studies, recall that almost two-thirds of the exercise training-induced cardiovascular protection in humans is not explained by changes in traditional risk factors (81). In the studies on diabetic dyslipidemic swine reviewed here no component of the “diabetic milieu” (body weight, glucose, lipids, etc.) was changed by exercise training (Table 2). While all conceivable hormones and metabolic factors were not measured, these carefully controlled studies in swine are highly consistent with the conclusion that exercise effects are largely due to direct actions on the vasculature.

**Exercise training effects on CSM Ca$^{2+}$ regulation in metabolic syndrome.** Similar to the above discussion of diabetic dyslipidemia, I show the spatial relationships in Fig. 3A and list the changes in MetS Ossabaw swine in Table 2, top (Metabolic syndrome, type 2 diabetes) because there are several Ca regulatory processes, proteins, and membrane domains that influence Ca signaling and the MetS milieu is critical in these studies. Although Yucatan do not meet criteria to be defined as having MetS, data are included in Table 2 because Yucatans have the major component of dyslipidemia. MetS Ossabaw swine provide strong evidence for increased functional and molecular expression of TRPC channels in CSM (29). TRPC channels are thought to be one molecular form of “store-operated channels” (SOC) activated by signals from depletion of Ca$^{2+}$ from the SR Ca$^{2+}$ store (Fig. 3A, dashed arrow; 7, 57, 69). The SR Ca$^{2+}$ depletion is sensed by the stromal interaction molecule type 1 (Fig. 3, STIM1) to couple to TRPC channel opening. Increased Ca$_{in}$ and Ca$_{cyt}$ responses to mitogens are key findings in alloxan diabetic and dyslipidemic Yucatan swine (67, 123), but unlike the Ossabaw MetS, we have never in ~20 years of studying CSM from healthy or diabetic dyslipidemic Yucatan pigs found activation of TRPC/SOC Ca$^{2+}$ influx in CSM of Yucatan pigs (e.g., Fig. 2A in Ref. 132).

In our hands SOC-mediated Ca$^{2+}$ influx is absent from Yucatan and the presence SOC is unique to Ossabaw CSM. Importantly, SOC-mediated Ca$^{2+}$ entry contributes to vascular smooth muscle phenotypic switching and vascular disease (57, 59, 111). Although there are six isoforms of TRPC (TRPC1, 3, 4, 5, 6, 7), TRPC1 mediated neointimal hyperplasia in human arteries in vitro (57). Indeed, the pivotal study of Kumar et al. (57) showed definitively a causal role of TRPC1, as inhibition of TRPC1 in organ culture with a highly specific blocking antibody that binds an extracellular epitope elicited about a 50% decrease in neointima formation. However, many details remain to be determined regarding the sarcolemmal receptors for TRPC activation in MetS-induced CAD and restenosis and these purely in vitro studies did not address TRPC channel regulation in culture media simulating the MetS milieu or exercise milieu.

Edwards et al. (29) found that SR Ca$^{2+}$ store depletion activated Ca$^{2+}$ influx in CSM, which was increased in MetS vs. lean pigs and attenuated by 7 wk of exercise training (Fig. 3, Table 2). These data were from unidirectional divalent cation (Ca$^{2+}$, Mn$^{2+}$) influx measures using the sensitive Mn$^{2+}$ quench method. The voltage dependence of SOC current in patch-clamp studies showed the characteristic nonselectivity of TRPC1, which was supported by increased TRPC1 mRNA and protein, but not Orai1 protein. The accessory protein STIM1 also showed parallel increases with TRPC1 in MetS and attenuation after exercise training (Fig. 3). A provocative possibility is whether intermediate conductance Ca-activated K channel (I$_{KCa}$) expression is increased in MetS pigs, similar to that noted in cultured, proliferating CSM, in CSM from atherosclerotic pigs (112), and after coronary restenosis (113). The result should be hyperpolarization and increased Ca$^{2+}$ influx through TRPC1 due to the increased driving force for Ca$^{2+}$ (Fig. 3, + and dashed line). The decreased large conductance K$_{Ca}$ in CSM of MetS pigs (8) would suggest highly specific expression of these K$_{Ca}$ isoforms. The fine tuning of K$_{Ca}$ dysregulation in hypertension and metabolic disease is entirely possible and provides impetus for more work in the area (97), especially with regard to effects of exercise training. These data further argue for the specificity of the diabetic milieu, as the decrease in K$_{Ca}$ in MetS (8) is opposite of the upregulation noted in conduit CSM in diabetic dyslipidemia (77, 78) (Table 2).

The degree of hyperglycemia, obesity, plasma cholesterol, insulin, aldosterone, and other hormones differed between the studies (8, 77, 78); thus, differential signals for vascular adaptations could have a major influence. The SERCA pump in MetS is apparently more responsive to its milieu, as SERCA is upregulated in CSM of lean Ossabaw pigs compared with lean Yucatan and SERCA decreases in MetS (84). This suggests a propensity toward SERCA dysfunction in Ossabaw swine predisposed to MetS. Sarcolemmal Ca$^{2+}$ extrusion is also impaired in CSM of lean Ossabaw vs. Yucatan swine and there have been no exercise training studies to assess the impact on SERCA or sarcolemmal Ca$^{2+}$ transporters (84). Finally, the available data on MetS again support the finding that exercise training-induced cardioprotection is not due exclusively to changes in traditional risk factors (81). Only LDL/HDL ratio
was improved by exercise training of MetS swine, while seven other metabolic parameters were unchanged (Table 1), despite improved CSM Ca\(^{2+}\) regulation by TRPC1 and attenuated CAD after exercise training (29).

Although much of the evidence provided does not definitely show whether altered Ca\(^{2+}\) signaling in MetS and diabetes causes native CAD and restenosis, several lines of evidence are consistent with this interpretation based on longstanding logic from environmental medicine (43). First, the varied degree of CAD within a single artery and between arteries allows one to determine whether underlying Ca\(^{2+}\) regulatory mechanisms (mRNA, protein, and activity) are proportional to the degree of CAD, i.e., assess causation by the strength of the association (123). Second, studying CAD at varying durations of MetS, diabetes, and after stenting allows one to determine whether Ca\(^{2+}\) signaling mechanisms occur before CAD, i.e., assesses causation by the temporality of the relationship (85). For example, TRPC channels increased in MetS before CAD (29, 85). Assessment of causation by specificity (selective antagonism) is very difficult in coronary arteries in vivo because it would require chronic use of systemic pharmacological inhibitors or transgenic technology (e.g., 58). Selective antagonism with pharmacological inhibitors and molecular tools (siRNA, antisense oligonucleotides) to dissect mechanistic pathways may require the use of in vitro organ culture approach (e.g., 121) and more refined catheter delivery methods for local targeting in vivo (54).

The collective interpretation of data from CSM Ca\(^{2+}\) signaling in diabetic dyslipidemic Yucatan pigs and MetS Os-sawbaw pigs is that Ca\(^{2+}\) influx via VGCC promotes CSM differentiation, while Ca\(^{2+}\) release and Ca\(^{2+}\) influx via SOC (TRPC1) promote CSM dedifferentiation and proliferation. Exercise training prevents/reverses these the Ca\(^{2+}\) signaling abnormalities.

**CONCLUSIONS, PERSPECTIVES, AND FUTURE DIRECTIONS**

Some of the most therapeutically beneficial drugs have multiple actions. For example, statin drugs have pleiotropic effects beyond their lipid-lowering ability, which may account for their convincing success in prevention (25), regression (88), and stabilization of CAD (76, 127). Exercise may very well rival statins, because it is difficult to fathom that a single drug or therapy could elicit such widespread adaptations in Ca\(^{2+}\) signaling as exercise training. Characterization of the multiple Ca\(^{2+}\) signaling mechanisms, even if “only” associated with exercise, provide a glimpse of many Ca\(^{2+}\) regulatory pathways that may be targeted by pharmacological therapy. While characterization of Ca\(^{2+}\) signaling is a major advance, there are key priorities for future directions: 1) target key molecules in the Ca\(^{2+}\) signaling pathways for over- or underexpression to determine with more confidence the causal role, instead of only the association, of these molecules in mechanisms of exercise protection against CAD; 2) combine mechanistic studies with hard clinical outcomes, e.g., reduction of spontaneous myocardial infarction and mortality. Even precise intravascular imaging methods and histology measures of atherosclerosis are considered surrogate endpoints. Studies of hard clinical endpoints would facilitate translation of cellular and molecular mechanisms of Ca\(^{2+}\) regulation to true cardioprotection.

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First and foremost, I thank many colleagues who contributed to this work over the past nearly 20 years since our first publication on the effects of exercise training on coronary smooth muscle Ca\(^{2+}\) regulation. I thank Dr. M. Richardson for his work on Fig. 1. Although every attempt was made to cite many studies on swine metabolic derangements and atherosclerosis, the space and reference limit in this brief review format precluded citation of all the excellent work in the field.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


Ca\(^{2+}\) REGULATION IN EXERCISE, CAD, AND DIABETES


