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Metabolic Syndrome Abolishes Glucagon-Like Peptide 1 Receptor Agonist Stimulation of SERCA in Coronary Smooth Muscle

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Metabolic syndrome (MetS) doubles the risk of adverse cardiovascular events. Glucagon-like peptide 1 (GLP-1) receptor agonists induce weight loss, increase insulin secretion, and improve glucose tolerance. Studies in healthy animals suggest cardioprotective properties of GLP-1 receptor agonists, perhaps partially mediated by improved sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity. We examined the acute effect of GLP-1 receptor agonists on coronary smooth muscle cells (CSM) enzymatically isolated from lean, healthy Ossabaw miniature swine. Intracellular Ca^{2+} handling was interrogated with fura-2. The GLP-1 receptor agonist exenatide activated SERCA but did not alter other Ca^{2+} transporters. Further, we tested the hypothesis that chronic, in vivo treatment with GLP-1 receptor agonist AC3174 would attenuate coronary artery disease (CAD) in swine with MetS. MetS was induced in 20 swine by 6 months' feeding of a hypercaloric, atherogenic diet. Swine were then randomized (*n* = 10/group) into placebo or AC3174 treatment groups and continued the diet for an additional 6 months. AC3174 treatment attenuated weight gain, increased insulin secretion, and improved glucose tolerance. Intravascular ultrasound and histology showed no effect of AC3174 on CAD. MetS abolished SERCA activation by GLP-1 receptor agonists. We conclude that MetS confers vascular resistance to GLP-1 receptor agonists, partially through impaired cellular signaling steps involving SERCA.

Metabolic syndrome (MetS) is defined as the presence of three or more of the following five risk factors: obesity, hypertension, glucose intolerance, insulin resistance, and dyslipidemia (1). Obesity, MetS, and type 2 diabetes are all independent risk factors for cardiovascular disease. The presence of MetS doubles the risk of experiencing an adverse cardiovascular event (2). Thus, it is important to understand the effect of diabetes and MetS treatment modalities on cardiovascular health.

Insulin-sensitizing drugs and those that enhance insulin secretion from pancreatic β-cells have been used in the treatment of type 2 diabetes and MetS. Others, such as dipeptidyl peptidase-4 inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists (exenatide, liraglutide, AC3174, etc.), increase both insulin sensitivity and insulin secretion (3,4). Recently, insulin-sensitizing thiazolidinediones have been implicated in increased risk of fluid retention, LDL and triglyceride accumulation, heart failure, and myocardial infarction (5–7). Hence, there is great attention on cardiovascular outcomes of antidiabetic agents, and the U.S. Food and Drug Administration requires cardiovascular safety profiles for all new agents (8).

GLP-1 receptor agonists are attractive treatment options for MetS because GLP-1 is an endogenous hormone that functions in normal physiology to increase insulin sensitivity, biosynthesis, and secretion (3). Additionally, GLP-1 receptor agonists reduce myocardial infarct size in ischemia/reperfusion injury (9), improve cardiac function in chronic heart failure (10), and attenuate neointimal formation after vascular injury (11). At the cellular level, GLP-1 receptor agonists improve endothelial calcium homeostasis after simulated ischemia/reperfusion (12). Further, GLP-1 receptor agonists improve sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity in multiple cell types (13,14). These

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studies provide rationale for a protective role of GLP-1 in the coronary vasculature.

A recent study revealed impaired GLP-1 stimulation of myocardial glucose uptake in human patients with type 2 diabetes and in MetS swine (15). This finding raises the question of potential resistance to GLP-1 in MetS and type 2 diabetes and highlights the need for rigorous examination of GLP-1 action on coronary artery disease (CAD), specifically in MetS. Given the stimulation of SERCA in macrophages and ventricular myocytes (13,14) and altered SERCA in coronary smooth muscle cells (CSM) in MetS and diabetes (16,17), potentially vasoprotective actions of GLP-1 could be mediated through cellular Ca^{2+} signaling. This study investigated the effect of GLP-1 receptor agonists on SERCA stimulation in CSM from lean swine and on CAD and CSM SERCA stimulation in MetS.

RESEARCH DESIGN AND METHODS

Animals

All protocols involving animals were approved by the Institutional Animal Care and Use Committee at Indiana University School of Medicine and fully complied with animal use standards (18,19). Ossabaw miniature swine were separated into two treatment groups: placebo (n = 10) and GLP-1 receptor agonist AC3174 treated (n = 10; 0.25 μg/kg body weight subcutaneously, twice daily; Amylin Pharmaceuticals, San Diego, CA), which displays nearly identical pharmacokinetic and pharmacodynamics profiles as the marketed GLP-1 receptor agonist exenatide (4). CAD and MetS were induced in both treatment groups as described in the Supplementary Material. One animal in the placebo group died prior to the collection of end point data.

Isolation of CSM

Epicardial coronary arteries were cleaned of adherent tissue and CSM were isolated with a collagenase solution as previously described (16,17).

Measurement of Intracellular Ca^{2+}

CSM were loaded with fura-2/AM, and whole-cell intracellular Ca^{2+} levels were measured as described in the Supplementary Material.

Acute In Vitro Exenatide Treatment

CSM from lean, healthy Ossabaw swine or a separate group of swine with MetS and CAD were treated with 100 nmol/L exenatide during assessment of intracellular Ca^{2+}. The selective SERCA inhibitor cyclopiazonic acid (CPA; 10 μmol/L) was used as a negative control for SERCA function.

Intravenous Glucose Tolerance Testing

Intravenous glucose tolerance testing (IVGTT) was performed as previously described (16,20) and in the Supplementary Material.

Plasma Lipid, Electrolyte, and Enzyme Assays

Blood samples were obtained at time of IVGTT prior to intravenous injection of glucose. Lipid electrolyte and enzyme content were measured offsite (Antech Diagnostics, West Lafayette, IN).

Intravascular Ultrasound

After 12 months on the diet, intravascular ultrasound (IVUS) pullbacks were performed as described in the Supplementary Material.

Histology

Coronary arterial rings were placed in phosphate-buffered formalin at the time of euthanasia. Hematoxylin and eosin, Verhoeff-van Gieson, and trichrome staining were performed on sections of these rings. Plaque burden and collagen content were determined using commercially available software (ImageJ 1.48v; National Institutes of Health) as previously described (16).

RESULTS

Acute In Vitro Exenatide Treatment in CSM From Lean, Healthy Swine

We investigated the acute effect of the GLP-1 receptor agonist, exenatide, on intracellular Ca^{2+} handling in CSM isolated from lean, healthy Ossabaw swine. Sarcoplasmic reticulum (SR) Ca^{2+} stores were released with caffeine in the absence of extracellular Ca^{2+} in the presence and absence of 100 nmol/L exenatide (Fig. 1*A*), and the subsequent undershoot of cytosolic Ca^{2+} during recovery was assessed. Exenatide treatment did not alter resting cytosolic Ca^{2+} (Fig. 1*B*) or Ca^{2+} influx through voltage-gated Ca^{2+} channels (Fig. 1*C*). Exenatide elicited improved recovery of cytosolic Ca^{2+} below baseline levels, and this effect was completely ablated in the presence of the SERCA inhibitor CPA (Fig. 1*D*). The GLP-1 receptor agonist liraglutide also demonstrated improved recovery of cytosolic Ca^{2+}, which was prevented by the GLP-1 receptor antagonist, exendin (9-39) (Supplementary Fig. 1). In the presence of caffeine, SERCA buffering of cytosolic Ca^{2+} is functionally inhibited because of the much more rapid release of Ca^{2+} through ryanodine receptors on the SR membrane. Therefore, we assessed the rate of recovery to 50% recovery to baseline (t_{50}) in the presence of caffeine as a measure of Ca^{2+} extrusion and/or Ca^{2+} uptake into caffeine-insensitive intracellular stores, and to 100% recovery to baseline (t_{100}) in the absence of caffeine as another measure of SERCA activation. Exenatide significantly decreased time to 100%, but not 50%, recovery to baseline (Fig. 1*E*).

Clinical Measurement of MetS in Ossabaw Swine

We then investigated the chronic effect of GLP-1 receptor agonism in vivo. To examine the effects of diet and GLP-1 receptor treatment on glucose metabolism, kidney function, and plasma electrolytes, blood profiles of these parameters were obtained (Supplementary Table 1) and IVGTTs were performed. Overall, feeding of an excess-calorie, atherogenic diet increased body weight, which was significantly attenuated by AC3174 treatment (Fig. 2*A*). Blood pressure was not altered by diet or AC3174 treatment. Whereas fasting plasma glucose was not altered by diet or AC3174 treatment (Supplementary Table 1), IVGTT assessment of glucoregulation revealed

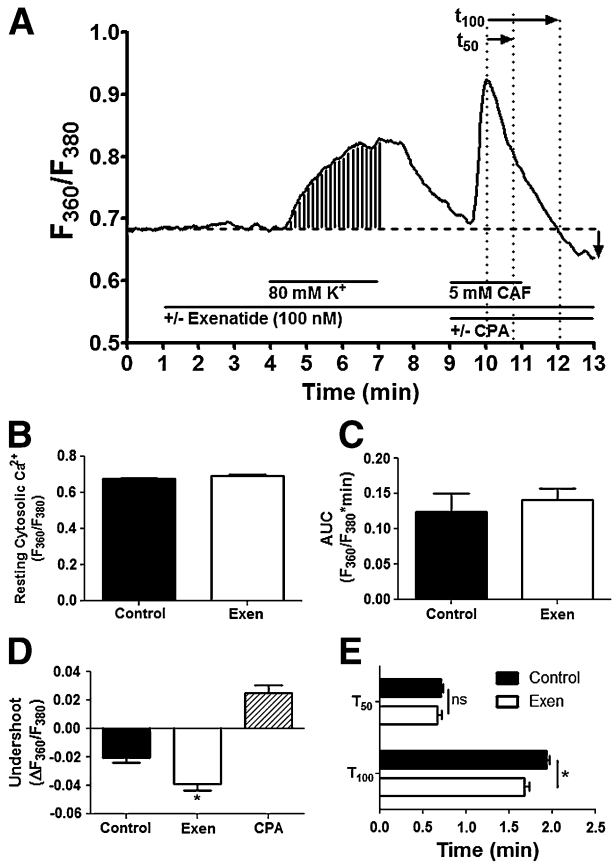

Figure 1—Effect of acute exenatide treatment on CSM Ca^{2+} handling in CSM isolated from lean, healthy Ossabaw swine. *A*: Representative tracing of data from Ca^{2+} imaging protocol. Horizontal dashed line indicates baseline. (CAF, caffeine; control, n = 114 CSM; exenatide [Exen], n = 82 CSM; CPA, n = 68 CSM.) *B*: Resting cytosolic Ca^{2+}. *C*: Area under the curve (AUC) for Ca^{2+} influx during high K^+, corresponding to the shaded region in panel *A*. *D*: Undershoot of Ca^{2+} below resting levels, corresponding to the black vertical arrow in panel *A*. *E*: Time to half (t_{50}) and total (t_{100}) recovery from caffeine-induced rise in cytosolic Ca^{2+}, corresponding to the horizontal black arrows in panel *A*. *, different from other treatments. $P < 0.001$.

improved glucose handling in AC3174-treated swine, compared with placebo (Fig. 2*B–D*). This was corroborated by augmented plasma insulin levels after intravenous glucose challenge in AC3174-treated animals, compared with placebo (Fig. 2*E–G*).

Intravascular Ultrasound and Histology Measurement of CAD

IVUS was used to examine severity of CAD in vivo and was confirmed by histology. Both wall coverage and percent plaque burden were assessed as previously described (16) in placebo and AC3174-treated pigs. AC3174 treatment did not alter CAD severity, as indicated by either wall coverage or percent plaque burden (Fig. 3*C* and *D*). In vitro, histological examination of coronary arterial rings revealed no effect of AC3174 treatment on plaque burden (Fig. 3*E*, *F*, *I*, *J*, and *H*) or collagen deposition (Fig. 3*G*, *K*, and *L*).

Effect of Chronic, In Vivo GLP-1 Receptor Agonist Treatment on CSM Ca^{2+} Handling in MetS-Induced CAD

We have previously demonstrated alterations in CSM Ca^{2+} handling in MetS-induced CAD (16,17,21). We therefore investigated whether chronic, in vivo AC3174 treatment resulted in improved intracellular Ca^{2+} handling in CSM. Figure 4*A* shows the Ca^{2+} signaling protocol to assess baseline Ca^{2+} levels, SR Ca^{2+} store release assessed by the caffeine-induced Ca^{2+} peak in the absence of extracellular Ca^{2+}, and SERCA activity assessed by subsequent recovery of cytosolic Ca^{2+} levels and undershoot of cytosolic [Ca^{2+}] below baseline levels. Chronic AC3174 treatment did not alter intracellular Ca^{2+} handling in CSM from swine with MetS-induced CAD (Fig. 4*B–D*).

Effect of Acute, In Vitro Exenatide Treatment on CSM in MetS and CAD

Further, we assessed the acute, direct effect of GLP-1 receptor agonists on Ca^{2+} handling in CSM isolated from swine with MetS and CAD. Cells were exposed to the GLP-1 receptor agonist exenatide (100 nmol/L), which has an identical pharmacological profile as AC3174 (4) (Fig. 4*A*), for 3 min prior to treatment with high K^+. We again assessed caffeine-induced SR Ca^{2+} store release by peak Ca^{2+} response to caffeine and SERCA activity by the subsequent undershoot of cytosolic Ca^{2+} during recovery. Exenatide did not alter either SR Ca^{2+} store release or the undershoot of cytosolic Ca^{2+} below baseline (Fig. 4*E* and *F*).

DISCUSSION

The principle finding of this study is that MetS confers vascular resistance to the effects of GLP-1 receptor agonists. AC3174 did improve several cardiovascular risk factors, such as the metabolic factors, glucose tolerance, and insulin secretion, indicating that longer-term treatment may have indirect benefits on cardiovascular health. Treatment of MetS and type 2 diabetes is confounded by potential secondary and detrimental cardiovascular effects. Previous studies in lean, healthy animals indicate that GLP-1 receptor agonists provide a potential cardioprotective treatment for type 2 diabetes and MetS (9–11). In the current study, we examined possible Ca^{2+} regulatory mechanisms for GLP-1 receptor agonist action in CSM from lean, healthy Ossabaw swine. Our finding that GLP-1 receptor agonists exert a positive effect on SERCA activity in CSM from lean Ossabaw swine is in agreement with findings that GLP-1 receptor agonists enhance SERCA activity in other cell types, including endothelial cells (12), macrophages (13), and cardiomyocytes (14), and is the first study examining the effect of GLP-1 receptor agonists in CSM. The kinetics of the Ca^{2+} transient shown in Fig. 1*E* demonstrate a lack of exenatide effect on Ca^{2+} extrusion. We have previously shown these kinetic measurements to be an appropriate assay of Ca^{2+} extrusion (17). Reduced SERCA activity has been implicated in CSM proliferation and neointimal formation in

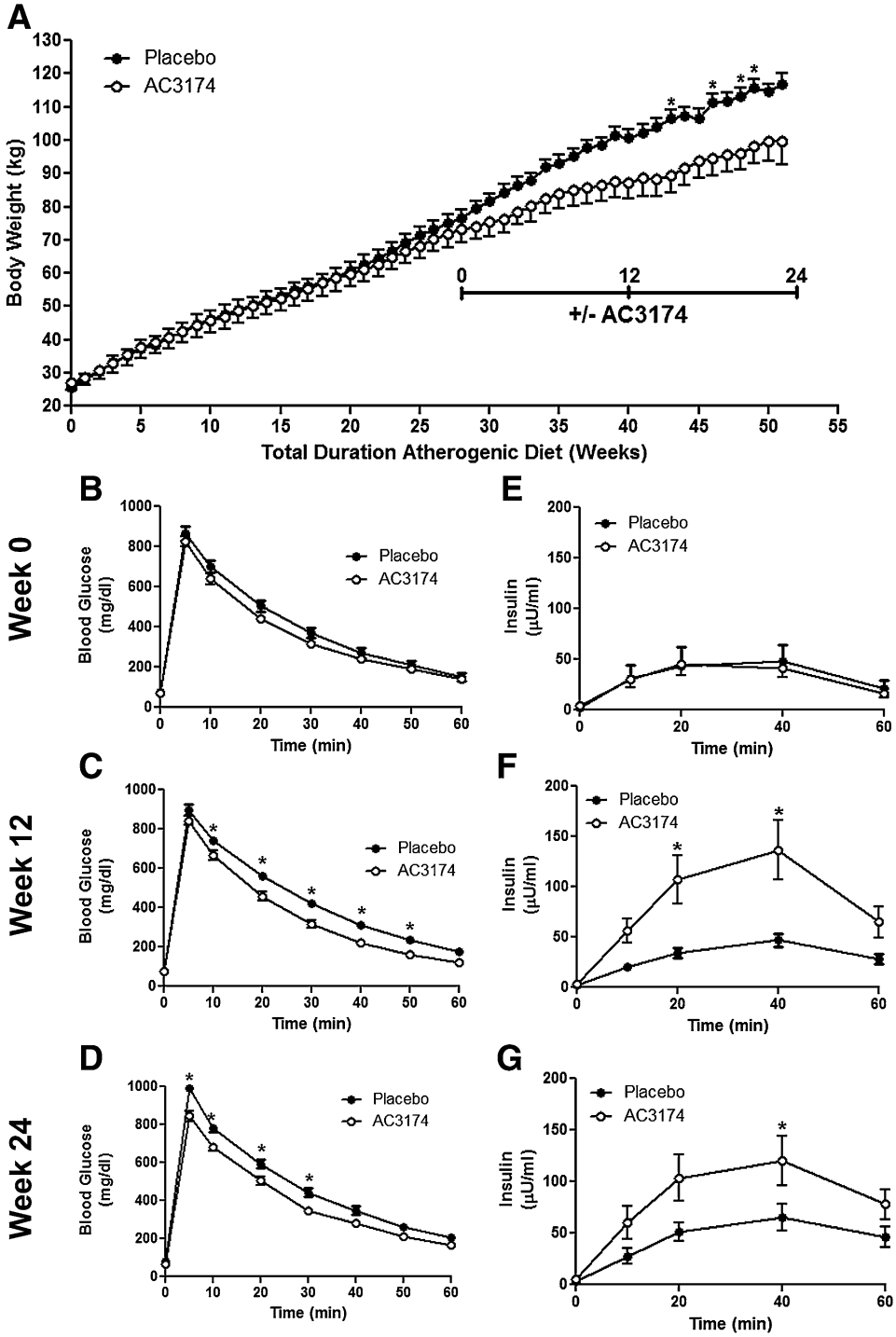

Figure 2—AC3174 attenuates weight gain and improves glucose handling in Ossabaw miniature swine. Closed circles, placebo treatment group; open circles, AC3174 treatment group. *A*: Weight gain in Ossabaw miniature swine. Inset timeline indicates time on placebo or AC3174 treatment, which corresponds with times in *B–D* and Supplementary Table 1. Time course of plasma glucose responses during IVGTT at beginning (*B*), 12 weeks (*C*), and 24 weeks (*D*) of AC3174 treatment. Time course of plasma insulin responses during IVGTT at beginning (*E*), 12 weeks (*F*), and 24 weeks (*G*) of AC3174 treatment. *, different from placebo. $P < 0.05$.

the progression of CAD (22,23). One possible mechanism explaining this phenomenon is induction of endoplasmic reticulum (ER) stress. As the single means by which Ca^{2+} may enter the ER, SERCA is a crucial regulator of ER Ca^{2+} homeostasis, and SERCA inhibition elicits an ER stress response through depletion of ER Ca^{2+} (24). ER stress is associated with the development of atherosclerosis (13). Inhibition of ER stress through heightened

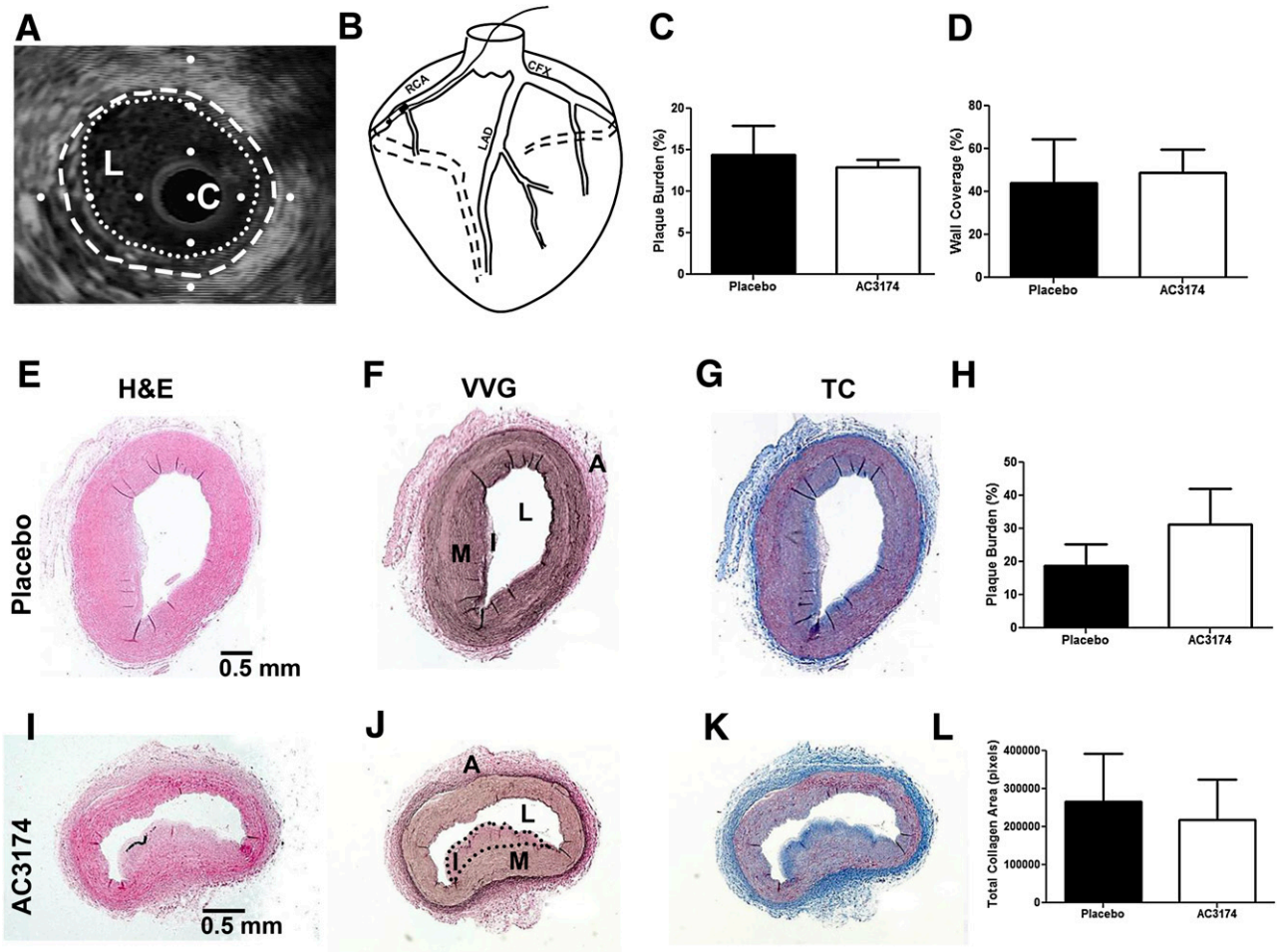

Figure 3—AC3174 treatment does not attenuate CAD progression as measured by IVUS and histology. *A*: IVUS image of artery with CAD. C, catheter; L, lumen. Distance between dots is 1 mm. Original lumen traced in white dashed line. Actual lumen traced in white dotted line. Atherosclerotic plaque is indicated between dotted and dashed lines. *B*: The heart and major epicardial coronary arteries. CFX, circumflex; LAD, left anterior descending; RCA, right coronary artery. The RCA and CFX on the anterior aspect of the heart are shown by the solid lines and the arteries traversing to the posterior aspect of the heart are shown by the dashed lines. The IVUS catheter is shown in the RCA positioned for a pullback. *C* and *D*: Quantification of IVUS pullbacks performed in the RCA in both placebo (n = 6) and AC3174-treated (n = 3) swine. AC3174 did not attenuate CAD as measured by percent plaque burden (*C*) or percent wall coverage (*D*). Coronary arterial rings from placebo (n = 4; *E–G*) and AC3174-treated (n = 5; *I–K*) swine. A, adventitia; I, intima; L, lumen; M, media. *A* and *E*: Coronary artery rings stained with hematoxylin and eosin (H&E). *F* and *J*: Coronary artery rings stained with Verhoeff-van Gieson (VVG) stain for elastin. An overt atherosclerotic plaque is traced in panel *H*. *G* and *K*: Coronary artery rings stained with Masson's trichrome (TC) for collagen. *H*: Graphical representation of plaque burden (P = 0.35). *L*: Graphical representation of total collagen area (P = 0.79).

activation of SERCA could provide a novel means by which to treat CAD.

We also examined the effect of GLP-1 receptor agonists on CAD progression in MetS. This study was needed because of recent evidence of MetS-induced resistance to the cardioprotective effects of GLP-1 (15). The improvement of systemic glucoregulation with AC3174 treatment provides essential positive evidence for GLP-1 receptor agonist action in the Ossabaw swine model of MetS and CAD. Further, we demonstrated that GLP-1 receptor agonist AC3174 has no effect on MetS-induced CAD. This is in contrast with the recent finding that GLP-1 prevents myocardial ischemia–reperfusion injury (9) and injury-induced neointimal hyperplasia (11), providing evidence that the MetS phenotype itself confers cardiovascular resistance to the beneficial effects of GLP-1. It is important to note that although AC3174 treatment did not attenuate CAD progression, the absence of adverse effects of AC3174 provide a cardiovascular safety profile for GLP-1 receptor agonists.

Additionally, we examined the acute, direct effect of GLP-1 receptor agonists on intracellular Ca^{2+} regulation in CSM from Ossabaw swine with MetS and CAD who had not received any treatment with GLP-1 receptor agonists. Here, we found that exenatide had no effect on Ca^{2+} regulation in CSM from swine with MetS and CAD. This finding is in contrast with other studies in which GLP-1 receptor agonism resulted in increased SERCA activity (12–14). A study in humans with type 2 diabetes revealed that exenatide treatment enhanced endothelial-dependent

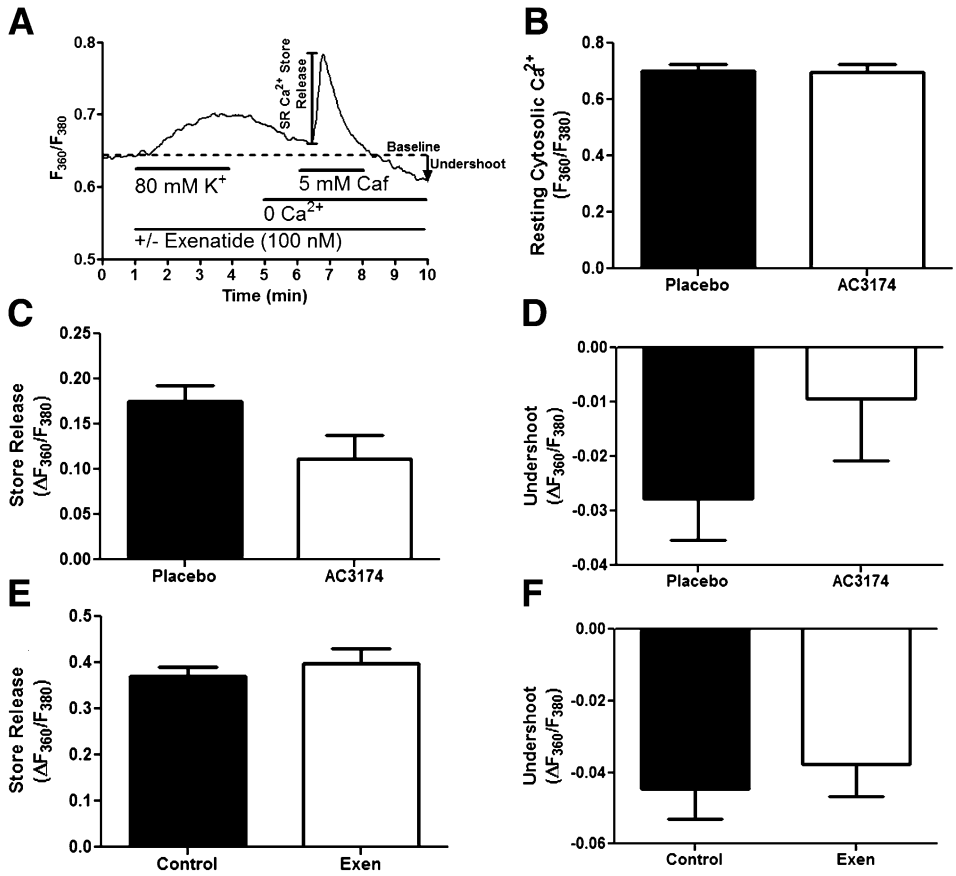

Figure 4—Effect of GLP-1R agonists on CSM Ca^{2+} handling in MetS Ossabaw swine. *A*: Representative tracing of data from Ca^{2+} imaging protocol. Dashed line indicates baseline. *B–D*: Effect of chronic in vivo AC3174 treatment on CSM Ca^{2+} handling (Caf, caffeine; placebo, $n = 4$ swine; AC3174, $n = 6$ swine). *B*: Resting cytosolic Ca^{2+} levels. *C*: Caffeine-induced SR Ca^{2+} store release, corresponding to brackets in panel *A*. *D*: Undershoot of cytosolic Ca^{2+} levels below baseline, corresponding to black arrow in panel *A*. *E* and *F*: Effect of acute ex vivo exenatide treatment on Ca^{2+} handling in CSM from MetS Ossabaw swine (0 Ca, $n = 38$ CSM; exenatide [Exen], $n = 24$ CSM). *E*: Caffeine-induced SR Ca^{2+} store release, corresponding to brackets in panel *A*. *F*: Undershoot of cytosolic Ca^{2+} levels below baseline, corresponding to black arrow in panel *A*.

vasodilation (25), although it is important to note that blood cholesterol levels were controlled in these patients. The current study, revealing a lack of effect of GLP-1 receptor agonism on SERCA activity in the setting of MetS, corroborates the earlier finding that MetS ablated the effect of GLP-1 receptor agonists in myocardium and highlights the need for additional studies to investigate specific aspects of MetS (obesity, hyperinsulinemia, glucose intolerance, hypertension, and dyslipidemia) that may underlie resistance of the coronary vasculature to GLP-1 receptor agonists.

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References

1. Go AS, Mozaffarian D, Roger VL, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. Circulation 2014;129:e28–e292

2. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 2007;49:403–414

3. Drucker DJ. The biology of incretin hormones. Cell Metab 2006;3:153–165

4. Hargrove DM, Kendall ES, Reynolds JM, et al. Biological activity of AC3174, a peptide analog of exendin-4. Regul Pept 2007;141:113–119
5. Masoudi FA, Inzucchi SE, Wang Y, Havranek EP, Foody JM, Krumholz HM. Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. Circulation 2005;111:583–590
6. Nesto RW, Bell D, Bonow RO, et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Diabetes Care 2004;27: 256–263
7. Masoudi FA. Improving drug safety surveillance: lessons from rosiglitazone. Circ Cardiovasc Qual Outcomes 2010;3:444–446
8. Center for Drug Evaluation and Research. Guidance for industry: diabetes mellitus - evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes, 2008. Available from http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071627.pdf. Accessed 15 October 2014
9. Timmers L, Henriques JPS, de Kleijn DPV, et al. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. J Am Coll Cardiol 2009;53:501–510
10. Liu Q, Anderson C, Broyde A, et al. Glucagon-like peptide-1 and the exenatide analogue AC3174 improve cardiac function, cardiac remodeling, and survival in rats with chronic heart failure. Cardiovasc Diabetol 2010;9:76
11. Hirata Y, Kurobe H, Nishio C, et al. Exendin-4, a glucagon-like peptide-1 receptor agonist, attenuates neointimal hyperplasia after vascular injury. Eur J Pharmacol 2013;699:106–111
12. Dokken BB, Weber CS, Mitchell JL, Gold JM, Lynch RM. Glucagon-like peptide-1 modulates calcium homeostasis in human coronary microvascular endothelial cells after ischemia and reperfusion. Journal of Endocrinology, Diabetes & Obesity 2014;2:1025–1031
13. Liang CP, Han S, Li G, Tabas I, Tall AR. Impaired MEK signaling and SERCA expression promote ER stress and apoptosis in insulin-resistant macrophages and are reversed by exenatide treatment. Diabetes 2012;61: 2609–2620
14. Younce CW, Burmeister MA, Ayala JE. Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a. Am J Physiol Cell Physiol 2013; 304:C508–C518
15. Moberly SP, Mather KJ, Berwick ZC, et al. Impaired cardiometabolic responses to glucagon-like peptide 1 in obesity and type 2 diabetes mellitus. Basic Res Cardiol 2013;108:365
16. Neeb ZP, Edwards JM, Alloosh M, Long X, Mokelke EA, Sturek M. Metabolic syndrome and coronary artery disease in Ossabaw compared with Yucatan swine. Comp Med 2010;60:300–315
17. Witczak CA, Wamhoff BR, Sturek M. Exercise training prevents Ca^{2+} dysregulation in coronary smooth muscle from diabetic dyslipidemic Yucatan swine. J Appl Physiol (1985) 2006;101:752–762
18. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*. Washington, D.C., National Academy Press, 2010
19. AVMA Panel on Euthanasia. American Veterinary Medical Association. 2000 Report of the AVMA Panel on Euthanasia. J Am Vet Med Assoc 2001;218: 669–696
20. Sturek M, Alloosh M, Wenzel J, et al. Ossabaw Island miniature swine: cardiometabolic syndrome assessment. In *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*. 2nd ed. Swindle MM, Ed. Boca Raton, FL, CRC Press, 2007, p. 397–402
21. Sturek M. Ca^{2+} regulatory mechanisms of exercise protection against coronary artery disease in metabolic syndrome and diabetes. J Appl Physiol (1985) 2011;111:573–586
22. Lipskaia L, del Monte F, Capiod T, et al. Sarco/endoplasmic reticulum Ca2+-ATPase gene transfer reduces vascular smooth muscle cell proliferation and neointima formation in the rat. Circ Res 2005;97:488–495
23. Lipskaia L, Hadri L, Le PP, et al. SERCA2a gene transfer prevents intimal proliferation in an organ culture of human internal mammary artery. Gene Ther 2013;20:396–406
24. Yoshida I, Monji A, Tashiro K, Nakamura K, Inoue R, Kanba S. Depletion of intracellular Ca2+ store itself may be a major factor in thapsigargin-induced ER stress and apoptosis in PC12 cells. Neurochem Int 2006;48:696–702
25. Nyström T, Gutniak MK, Zhang Q, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. Am J Physiol Endocrinol Metab 2004;287:E1209–E1215