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Targeting mitochondria to restore failed adaptation to exercise in diabetes

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Abstract

Our translational research group focuses on addressing the problem of exercise defects in diabetes with basic research efforts in cell and rodent models and clinical research efforts in subjects with diabetes mellitus. CREB (cAMP-response-element-binding protein) regulates cellular differentiation of neurons, β-cells, adipocytes and smooth muscle cells; it is also a potent survival factor and an upstream regulator of mitochondrial biogenesis. In diabetes and cardiovascular disease, CREB protein content is decreased in the vascular media, and its regulation in aberrant in β-cells, neurons and cardiomyocytes. Loss of CREB content and function leads to decreased vascular target tissue resilience when exposed to stressors such as metabolic, oxidative or sheer stress. This basic research programme set the stage for our central hypothesis that diabetes-mediated CREB dysfunction predisposes the diabetes disease progression and cardiovascular complications. Our clinical research programme revealed that diabetes mellitus leads to defects in functional exercise capacity. Our group has determined that the defects in exercise correlate with insulin resistance, endothelial dysfunction, decreased cardiac perfusion and diastolic dysfunction, slowed muscle perfusion kinetics, decreased muscle perfusion and slowed oxidative phosphorylation. Combined basic and clinical research has defined the relationship between exercise and vascular function with particular emphasis on how the signalling to CREB and eNOS [endothelial NOS (nitric oxide synthase)] regulates tissue perfusion, mitochondrial dynamics, vascular function and exercise capacity. The present review summarizes our current working hypothesis that restoration of eNOS/NOS dysfunction will restore cellular homoeostasis and permit an optimal tissue response to an exercise training intervention.

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Keywords

cAMP-response-element-binding protein (CREB); cardiovascular disease; diabetes; exercise; mitochondrion; nitric oxide synthase (NOS)

Introduction

Diabetes is a leading cause of death and disability worldwide and a leading cause of blindness, end-stage renal disease, non-traumatic amputation and CVD (cardiovascular disease). Interventions to optimize glucose control, blood pressure and lipids dramatically decrease microvascular and macrovascular events, yet excess burden remains. For example, even with optimal risk factor reduction, cardiovascular events and death is 3–5-fold higher in people with diabetes [1]. The goal of the research ongoing in our laboratory is to define novel strategies for reduction of CVD in diabetes. In our larger clinical research groups, we made the concerning observation that people with diabetes have decreased functional exercise capacity [2]. Specifically, adults and adolescents with either Type 2 or Type 1 diabetes have decreased cardiovascular fitness as measured by O_2 consumption ($\dot{V}o_2$ max) and slowed oxygen uptake kinetics [2–5]. The decreased $\dot{V}o_2$ peak is associated with excess CVD and all-cause mortality [6,7]. $\dot{V}o_2$ kinetics measures the acceleration of oxygen uptake and consumption at the onset of exercise and, when slowed, represents a defect in submaximal exercise function (relevant for activities of daily living). These defects in functional exercise capacity have implications for day-to-day life and longevity.

Our translational research group focuses on addressing the problem of exercise defects in diabetes with basic research efforts in cell and rodent models and clinical research efforts in subjects with diabetes mellitus. The present review outlines the basic observations that have led to our current working model starting with our observation that the transcription factor CREB (cAMP-response-element-binding protein), is a crucial modulator of cellular homoeostasis. CREB regulates cellular differentiation of neurons, β-cells, adipocytes and SMCs (smooth muscle cells); it is also a potent survival factor and an upstream regulator of mitochondrial biogenesis. In diabetes and CVD, CREB protein content is decreased in the vascular media and its regulation in aberrant in β-cells, neurons and cardiomyocytes [8–12]. Loss of CREB content and function leads to decreased vascular target tissue resilience when exposed to stressors such as metabolic, oxidative or sheer stress. This basic research programme set the stage for our central hypothesis that diabetes-mediated CREB dysfunction predisposes the diabetes disease progression and cardiovascular complications. The second line of research revealed that diabetes mellitus (Type 1 and Type 2 in youth and adults) leads to defects in functional exercise capacity [13]. This defect manifests in a 20–30% decrease in maximal O_2 consumption and slowed oxygenation and heart rate kinetics at submaximal exercise. Our group has determined that the defects in exercise correlate with insulin resistance, endothelial dysfunction, decreased cardiac perfusion and diastolic dysfunction, slowed muscle perfusion kinetics, decreased muscle perfusion [4,13–15] and slowed oxidative phosphorylation (M. Cree-Green and K.J. Nadeau, unpublished work). In the last few years, we have begun to weave these two threads together to define the relationship between exercise and vascular function with particular emphasis on how the

signalling to CREB and eNOS [endothelial NOS (nitric oxide synthase)] regulates tissue perfusion, mitochondrial dynamics, vascular function and exercise capacity. This review summarizes our published work on CREB, mitochondrial function, cell-specific eNOS/NOS and diabetes mediated exercise impairments to set the stage for our current working hypothesis that restoration of eNOS/NOS dysfunction will restore cellular homoeostasis and permit optimal response to an exercise training intervention.

CREB regulates vascular SMC phenotype and is degraded in vascular disease

CREB is a transcription factor that plays an integral role in cellular proliferation, differentiation and adaptive responses [16–18]. CREB is regulated by G-protein-coupled receptors, tyrosine kinase receptors, oxidant and osmotic stress and Ca^{2+}. Pivotal targets of CREB include genes important for survival, differentiation and metabolic adaptation (Figure 1). CREB is an integrator or hub protein that plays a role in life/death decisions of the cell and it is dysfunctional in the vasculature, heart, brain and β-cell in diabetes [8,11,12,20]. Our laboratory first identified CREB as a determinant of the quiescent SMC phenotype in a series of companion papers [10,11]. We demonstrated that overexpression of active CREB in SMCs decreased proliferative potential, whereas dominant-negative CREB increased SMC activation [10]. Diabetes mellitus induces phenotypic modulation of SMCs in the vasculature, leading to aberrant proliferation and migration of SMCs and vascular dysfunction [11]. Vascular CREB content is decreased in a spectrum of rodent models of CVD risk (hypertension, obesity, diabetes, the metabolic syndrome, pulmonary hypertension and dyslipidaemia) [21]. Using an atherosclerosis model of LDL (low-density lipoprotein) receptor-null mice and *in vitro* studies of SMCs exposed to LDL and oxLDL (oxidized LDL), we showed that both forms of LDL induce an acute activation of CREB. However, only oxLDL leads to CREB down-regulation [21]. We showed further that SMCs exposed to a panel of non-esterified (‘free’) fatty acids exhibited an acute activation of CREB via PKC (protein kinase C) activation. Only saturated fatty acids triggered the down-regulation of CREB [22]. CREB protein content is also reduced in the SMCs of hypertensive pulmonary arteries (PA SMCs) in animals exposed to chronic hypoxia. Hypoxia-induced PA SMCs produce a growth factor called PDGF (platelet-derived growth factor)-BB. We defined that CREB down-regulation by chronic PDGF-BB is mediated through chronic activation of PI3K (phosphoinositide 3-kinase)/Akt and induction of a novel downstream target: protein kinase CK2 [23]. CK2 augments CREB phosphorylation at Ser^{103} and Ser^{107}, enhancing the nuclear export and proteasomal degration of CREB [23]. In the systemic vasculature, TZDs (thiazolidinediones) prevent arterial remodelling and vasoconstriction. TZDs block induction of CK2 and interfere with PDGF-mediated CREB degradation [24]. The physiological relevance of the TZD/Akt/CK2/CREB SMC protection pathway is supported by our recent publications demonstrating the ability of rosiglitazone, PI3K inhibitors and antioxidants to block the proliferation of PA SMCs and stimulate regression of arterial remodelling [24–26]. Collectively, these data support a model wherein CREB serves as a regulator of the quiescent SMC phenotype. Models of vascular disease including diabetes mellitus, hyperlipidaemia, aging and pulmonary hypertension consistently show that loss of

SMC CREB via degradation or nuclear export is permissive for the proliferative SMC phenotype, ultimately promoting disease progression.

CREB regulation of mitochondrial function

Mitochondria are critical sensors of cellular environment involved in cellular homoeostatic decision making. In the context of cellular stress (either toxic or physiological), mitochondrial adaptation is at the centre of cell fate. The decision to increase or decrease metabolism, adjust fuel partitioning and efficiency, and support survival are each, in part, regulated by the mitochondria. Early work from our group and others demonstrated that CREB is a critical regulator of cell survival and mitochondrial integrity via stimulation of Bcl-2 expression [27]. We reported redundant signalling downstream of the insulin receptor via p38 MAPK (mitogen-activated protein kinase), Akt and ERK (extracellular-signal-regulated kinase) to CREB and Bcl-2 [27–29]. In addition, we demonstrated a potent role for CREB in the context of diabetes-mediated oxidant or cytokine stress [8,20,30]. More recently, we and others reported that CREB is an upstream regulator of mitochondrial biogenesis via regulation of PGC1α (peroxisome-proliferator-activated receptor γ co-activator 1α) and ERRα (oestrogen-related receptor α) [31]. We demonstrated that exercise stimulated CREB activity and Bcl-2 decreased caspase 3 activation and decreased heart failure in the SHHF (spontaneous hypertensive heart failure) rat [12]. Consistent with this observation, CREB activity (not protein content) is decreased across a spectrum of cardiac heart failure models [32]. Fentzke et al. [33] generated a cardiac-specific transgenic mouse with dominant-negative CREB under the *MHC* promoter (MHC DNCREB). This mouse develops dilated cardiomyopathy [33]. Watson et al. [31] showed abnormal mitochondrial structure and function plus abnormal fuel partitioning in the MHC DNCREB mouse. In addition, this mouse fails to respond to exercise training with physiological remodelling [34]. Taken together, this study indicates that CREB is essential for heart mitochondrial function and contributes to physiological remodelling. These observations led us to postulate that CREB dysregulation in the diabetic heart and vasculature may contribute to, or exacerbate, the well-established mitochondrial dysfunction in diabetes. We are currently pursuing aspects of this question in our basic and clinical studies. In the next section, we provide an overview of our work in the diabetic vasculature evaluating the mitochondrial adaptation to exercise intervention and close with a summary of our ongoing and published clinical trials targeting insulin action, CREB and the mitochondria as strategies to improve exercise capacity in diabetes and enhance the physiological impact of exercise in the diabetes mellitus host.

Mitochondrial adaptation

CREB content and nuclear localization are decreased in vascular diseases including diabetes, dyslipidaemia, hypertension, aging and pulmonary hypertension [9,21,35]. In the light of the impact of CREB on mitochondrial function, we evaluated the mitochondrial content and function in diabetic rodent models and observed decreased mitochondrial protein profiles and enzyme activities [30]. We next tested whether we could restore vascular CREB content or CREB mitochondrial targets with exercise intervention [36]. In control rats, we observed a significant increase in mitochondrial protein profiles with a

short-term exercise intervention which was, unexpectedly, absent from hypertensive or hypertensive diabetes mellitus rats [36,37] (Figure 2). These unanticipated results led to a series of studies to define the molecular determinants of vascular mitochondrial response to exercise and to evaluate these targets in diabetes mellitus.

Mitochondrial dynamics

Mitochondrial content, structure and function are coordinated by an agile intracellular machinery regulating mitochondrial biogenesis, mitochondrial dynamics (fission and fusion), mitochondrial localization and autophagy (reviewed in [38]). This machinery enables the homoeostatic plasticity needed for rapid adaptation to physiological challenges. In the vasculature, mitochondria serve a number of functions in addition to ATP production, including the regulation of Ca^{2+} sparks and currents, regulation of ROS (reactive oxygen species), and regulation of cell proliferation and survival [39,40]. Early studies by Taggart and Wray [41] and Sward et al. [42] demonstrated a seminal role for mitochondria in control of vascular contractile function (constriction and relaxation); more recent work defined their importance for calcium regulation and control of vascular tone (stiffness) [43]. We were interested in defining the determinants of basal and adaptive mitochondrial function in the vasculature.

Role of NOS in vascular mitochondrial content and adaptation

To better understand the underpinnings of decreased vascular mitochondrial content and adaptation in diabetes, we explored the connections between known mitochondrial regulatory pathways and established vascular consequences of diabetes. Endothelial dysfunction, defined by decreased flow-mediated vasodilatation and vascular insulin resistance (specifically to eNOS regulation) are established vascular abnormalities in diabetes [44]. The work of Nisoli's group firmly established the role of NO [generated by eNOS and nNOS (neuronal NOS)] as an upstream regulator of mitochondrial biogenesis [45]. We therefore examined the impact of eNOS deletion or pharmacological inhibition of NOS on baseline and exercise-stimulated vascular mitochondrial biogenesis and dynamics. Miller et al. [37] reported decreased aortic mitochondrial protein profiles in aortic tissues of eNOS-null and L-NAME (N^G-nitro-L-arginine methyl ester)-treated rodents (similar to our observations in diabetes mellitus) [37]. In addition to decreased CREB and PGC1α in these models, we also observed increased expression of fission proteins (Fis1 and Drp1) and decreased expression of fusion proteins (Opa1, Mfn1 and Mfn2) [37] (Figure 3). These data are consistent with NOS as a biologically important modulator of basal vascular mitochondrial function and structure. In addition, we demonstrated that exercise increased Mfn2 and decreased Fis1 in control rats [37]. This pattern would predict a more fused and networked mitochondrial structure. In contrast, L-NAME prohibited the increase in mitochondrial protein expression and the changes in Mfn2 and Fis1 with exercise [37]. These findings have informed our current working hypothesis that diabetes mellitus-mediated vascular NOS dysfunction (particularly with exercise) contributes to failed mitochondrial plasticity and vascular dysfunction.

Bridging clinical therapeutics to new molecular targets

Type 2 diabetes affects more than 300 million people worldwide and its pathogenesis is a combination of insulin resistance and relative β-cell deficiency. Many agents exist to manage Type 2 diabetes, including agents that augment or replace defective insulin secretion and agents that improve or mimic insulin action on target tissues (decrease hepatic glucose output or improve glucose utilization). We are intrigued that the incretin class of insulin secretogogues, GLP1 (glucagon-like peptide 1) receptor analogues or DPP4 (dipeptidyl peptidase 4) inhibitors act via G-protein-coupled receptors that are highly expressed in the vasculature [46,47]. In addition, we closely follow the work of Lui's group who has demonstrated that incretin agents stimulate eNOS and PKA (protein kinase A) and enhance endothelial function and tissue perfusion leading to improved muscle glucose utilization [48,49]. We therefore examined the impact of the DPP4 inhibitor saxagliptin on vascular mitochondrial adaptation in diabetes. In these studies, which have been published only in abstract form to date [50], we observed restoration of the exercise-mediated increase in mitochondrial protein profiles in the diabetes mellitus aorta, increased expression of eNOS, nNOS and PGC1α. Studies are underway to examine further the impact of the incretins on target tissue response to exercise in health and diabetes.

Clinical implications

People with diabetes have decreased functional exercise capacity that may be important in the premature mortality in diabetes. We have defined a set of physiological parameters that predict or correlate with exercise defects. As outlined in the Introduction, our groups have determined that the defects in exercise correlate with insulin resistance, endothelial dysfunction, diastolic dysfunction, decreased cardiac perfusion, slowed muscle perfusion kinetics [3–5,14,15] and slowed oxidative phosphorylation (J. Reusch, K. Nadeau and M. Cree-Green, unpublished work). Over the last decade, we have sought to target physical inactivity, insulin-sensitivity and excess oxidant burden as ways to improve exercise function in people with diabetes. As expected, exercise training can improve fitness in people with diabetes, but it is not normalized to the level of their matched non-diabetic counterparts [3]. In a series of proof-of-concept studies, we and others demonstrated that improving insulin-sensitivity and endothelial function with TZDs enhances exercise capacity in Type 2 diabetes [50]. Similarly, vitamin E plus C also improved endothelial function and exercise capacity (published so far only in abstract form [52]). Ongoing investigations (see http://www.clintrials.gov) are examining the impact of the incretin class of drugs on these end points.

Summary

Diabetes, via many pathways including glucose elevation, insulin resistance, oxidant burden and low-grade inflammation, generates persistent target organ stress that overwhelms the innate homoeostatic machinery and leads to target organ dysfunction. Our work has defined a unique consequence of this homoeostatic failure: loss of adaptive mitochondrial plasticity in the vasculature. Exploration of this defect has revealed the interesting roles of eNOS and nNOS that may be amenable to pharmacological targeting.

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Abbreviations

CREB	cAMP-response-element-binding protein
CVD	cardiovascular disease
DPP4	dipeptidyl peptidase 4
eNOS	endothelial nitric oxide synthase
LDL	low-density lipoprotein
L-NAME	N^G-nitro-L-arginine methyl ester
MHC DNCREB	dominant-negative CREB under the *MHC* promoter
nNOS	neuronal NOS
NOS	nitric oxide synthase
oxLDL	oxidized LDL
PA SMC	SMC of hypertensive pulmonary arteries
PDGF	platelet-derived growth factor
PGC1α	peroxisome-proliferator-activated receptor γ co-activator 1α
PI3K	phosphoinositide 3-kinase
SHHF	spontaneous hypertensive heart failure
SMC	smooth muscle cell
TZD	thiazolidinedione.

References

1. Vamos EP, Millett C, Parsons C, Aylin P, Majeed A, Bottle A. Nationwide study on trends in hospital admissions for major cardiovascular events and procedures among people with and without diabetes in England 2004–2009. Diabetes Care. 2012; 35:265–272. [PubMed: 22210568]
2. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. Med Sci. Sports Exerc. 1995; 27:661–667. [PubMed: 7674869]
3. Brandenburg SL, Reusch JE, Bauer TA, Jeffers BW, Hiatt WR, Regensteiner JG. Effects of exercise training on oxygen uptake kinetic responses in women with type 2 diabetes. Diabetes Care. 1999; 22:1640–1646. [PubMed: 10526728]
4. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, Reusch JE, Regensteiner JG. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. J. Clin. Endocrinol. Metab. 2009; 94:3687–3695. [PubMed: 19584191]

5. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, Hull A, Zeitler P, Draznin B, Reusch JE. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J. Clin. Endocrinol. Metab. 2010; 95:513–521. [PubMed: 19915016]
6. Church TS, Thomas DM, Tudor-Locke C, Katzmarzyk PT, Earnest CP, Rodarte RQ, Martin CK, Blair SN, Bouchard C. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. PLoS ONE. 2012; 6:e19657. [PubMed: 21647427]
7. Katzmarzyk PT, Church TS, Janssen I, Ross R, Blair SN. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. Diabetes Care. 2005; 28:391–397. [PubMed: 15677798]
8. Jambal P, Masterson S, Nesterova A, Bouchard R, Bergman B, Hutton JC, Boxer LM, Reusch JE, Pugazhenthi S. Cytokine-mediated downregulation of the transcription factor CREB in pancreatic β-cells. J. Biol. Chem. 2003; 278:23055–23065. [PubMed: 12679364]
9. Klemm DJ, Leitner JW, Watson P, Nesterova A, Reusch JE, Goalstone ML, Draznin B. Insulin-induced adipocyte differentiation: activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation. J. Biol. Chem. 2001; 276:28430–28435. [PubMed: 11375992]
10. Klemm DJ, Watson PA, Frid MG, Dempsey EC, Schaack J, Colton LA, Nesterova A, Stenmark KR, Reusch JE. cAMP response element-binding protein content is a molecular determinant of smooth muscle cell proliferation and migration. J. Biol. Chem. 2001; 276:46132–46141. [PubMed: 11560924]
11. Watson PA, Nesterova A, Burant CF, Klemm DJ, Reusch JE-B. Diabetes-related changes in cAMP response element-binding protein content enhance smooth muscle cell proliferation and migration. J. Biol. Chem. 2001; 276:46142–46150. [PubMed: 11560925]
12. Watson PA, Reusch JE, McCune SA, Leinwand LA, Luckey SW, Konhilas JP, Brown DA, Chicco AJ, Sparagna GC, Long CS, Moore RL. Restoration of CREB function is linked to completion and stabilization of adaptive cardiac hypertrophy in response to exercise. Am J. Physiol. Heart Circ. Physiol. 2007; 293:H246–H259. [PubMed: 17337597]
13. Regensteiner JG, Bauer TA, Reusch JE, Brandenburg SL, Sippel JM, Vogelsong AM, Smith S, Wolfel EE, Eckel RH, Hiatt WR. Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. J. Appl. Physiol. 1998; 85:310–317. [PubMed: 9655791]
14. Bauer TA, Reusch JE, Levi M, Regensteiner JG. Skeletal muscle deoxygenation following the onset of moderate exercise suggests slowed microvascular blood flow kinetics in Type 2 diabetes. Diabetes Care. 2007; 11:2880–2885. [PubMed: 17675540]
15. Regensteiner JG, Bauer TA, Reusch JE, Quaife RA, Chen MY, Smith SC, Miller TM, Groves BM, Wolfel EE. Cardiac dysfunction during exercise in uncomplicated type 2 diabetes. Med. Sci. Sports Exerc. 2009; 41:977–984. [PubMed: 19346991]
16. Dworkin S, Mantamadiotis T. Targeting CREB signalling in neurogenesis. Expert Opin. Ther. Targets. 2010; 14:869–879. [PubMed: 20569094]
17. Knoll B, Nordheim A. Functional versatility of transcription factors in the nervous system: the SRF paradigm. Trends Neurosci. 2009; 32:432–442. [PubMed: 19643506]
18. Lopez-Lluch G, Irusta PM, Navas P, de Cabo R. Mitochondrial biogenesis and healthy aging. Exp. Gerontol. 2008; 43:813–819. [PubMed: 18662766]
19. Reference deleted.
20. Pugazhenthi S, Nesterova A, Jambal P, Audesirk G, Kern M, Cabell L, Eves E, Rosner MR, Boxer LM, Reusch JE. Oxidative stress-mediated down-regulation of bcl-2 promoter in hippocampal neurons. J. Neurochem. 2003; 84:982–996. [PubMed: 12603823]
21. Schauer IE, Knaub LA, Lloyd M, Watson PA, Gliwa C, Lewis KE, Chait A, Klemm DJ, Gunter JM, Bouchard R, et al. CREB downregulation in vascular disease: a common response to cardiovascular risk. Arterioscler. ThrombVasc. Biol. 2010; 30:733–741.
22. Schauer IE, Reusch JE. Nonesterified fatty acid exposure activates protective and mitogenic pathways in vascular smooth muscle cells by alternate signaling pathways. Metabolism. 2009; 58:319–327. [PubMed: 19217446]
23. Garat CV, Fankell D, Erickson PF, Reusch JE, Bauer NN, McMurtry IF, Klemm DJ. Platelet-derived growth factor BB induces nuclear export and proteasomal degradation of CREB via

phosphatidylinositol 3-kinase/Akt signaling in pulmonary artery smooth muscle cells. Mol. Cell. Biol. 2006; 26:4934–4948. [PubMed: 16782881]

24. Crossno JT Jr, Garat CV, Reusch JE, Morris KG, Dempsey EC, McMurtry IF, Stenmark KR, Klemm DJ. Rosiglitazone attenuates hypoxia-induced pulmonary arterial remodeling. Am. J. Physiol. Lung Cell. Mol. Physiol. 2007; 292:L885–L897. [PubMed: 17189321]
25. Klemm DJ, Majka SM, Crossno JT Jr, Psilas JC, Reusch JE, Garat CV. Reduction of reactive oxygen species prevents hypoxia-induced CREB depletion in pulmonary artery smooth muscle cells. J. Cardiovasc. Pharmacol. 2011; 58:181–191. [PubMed: 21562428]
26. Garat CV, Crossno JT Jr, Sullivan TM, Reusch JE, Klemm DJ. Inhibition of phosphatidylinositol-3-kinase/Akt signaling attenuates hypoxia-induced pulmonary artery remodeling and suppresses CREB depletion in arterial smooth muscle cells. J. Cardiovasc. Pharmacol. 2013; 6:539–548. [PubMed: 24084215]
27. Pugazhenthi S, Miller E, Sable C, Young P, Heidenreich KA, Boxer LM, Reusch JE. Insulin-like growth factor-I induces bcl-2 promoter through the transcription factor cAMP-response element-binding protein. J. Biol. Chem. 1999; 274:27529–27535. [PubMed: 10488088]
28. Pugazhenthi S, Boras T, O'Connor D, Meintzer MK, Heidenreich KA, Reusch JE. Insulin-like growth factor I-mediated activation of the transcription factor cAMP response element-binding protein in PC12 cells: involvement of p38 mitogen-activated protein kinase-mediated pathway. J. Biol. Chem. 1999; 274:2829–2837. [PubMed: 9915817]
29. Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J. Biol. Chem. 2000; 275:10761–10766. [PubMed: 10753867]
30. Sarkar SA, Gunter J, Bouchard R, Reusch JE, Wiseman A, Gill RG, Hutton JC, Pugazhenthi S. Dominant negative mutant forms of the cAMP response element binding protein induce apoptosis and decrease the anti-apoptotic action of growth factors in human islets. Diabetologia. 2007; 50:1649–1659. [PubMed: 17593347]
31. Watson PA, Birdsey N, Huggins GS, Svensson EC, Heppe DB, Knaub LA. Cardiac-specific overexpression of dominant-negative CREB leads to increased mortality and mitochondrial dysfunction in female mice. Am. J. Physiol. Heart Circ. Physiol. 2010; 293:H246–H259. [PubMed: 17337597]
32. Konhilas JP, Watson PA, Maass A, Boucek DM, Horn T, Stauffer BL, Luckey SW, Rosenberg P, Leinwand LA. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. Circ. Res. 2006; 98:540–548. [PubMed: 16439687]
33. Fentzke RC, Korcarz CE, Lang RM, Lin H, Leiden JM. Dilated cardiomyopathy in transgenic mice expressing a dominant-negative CREB transcription factor in the heart. J. Clin. Invest. 1998; 101:2415–2426. [PubMed: 9616213]
34. Spencer KT, Collins K, Korcarz C, Fentzke R, Lang RM, Leiden JM. Effects of exercise training on LV performance and mortality in a murine model of dilated cardiomyopathy. Am. J. Physiol. Heart Circ. Physiol. 2000; 279:H210–H215. [PubMed: 10899058]
35. Garat CV, Crossno JT Jr, Sullivan TM, Reusch JE, Klemm DJ. Thiazolidinediones prevent PDGF-BB-induced CREB depletion in pulmonary artery smooth muscle cells by preventing upregulation of casein kinase 2α' catalytic subunit. J. Cardiovasc. Pharmacol. 2010; 55:469–480. [PubMed: 20147842]
36. Knaub LA, McCune S, Chicco AJ, Miller M, Moore RL, Birdsey N, Lloyd MI, Villarreal J, Keller AC, Watson PA, Reusch JE. Impaired response to exercise intervention in the vasculature in metabolic syndrome. Diab. Vasc. Dis. Res. 2013; 10:222–238. [PubMed: 23162060]
37. Miller MW, Knaub LA, Olivera-Fragoso LF, Keller AC, Balasubramaniam V, Watson PA, Reusch JE. Nitric oxide regulates vascular adaptive mitochondrial dynamics. Am. J. Physiol. Heart Circ. Physiol. 2013; 304:H1624–H1633. [PubMed: 23585138]
38. Dorn GW 2nd. Mitochondrial dynamics in heart disease. Biochim. Biophys. Acta. 2013; 1833:233–241. [PubMed: 22450031]
39. Chalmers S, Saunter C, Wilson C, Coats P, Girkin JM, McCarron JG. Mitochondrial motility and vascular smooth muscle proliferation. Arterioscler. Thromb. Vasc. Biol. 2012; 32:3000–3011. [PubMed: 23104850]

40. McCarron JG, Olson ML, Wilson C, Sandison ME, Chalmers S. Examining the role of mitochondria in Ca^{2+} signaling in native vascular smooth muscle. Microcirculation. 2013; 20:317–329. [PubMed: 23305516]
41. Taggart MJ, Wray S. Hypoxia and smooth muscle function: key regulatory events during metabolic stress. J. Physiol. 1998; 509:315–325. [PubMed: 9575282]
42. Sward K, Dreja K, Lindqvist A, Persson E, Hellstrand P. Influence of mitochondrial inhibition on global and local $[Ca^{2+}]_I$ in rat tail artery. Circ. Res. 2002; 90:792–799. [PubMed: 11964372]
43. Katakam PV, Wappler E, Katz P, Rutkai I, Institoris A, Domoki F, Gaspar T, Grovenburg SM, Snipes JA, Busija DW. Depolarization of mitochondria in endothelial cells promotes cerebral vascular vasodilation by activation of nitric oxide synthase. Arterioscler. Thromb. Vasc. Biol. 2013; 33:752–759. [PubMed: 23329133]
44. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca^{2+} but requires phosphorylation by Akt at Ser^{1179}. J. Biol. Chem. 2001; 276:30392–30398. [PubMed: 11402048]
45. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science. 2003; 299:896–899. [PubMed: 12574632]
46. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation. 2008; 117:2340–2350. [PubMed: 18427132]
47. Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, Parker TG, Huang Q, Drucker DJ, Husain M. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. Endocrinology. 2003; 144:2242–2252. [PubMed: 12746281]
48. Chai W, Dong Z, Wang N, Wang W, Tao L, Cao W, Liu Z. Glucagon-like peptide 1 recruits microvasculature and increases glucose use in muscle via a nitric oxide-dependent mechanism. Diabetes. 2012; 61:888–896. [PubMed: 22357961]
49. Dong Z, Chai W, Wang W, Zhao L, Fu Z, Cao W, Liu Z. Protein kinase A mediates glucagon-like peptide 1-induced nitric oxide production and muscle microvascular recruitment. Am. J. Physiol. Endocrinol. Metab. 2013; 304:E222–E228. [PubMed: 23193054]
50. Keller AC, Knaub LA, Miller MW, Reusch JE. Saxagliptin restores vascular mitochondrial exercise response in diabetes. Arterioscler. Thromb. Vasc. Biol. 2013; 33:A165.
51. Regensteiner JG, Bauer TA, Reusch JE. Rosiglitazone improves exercise capacity in individuals with type 2 diabetes. Diabetes Care. 2005; 28:2877–2883. [PubMed: 16306548]
52. Gill E, Weil J, Reusch JEB, Wolfel E, Hiatt W, Lindenfeld J, Quaife R, Regensteiner JA. Administration of l-arginine and antioxidants increase vasodilator function in women with type 2 DM. Circulation. 1999; 100(Suppl.):I.757.

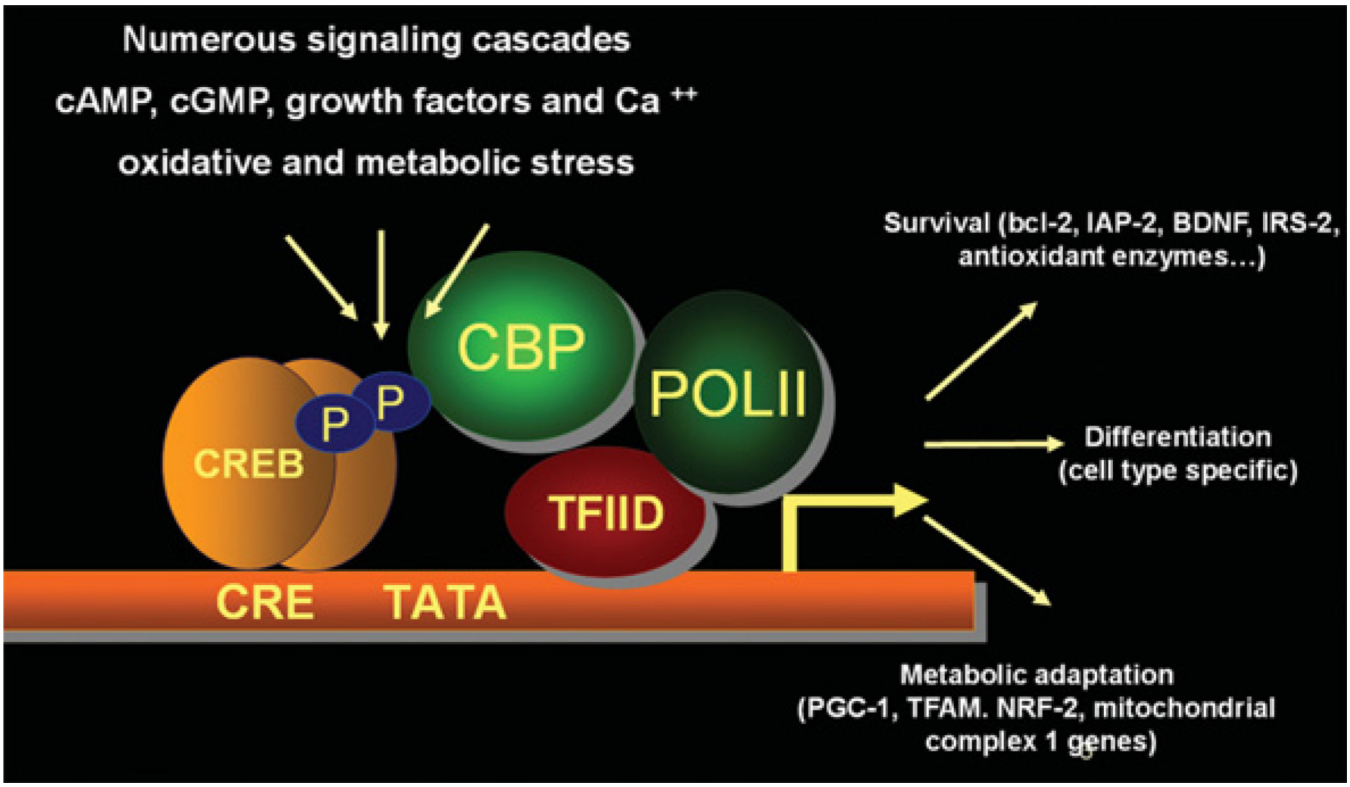

Figure 1. Targets of CREB regulation

CREB is a transcriptional hub integrating signals from G-protein-coupled receptors, tyrosine kinase receptors, Ca^{2+} and oxidant injury to regulate genes essential for survival, differentiation and metabolic adaptation. BDNF, brain-derived neurotrophic factor; CBP, CREB-binding protein; CRE, cAMP-response element; IAP-2, inhibitor of apoptosis 2; IRS-2, insulin receptor substrate 2; NRF-2, nuclear factor-erythroid 2-related factor 2; POLII, RNA polymerase II; TFAM, transcription factor A, mitochondrial; TFIID, transcription factor IID.

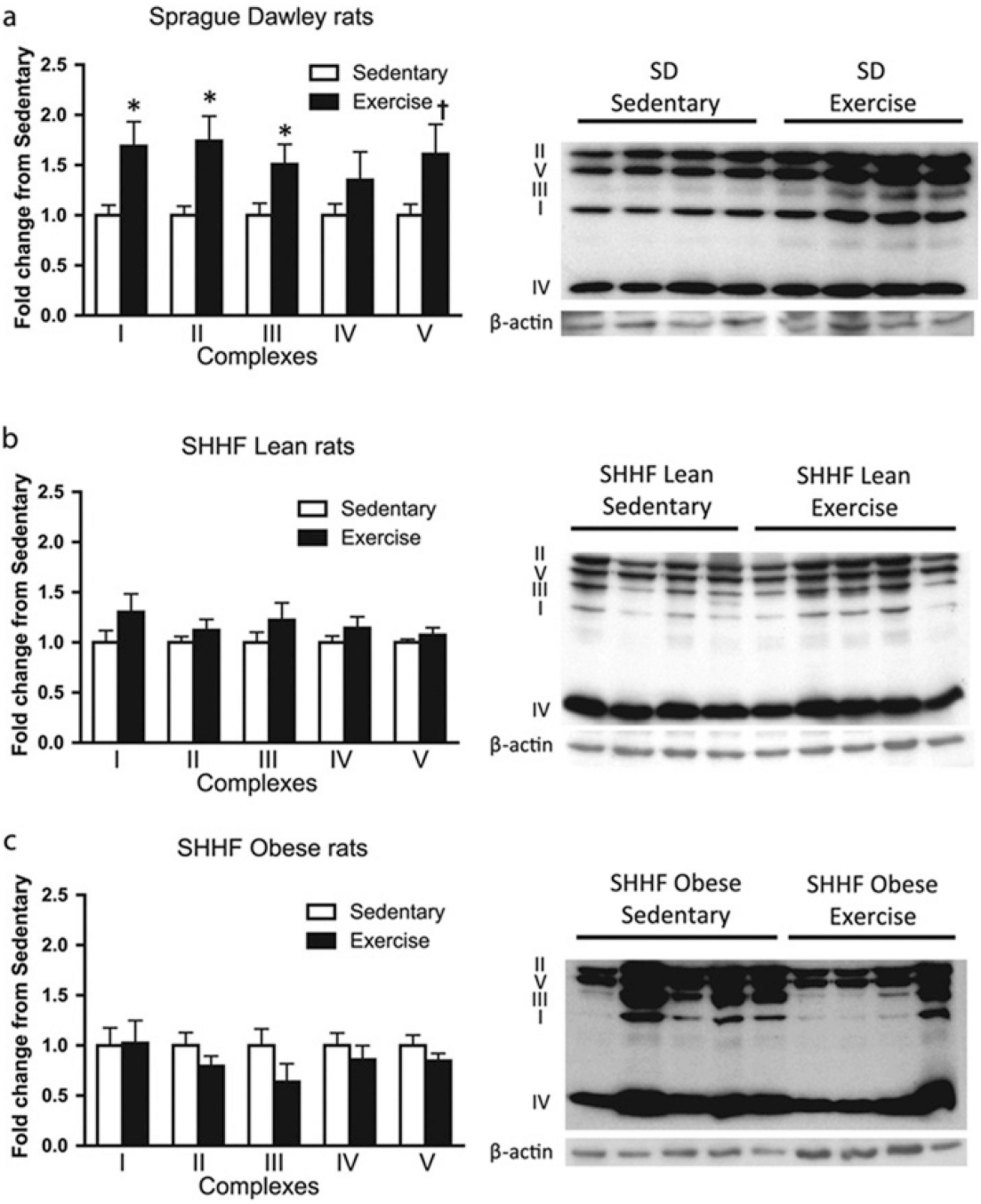

Figure 2. Aortic mitochondrial protein content with exercise intervention

Mitochondrial protein expression profile in SD (Sprague–Dawley) and SHHF lean and obese rats with and without exercise training. Aortic lysates were generated from SD (n = 8 and 9 for sedentary and exercise respectively), SHHF lean (n = 8 and 10 for sedentary and exercise respectively) and SHHF obese (n = 9 for both sedentary and exercise) rats as labelled: 30 μg of protein was run on SDS/PAGE (10% gels), transferred on to nitrocellulose membranes and Western blot analysis was carried out. Results are means±S.E.M. *P < 0.05; †P=0.05–0.10 (Student's t test). Reproduced with permission from [36]: Knaub, L.A.,

McCune, S., Chicco, A.J., Miller, M., Moore, R.L., Birdsey, N., Lloyd, M.I., Villarreal, J., Keller, A.C., Watson, P.A. and Reusch, J.E. (2013) Impaired response to exercise intervention in the vasculature in metabolic syndrome. Diab. Vasc. Dis. Res. **10**, 222–238. © 2013 SAGE Publications.

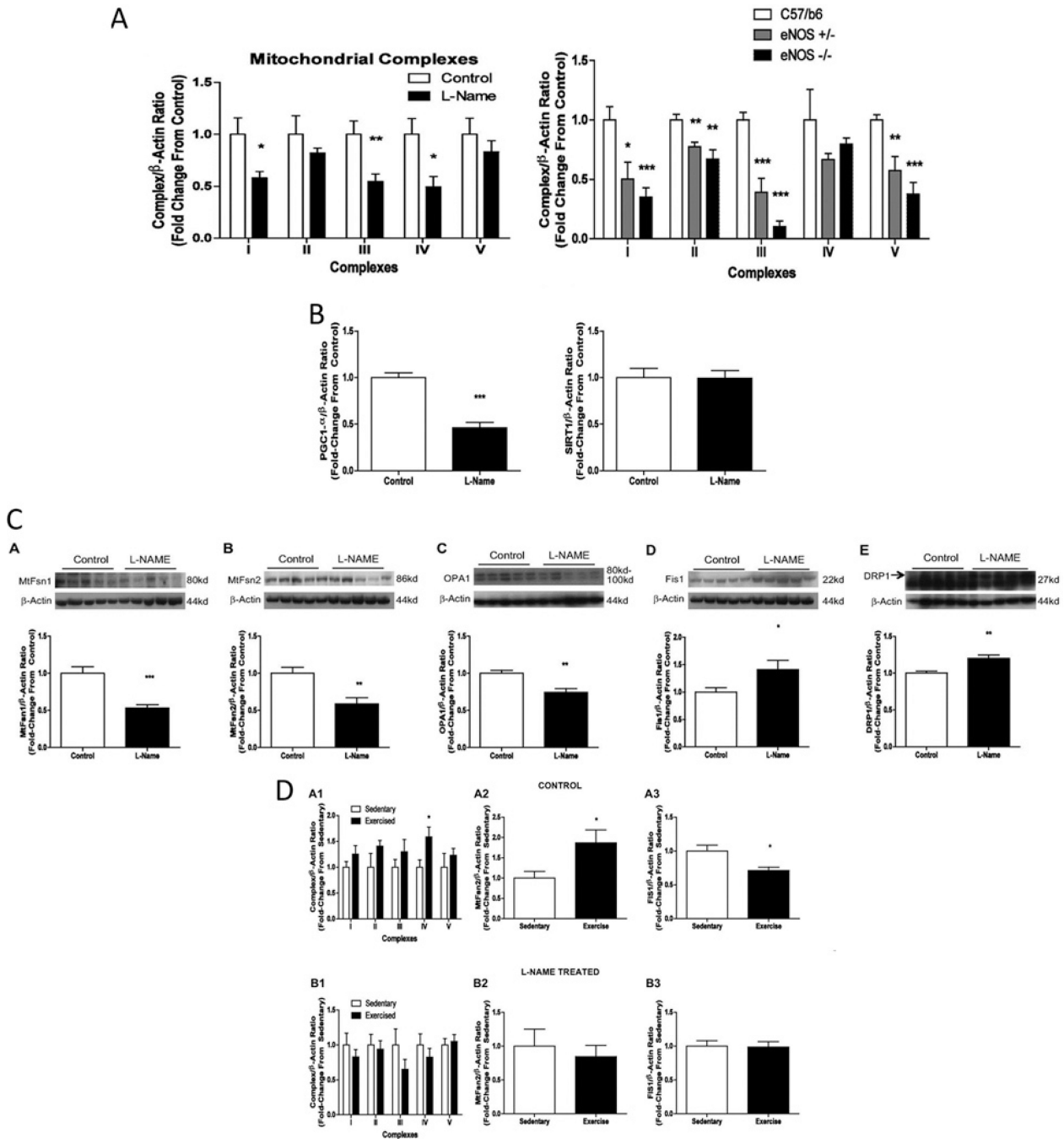

Figure 3. Impact of NOS/eNOS on aortic mitochondrial protein content and dynamics baseline and with exercise intervention

eNOS deletion and NOS disruption mimic diabetes and disrupt adaptive mitochondrial dynamics. (**A**) eNOS deletion or pharmacological NOS inhibition decrease aortic mitochondrial content. (**B**) NOS inhibition decreases mitochondrial regulator PGC1α without changing SIRT1 (sirtuin 1) protein content. (**C**) NOS inhibition decreases mitochondrial fusion proteins (Mfn1, Mfn2 and OPA1) and increases mitochondrial fission proteins (Fis1 and Drp1). (**D**) Exercise intervention increases mitochondrial protein and

Mfn2 and decreases Fis1 in control rat aorta, whereas no change is observed in rats exercising with the NOS inhibitor treatment. Adapted from [37]: Miller, M.W., Knaub, L.A., Olivera-Fragoso, L.F., Keller, A.C., Balasubramaniam, V., Watson, P.A. and Reusch, J.E. (2013) Nitric oxide regulates vascular adaptive mitochondrial dynamics. Am. J. Physiol. Heart Circ. Physiol. **304**, H1624–H1633. © 2013 The American Physiological Society.