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Rab4a signaling unmask a pivotal link between myocardial homeostasis and metabolic remodeling in the diabetic heart

Michael N. Sack

Center for Molecular Medicine, NHLBI, NIH, Bethesda, MD

It is projected that by 2025 one third of a billion individuals worldwide will be inflicted with type 2 diabetes mellitus (T2DM) [1]. This widespread rise in incidence is expected to exacerbate cardiovascular disease due to the strong link between T2DM with cardiac hypertrophy, heart failure, increased susceptibility to myocardial infarction, hypertension and stroke. Of the cardiovascular consequences of T2DM, the most perplexing condition is that of ‘diabetic cardiomyopathy’ (see recent reviews [2,3]). This syndrome arises where diabetic patients develop cardiac contractile dysfunction out of proportion to, and in excess of other underlying risk factors, such as hypertension and/or coronary artery disease. Although the pathophysiology has not been completely defined, its hallmarks include; i) cardiac insulin resistance, ii) metabolic remodeling with increased fatty acid utilization and associated mitochondrial dysfunction and iii) cardiac hypertrophy and contractile dysfunction. Our understanding of the pathophysiology of diabetic cardiomyopathy is a prerequisite to develop targeted therapeutics.

The inextricable link between disrupted insulin signaling and metabolic and mitochondrial perturbations is well established in other insulin responsive tissues such as skeletal muscle and the liver [4,5]. In these tissues canonical insulin signaling pathway inhibition has been shown to disrupt glucose uptake and mitochondrial metabolism [6] and conversely the disruption of mitochondrial function has been shown to result in the disruption of insulin signaling [7]. In light of its high energy demand and continuous contractile functioning, an addition dimension of complexity is evident in the heart where the fidelity of insulin signaling and mitochondrial energy efficiency is required to sustain normal cardiac function. The imbalance between the triad of insulin signaling, metabolic functioning and contractile homeostasis are proposed to predispose to diabetic cardiomyopathy and investigations into how these integrate to control cardiac function is being actively pursued.

Genetic and dietary models that predispose to cardiac lipid overload replicate the diabetic cardiomyopathy phenotype [2,3]. In general these murine models display impaired insulin signaling, enhanced fatty acid oxidation, mitochondrial dysfunction and a gradual progression from normal to diastolic and then to combined diastolic and systolic dysfunction. These models highlight the probability that toxic lipid intermediates exacerbate multiple aspects of this cardiomyopathic phenotype but have not, in their own right, delineated the mechanisms linking

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Address for Correspondence: Michael N. Sack, NHLBI, NIH, 10 Center Drive, Bethesda, MD, 20892-1454, USA, sackm@nhlbi.nih.gov.

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Conflicts:

None.

disrupted insulin signaling, perturbed mitochondrial homeostasis and progressive disruption in cardiac contractile function.

To delineate these mechanisms, investigators have employed genetic models to manipulate distinct components of this overall phenotype to dissect out their relative interactions. The Abel laboratory has genetically depleted the cardiac insulin receptor in mice, the so called CIRKO (cardiac insulin receptor knockout) mouse [8,9]. These mice show that the exclusive disruption of insulin signaling in the heart results in disrupted glucose uptake, impaired glucose and fat oxidation, impaired mitochondrial homeostasis with decreased mitochondrial efficiency and increased reactive oxygen species generation. In addition, cardiac contractile dysfunction develops as these mice age. This mouse model directly confirms that disruption of the insulin signaling pathway can replicate the diabetic cardiomyopathy phenotype and implicates ROS signaling as a possible mediator of this syndrome, at least with respect to the disruption of mitochondrial function [10]. The role of insulin signaling in this cardiomyopathic condition is further supported in the whole body knockout of the insulin signaling intermediate serine-threonine kinase Akt2 [11]. In these mice glucose metabolism is disrupted, a *sine quo non* of insulin resistance, and their hearts exhibit increased susceptibility to cell death in response to ischemic stress [12]. The cardiomyopathic phenotype is not completely replicated as the Akt2 knockout mice do not have an altered growth response to pressure overload and, at least at a young age, show no signs of cardiac contractile dysfunction. This incomplete phenotype may reflect in part that Akt2 is one of three Akt family members expressed in the myocardium where the other isoforms may govern additional features of diabetic cardiomyopathy. The Kelly laboratory employed an alternative approach where they engineered mice to preferentially derived cardiac energy from fatty acid oxidation following cardiac-enriched overexpression of PPAR α in transgenic mice (MHC-PPAR α). The phenotype in these mice show increased fatty acid oxidation, decreased glucose utilization, increased cardiac hypertrophy and heart failure and increased susceptibility to ischemic injury [13,14]. Together these targeted manipulations in mice show that cardiac contractile dysfunction associate with disrupted insulin signaling and with metabolic perturbations associated with metabolic remodeling indicative of insulin resistance and T2DM. The mechanistic links between the triad of signaling and metabolic perturbations with contractile function are not, however, completely elucidated by these studies.

Investigation into direct effects of calcium homeostasis on the contractile apparatus in insulin resistance associated diabetic cardiomyopathy is also being actively explored. Potential mechanisms here include the: (i) disruption of sarcoplasmic/endoplasmic reticulum calcium ATPase functioning by impaired insulin signaling [15]; (ii) altered calcium handling due to perturbed mitochondrial function [10] and by perturbed myofilament calcium sensitivity due to post-translational modification of contractile proteins by, for example, phosphorylation or glycosylation [15]. This role of calcium control in this syndrome has not been extensively explored at the genetic level, but is likely to play an instrumental role in the development of diabetic cardiomyopathy. However, whether deficits in calcium handling are primary or result from disrupted insulin signaling and mitochondrial perturbations remains to be shown.

In this issue of the journal, a study from the Muslin laboratory has identified a possible direct link that potentially integrates the triad of features that exemplifies diabetic cardiomyopathy [Reference]. Using gene expression profiling comparing Akt2 knockout and wildtype mouse heart tissue, this laboratory found that the Ras related small GTP binding protein Rab4a is induced when Akt2 is depleted. The genetic deletion of Akt2 in mice, as described above, does develop an insulin-resistant/diabetic phenotype with some features of diabetic cardiomyopathy. In this context the authors investigated whether the known function of Rab4a in β -adrenergic receptor recycling [16,17] could be operational in the T2DM cardiac phenotype. They show that Rab4a induction in the Akt2 null mouse is restricted to the heart

and skeletal muscle, and that this regulation is reproducible in cell culture studies following the siRNA knockdown of Akt2 or by pharmacologic inhibition of Akt. In keeping with the β -adrenergic recycling function of Rab4a, the investigators show that the Akt2 knockout hearts are more sensitive to chronic β -adrenergic stimulation induced cardiac hypertrophy. Interestingly, this phenotype is replicated during aging in the knockout mice without exogenous adrenergic stimulation, and the administration of β -agonists prevents this aging-associated adverse phenotype. The induction of Rab4a appears to be a more generalized feature in the development of cardiac insulin resistance as the MHC-PPAR α mouse, which primarily drive metabolic remodeling as described above, and which phenocopies diabetic cardiomyopathy similarly have increased Rab4a levels in the heart. Similarly the overexpression of PPAR α in cardiomyocytes also shows the induction in Rab4a levels. As in the aging Akt2 knockout mouse, propranolol therapy reduces the extent of cardiac hypertrophy in MHC-PPAR α transgenic mice. Together these findings identify a potential regulatory link that could integrate insulin signaling, metabolic remodeling and cardiac functional impairment.

The identification of the regulation of Rab4a with its role in resensitization of the β -adrenergic receptor hypersensitivity [16,17] is an intriguing concept with respect to the diabetic heart as the activation of the neurohormonal systems in parallel with the deterioration in cardiac function leads to insulin resistance, and cardiac insulin resistance is proposed to lead to activation of neurohormonal system [18]. Excessive neurohormonal activation additionally exacerbates cardiac dysfunction by promoting progressive cardiac injury and fibrosis, hypertrophy, adverse remodeling which cumulatively also increases the risk of malignant cardiac arrhythmias [19]. The role of Rab4a in this pathophysiology, and its direct link with diabetic cardiomyopathy, has yet to be firmly established. However, the study by Etzion et al [Reference], unmasks this potential signaling link and affords us the opportunity to explore this pathology further with the genetic models available including mice with cardiac restricted overexpression of Rab4a [17].

Although this signaling link has been identified, additional research will need to be performed to validate the role of Rab4a in orchestrating the diabetic cardiomyopathy phenotype. Similarly, although the authors postulate that the insulin resistance induced regulation of metabolic remodeling then upregulates Rab4a via PPAR α , this regulatory program also needs to be directly demonstrated. The postulated interactions of these pathways in diabetic cardiomyopathy are schematized in Figure 1. In conclusion, the findings from the Muslin laboratory study, further highlights and provides mechanistic insight into the potential ameliorative effects of β -adrenergic receptor antagonist therapy. If this biology is validated, it would be further evidence to support the use of a currently available pharmacological class of agents, as a possibly therapeutic strategy to prevent or delay the development of diabetic cardiomyopathy.

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References

1. Hossain P, Kavar B, El NM. Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* 2007;356(3):213–215. [PubMed: 17229948]
2. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation* 2007;115(25):3213–3223. [PubMed: 17592090]
3. Sack MN. Type 2 diabetes, mitochondrial biology and the heart. *J Mol Cell Cardiol* 2009;46(6):842–849. [PubMed: 19217910]

4. Erion DM, Shulman GI. Diacylglycerol-mediated insulin resistance. *Nat Med* 2010 Apr;16(4):400–402. [PubMed: 20376053]
5. Pagel-Langenickel I, Bao J, Pang L, Sack MN. The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance. *Endocr Rev* 2010 Feb;31(1):25–51. [PubMed: 19861693]
6. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 2006;55 Suppl 2:S9–S15. [PubMed: 17130651]
7. Pagel-Langenickel I, Bao J, Joseph JJ, Schwartz DR, Mantell BS, Xu X, et al. PGC-1 α integrates insulin signaling, mitochondrial regulation, and bioenergetic function in skeletal muscle. *J Biol Chem* 2008;283(33):22464–22472. [PubMed: 18579525]
8. Belke DD, Betuing S, Tuttle MJ, Graveleau C, Young ME, Pham M, et al. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. *J Clin Invest* 2002 Mar;109(5):629–639. [PubMed: 11877471]
9. Boudina S, Bugger H, Sena S, O'Neill BT, Zaha VG, Ilkun O, et al. Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart. *Circulation* 2009;119(9):1272–1283. [PubMed: 19237663]
10. Bugger H, Abel ED. Mitochondria in the diabetic heart. *Cardiovasc Res.* 2010 Aug 11; In Press: doi: 10.1093/cvr/cvq239.
11. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). *Science* 2001 Jun 1;292(5522):1728–1731. [PubMed: 11387480]
12. DeBosch B, Sambandam N, Weinheimer C, Courtois M, Muslin AJ. Akt2 regulates cardiac metabolism and cardiomyocyte survival. *J Biol Chem* 2006 Oct 27;281(43):32841–32851. [PubMed: 16950770]
13. Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, et al. The cardiac phenotype induced by PPAR α overexpression mimics that caused by diabetes mellitus. *J Clin Invest* 2002;109(1):121–130. [PubMed: 11781357]
14. Sambandam N, Morabito D, Wagg C, Finck BN, Kelly DP, Lopaschuk GD. Chronic activation of PPAR α is detrimental to cardiac recovery after ischemia. *Am J Physiol Heart Circ Physiol* 2006 Jan;290(1):H87–H95. [PubMed: 16155108]
15. Lebeche D, Davidoff AJ, Hajjar RJ. Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. *Nat Clin Pract Cardiovasc Med* 2008 Nov;5(11):715–724. [PubMed: 18813212]
16. Odley A, Hahn HS, Lynch RA, Marreez Y, Osinska H, Robbins J, et al. Regulation of cardiac contractility by Rab4-modulated β 2-adrenergic receptor recycling. *Proc Natl Acad Sci U S A* 2004 May 4;101(18):7082–7087. [PubMed: 15105445]
17. Filipeanu CM, Zhou F, Lam ML, Kerut KE, Claycomb WC, Wu G. Enhancement of the recycling and activation of β -adrenergic receptor by Rab4 GTPase in cardiac myocytes. *J Biol Chem* 2006 Apr 21;281(16):11097–11103. [PubMed: 16484224]
18. Fonarow GC. An approach to heart failure and diabetes mellitus. *Am J Cardiol* 2005 Aug 22;96(4A):47E–52E.
19. Palatini P. Sympathetic overactivity in hypertension: a risk factor for cardiovascular disease. *Curr Hypertens Rep* 2001 Sep;3 Suppl 1:S3–S9. [PubMed: 11580882]

Figure 1.

Schematic of the possible interaction between insulin signaling, metabolic remodeling and cardiac function in T2DM incorporating a regulatory role for Rab4a. The three intransient features of diabetic cardiomyopathy are shown as the three sides of a triangle and the role of Rab4a in this triad is proposed. In brief, disrupted insulin signaling results, in part, through PPAR α signaling to metabolic remodeling. The features of metabolic remodeling are alongside the 'metabolic remodeling' side of the triangle. Protein levels of Rab4a are increased by disrupted insulin signaling and or by metabolic remodeling, via PPAR α , although the direct role of this transcription factor has not been proven. Rab4a promotes Glut4 translocation to the cardiomyocyte membrane as a putative adaptive response (see discussion in manuscript by Etzion et al in this volume of JMCC). Concurrently Rab4a promotes recycling of the β -adrenergic receptor to the cell surface to increase sensitivity to β -adrenergic signaling. This in turn may promote cardiomyopathic features such as hypertrophic growth, via the promotion of ventricular fibrosis and may predispose to an increased arrhythmogenic potential. Proposed functions are illustrated by hatched arrows. Abbreviations: PPAR α - peroxisome proliferator activated receptor alpha; Ox – oxidation; FAO – fatty acid β -oxidation.