Review

Oxidative stress in cardiac and skeletal muscle dysfunction associated with diabetes mellitus

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Diabetes mellitus increases the risk of heart failure independently of underlying coronary artery disease. It also causes skeletal muscle dysfunction, which is responsible for reduced exercise capacity commonly seen in heart failure. The underlying pathogenesis is partially understood. Several factors may contribute to the development of cardiac and skeletal muscle dysfunction in heart failure and diabetes mellitus. Based on the findings in animal models, this review discusses the role of oxidative stress that may be involved in the development and progression of cardiac and skeletal dysfunction associated with diabetes.

***Key Words*: oxidative stress, diabetes mellitus, cardiac dysfunction, skeletal muscle dysfunction, heart failure**

Diabetes mellitus often leads to heart failure (HF), even in the absence of any other risk factors such as coronary artery disease or hypertension, suggesting that diabetes itself causes a specific form of cardiomyopathic state.[1] Diabetes causes myocardial structural remodeling characterized by myocyte hypertrophy, interstitial fibrosis, and apoptosis,[2] which increases cardiac muscle stiffness and may contribute to diastolic dysfunction. Diastolic dysfunction has been regarded as a hemodynamic hallmark seen in diabetes and ultimately contributes to the development of HF.[3,4] Diabetes further worsens systolic dysfunction due to myocardial infarction (MI).[5–7] Previous clinical studies have shown that patients with diabetes have a worse outcome after MI than patients without diabetes despite similar left ventricular (LV) ejection fractions and coronary patency rates.[8,9]

The features of diabetic heart disease have been well identified, however, its pathogenesis and, in particular, the mechanisms underlying myocardial remodeling have not been fully elucidated. The excess production of reactive oxygen species (ROS), superoxide anion ($O_2^{\bullet-}$), H_2O_2, and hydroxyl radical ($^{\bullet}OH$), resulting in oxidative stress, is widely considered to be a cause of target organ damage such as the heart, kidney, and skeletal muscle, in diabetes.[10,11] Therefore, oxidative stress may contribute to the development of cardiac and skeletal muscle dysfunction in diabetes.

Diabetic Heart

Diabetes mellitus causes both diastolic and systolic cardiac dysfunction,[12] but an impairment of diastolic function usually occurs before systolic dysfunction develops. The impairment of diastolic function despite normal systolic function is thought to result from an increased myocardial stiffness.

A growing body of evidence suggests that the production of ROS is increased in the diabetic heart.[13] Specifically, ROS are produced within the mitochondria of the diabetic heart.[14] The first line of defense against ROS-mediated cardiac injury comprises several antioxidant enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSHPx). GSHPx is a key antioxidant that catalyzes the reduction of H_2O_2 and hydroperoxides. It not only scavenges H_2O_2 but also prevents the formation of other more toxic radicals such as $^{\bullet}OH$. GSHPx possesses a higher affinity for H_2O_2 than catalase. Furthermore, it is present in relatively high amounts within the heart, especially in the cytosolic and mitochondrial compartments.[15] These lines of evidence imply the primary importance of GSHPx as a defense mechanism within the heart. Moreover, GSHPx is expected to exert greater protective effects against oxidative damage than SOD because greater dismutation of $O_2^{\bullet-}$ by SOD may result in an increase of H_2O_2. Therefore, compared with SOD or catalase, GSHPx is thought to be more effective in protecting cells, tissues, and organs against oxidative damage.[16,17] In fact, the mice with GSHPx gene overexpression were more resistant to myocardial oxidative stress as well as remodeling and failure after MI.[18] In the diabetic heart, the reduction of myocardial oxidative stress by GSHPx overexpression was associated with the attenuation of diastolic dysfunction, myocyte hypertrophy, and interstitial fibrosis.[19] These findings are consistent with previous studies demonstrating that ROS are involved in the structural alterations of the extracellular matrix collagens.[20,21] The decline in myocardial fibrosis by GSHPx overexpression may contribute to the physiological improvement of diastolic properties.

There may be several factors whereby oxidative stress contributes to myocardial remodeling in diabetes. First, oxidative stress is involved in cardiac myocyte hypertrophy and apoptosis. A subtle increase in ROS caused by partial inhibition of SOD results in hypertrophy and apoptosis in isolated cardiac myocytes,[22] both of which are thought to contribute to diabetic myocardial damage. Specifically, the incidence of apoptosis is increased in the diabetic heart,[23] which may cause loss of myocytes, compensatory hypertrophy of residual myocytes, and interstitial infibrosis.[24] Myocyte necrosis may also be involved in the increased fibrosis from the diabetic heart. Second, oxidative stress induces the activation of matrix metalloproteinases (MMPs). We have demonstrated that MMP-2 activation plays an important role in the pathophysiology of cardiac remodeling.[25] Moreover, MMPs have been shown to be activated by ROS in cardiac fibroblasts.[26] On the basis of these findings, increased ROS may contribute to the activation of MMP and thus to the development of interstitial fibrosis in diabetes. Third, a possible role for growth factors in diabetes-related end-organ complications is increasingly being recognized. Transforming growth factor (TGF)-β and connective tissue growth factor (CTGF) can induce the production of collagen and fibronectin from cardiac fibroblasts and myocytes, and increased expression of both factors contributes to the development and progression of cardiac remodeling in diabetes.[27,28]

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Post-MI Diabetic Heart

Previous clinical findings demonstrated that patients with diabetes have a worse outcome after MI than that of patients without diabetes despite similar coronary patency rate and baseline LV ejection fraction.[8,9] Furthermore, in the clinical study of patients with HF, diabetes reduced long-term survival independent of the etiology of HF and baseline LV ejection fraction.[29] The poor outomes in patients with diabetes have been shown to be related with the progression of HF.[5–7]

Streptozotocin induces diabetes in rodents by chemical destruction of insulin-producing β-cells of the pancreatic islets, which thus models type 1 diabetes resulted from an auto immune-mediated loss of β-cell mass.[30] Hyperglycemia induced by streptozotocin exaggerates LV remodeling and failure after MI.[31,32] In contrast, type 2 diabetes is typically preceded by obesity and associated metabolic abnormalities such as insulin resistance and dyslipidemia. Type 2 diabetes can be modeled by feeding a high-fat diet (HFD) in mice.[33] A HFD leads to insulin resistance, impaired glucose tolerance, an increase in body fat and dyslipidemia, and eventually fasting hyperglycemia. We demonstrated that HFD induced type 2 diabetes characterized by insulin resistance and obesity in mice and exacerbated LV remodeling and failure after MI.[34] Similarly, in Goto Kakizaki rats, a nonobese type 2 diabetes model, the progression of HF was accelerated after MI.[35] Therefore, either type 1 or type 2 diabetes exaggerates post-MI LV remodeling and failure.

The production of ROS is increased in post-MI hearts, and pharmacological and genetic interventions to scavenge ROS can ameliorate this disease process.[36,37] Importantly, the activation of NAD(P)H oxidase enhances oxidative stress and plays an important role also in the progression of post-MI diabetic heart[34] as in other diabetic complications.[38–40]

Skeletal Muscle Dysfunction in Diabetes Mellitus

In patients with type 2 diabetes, the exercise capacity is limited[41] and the lower exercise capacity is a significant independent predictor for mortality.[42] Exercise intolerance is generally believed to be due to the abnormalities in the energy metabolism in skeletal muscle.[43] Moreover, mitochondrial fatty acid β-oxidation and mitochondrial respiration were impaired in skeletal muscle from patients with type 2 diabetes.[44] These results suggest that diabetes may adversely affect mitochondrial function in skeletal muscle and lead to exercise intolerance. However, the pathogenesis of skeletal muscle dysfunction in type 2 diabetes remains undefined. Systemic oxidative stress has been reported to be increased in type 2 diabetes.[45] Moreover, the mitochondrial structure and function were impaired in skeletal muscle from HFD-fed rodents with the altered oxidative stress markers.[46–48] Therefore, increased oxidative stress in skeletal muscle may impair mitochondrial function and limit the exercise capacity in type 2 diabetes.

We demonstrated that the exercise capacity was limited in the diabetic mice induced by HFD feeding.[49] This limitation of maximal exercise capacity was accompanied by a decrease of peak $\dot{V}O_2$. Coincident with these alterations, mitochondrial respiratory activity, electron transport chain (ETC) complex I and III activities and skeletal muscle mitochondrial content were decreased in the HFD mice. These findings are supported by the previous studies in patients with insulin resistance or type 2 diabetes, in which mitochondrial ATP production and state 3 respiration were decreased in skeletal muscle.[44] Moreover, mitochondrial oxidative phosphorylation genes including ubiquinol cytochrome *c* reductase binding protein, a subunit of complex III, were downregulated in skeletal muscle from patients with diabetes, and their expression levels were positively correlated with peak $\dot{V}O_2$.[50] Accordingly, the metabolic abnormalities in the mitochondria from skeletal muscle can well explain a decrease in the exercise capacity and peak $\dot{V}O_2$ in the diabetic mice.

Importantly, chronic administration of apocynin in the HFD mice significantly ameliorated the limited exercise capacity as well as mitochondrial dysfunction in skeletal muscle without affecting glucose metabolism or body fat. Therefore, increased oxidative stress causes mitochondrial dysfunction in skeletal muscle and contribute to exercise intolerance in diabetes. These findings are in agreement with our previous study that the exercise capacity was reduced in conditions in which $O_2^{\bullet-}$ was increased in heterozygous manganese SOD gene knockout mice.[51] The exercise capacity has been reported to be decreased also in rats fed vitamin E-deficient diets.[52] Therefore, oxidative stress and mitochondrial dysfunction in skeletal muscle play a crucial role not only in the development of insulin resistance but also exercise intolerance in type 2 diabetes.

The decrease of mitochondrial ETC complex I and III activities can potentially be explained by direct oxidative damage to mitochondrial complexes.[53] Mitochondria can be the primary target for oxidative damage when ROS production exceeds the capacity of the endogenous ROS scavenging system. $O_2^{\bullet-}$ can easily impair these ETC complexes because they include iron-sulfur center.[54] In addition, oxidative damage to mitochondrial DNA can also result in the decrease in ETC complex activities.[55] Furthermore, the impaired mitochondrial DNA may adversely affect mitochondrial biogenesis.

An explosive increase of patients with type 2 diabetes is a growing medical as well as public health problem in industrialized countries. Therefore, the development of novel therapeutic approaches for diabetes is an important goal for further investigation. The first line in the prevention and treatment of diabetes is the lifestyle modification including diet and physical exercise. Exercise training can increase mitochondrial oxidative capacity in skeletal muscle and delay or prevent diabetes by increasing insulin sensitivity. However, the limited exercise capacity might prevent the completion of proper exercise in patients with type 2 diabetes. Therefore, therapies designed to regulate oxidative stress and maintain mitochondrial function in skeletal muscle are expected to increase the exercise capacity, which may be beneficial in the treatment of diabetes and the prevention of cardiovascular diseases.

Conclusion

To improve the outcomes of patients with HF, the development of therapeutic strategies based on a novel insight into the pathophysiology of cardiac and skeletal muscle remodeling is critically needed.[56–58] Therapies designed to interfere with oxidative stress could be beneficial to prevent not only diabetic heart disease but also skeletal muscle dysfunction in diabetes.

Conflict of Interest

No Potential conflicts of interest were disclosed.

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References

1 Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287**: 2570–2581.

2 Fiordaliso F, Li B, Latini R, Sonnenblick EH, and *et al*. Myocyte death in streptozotocin-induced diabetes in rats in angiotensin II- dependent. *Lab Invest* 2000; **80**: 513–527.

3 Abe T, Ohga Y, Tabayashi N, and *et al*. Left ventricular diastolic dysfunction in type 2 diabetes mellitus model rats. *Am J Physiol Heart Circ Physiol* 2002; **282**: H138–H148.

4 Mizushige K, Yao L, Noma T, and *et al*. Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation* 2000; **101**: 899–907.

5 Aguilar D, Solomon SD, Køber L, and *et al*. Newly diagnosed and previously known diabetes mellitus and 1-year outcomes of acute myocardial infarction: the VALsartan In Acute myocardial iNfarcTion (VALIANT) trial. *Circulation* 2004; **110**: 1572–1578.

6 Stone PH, Muller JE, Hartwell T, and *et al*. The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis The MILIS Study Group. *J Am Coll Cardiol* 1989; **14**: 49–57.

7 Woodfield SL, Lundergan CF, Reiner JS, and *et al*. Angiographic findings and outcome in diabetic patients treated with thrombolytic therapy for acute myocardial infarction: the GUSTO-I experience. *J Am Coll Cardiol* 1996; **28**: 1661–1669.

8 Granger CB, Califf RM, Young S, and *et al*. Outcome of patients with diabetes mellitus and acute myocardial infarction treated with thrombolytic agents. The Thrombolysis and Angioplasty in Myocardial Infarction (TAMI) Study Group. *J Am Coll Cardiol* 1993; **21**: 920–925.

9 Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–234.

10 Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; **40**: 405–412.

11 Nishikawa T, Edelstein D, Du XL, and *et al*. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; **404**: 787–790.

12 Young ME, McNulty P, Taegtmeyer H. Adaptation and maladaptation of the heart in diabetes: Part II: potential mechanisms. *Circulation* 2002; **105**: 1861–1870.

13 Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. *Mol Cell Biochem* 1995; **151**: 113–119.

14 Kanazawa A, Nishio Y, Kashiwagi A, Inagaki H, Kikkawa R, Horiike K. Reduced activity of mtTFA decreases the transcription in mitochondria isolated from diabetic rat heart. *Am J Physiol Endocrinol Metab* 2002; **282**: E778–E785.

15 Le CT, Hollaar L, van der Valk EJ, van der Laarse A. Buthionine sulfoximine reduces the protective capacity of myocytes to withstand peroxide-derived free radical attack. *J Mol Cell Cardiol* 1993; **25**: 519–528.

16 Cohen G, Hochstein P. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry* 1963; **2**: 1420–1428.

17 Toussaint O, Houbion A, Remacle J. Relationship between the critical level of oxidative stresses and the glutathione peroxidase activity. *Toxicology* 1993; **81**: 89–101.

18 Shiomi T, Tsutsui H, Matsusaka H, and *et al*. Overexpression of glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 2004; **109**: 544–549.

19 Matsushima S, Kinugawa S, Ide T, and *et al*. Overexpression of glutathione peroxidase attenuates myocardial remodeling and preserves diastolic function in diabetic heart. *Am J Physiol Heart Circ Physiol* 2006; **291**: H2237–H2245.

20 Norton GR, Candy G, Woodiwiss AJ. Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation* 1996; **93**: 1905–1912.

21 Rosen P, Ballhausen T, Bloch W, Addicks K. Endothelial relaxation is disturbed by oxidative stress in the diabetic rat heart: influence of tocopherol as antioxidant. *Diabetologia* 1995; **38**: 1157–1168.

22 Siwik DA, Tzortzis JD, Pimental DR, and *et al*. Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes *in vitro*. *Circ Res* 1999; **85**: 147–153.

23 Cai L, Li W, Wang G, Guo L, Jiang Y, Kang YJ. Hyperglycemia-induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes* 2002; **51**: 1938–1948.

24 Fiordaliso F, Bianchi R, Staszewsky L, and *et al*. Antioxidant treatment attenuates hyperglycemia-induced cardiomyocyte death in rats. *J Mol Cell Cardiol* 2004; **37**: 959–968.

25 Kinugawa S, Tsutsui H, Hayashidani S, and *et al*. Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. *Circ Res* 2000; **87**: 392–398.

26 Siwik DA, Pagano PJ, Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol* 2001; **280**: C53–C60.

27 Jesmin S, Sakuma I, Hattori Y, Fujii S, Kitabatake A. Long-acting calcium channel blocker benidipine suppresses expression of angiogenic growth factors and prevents cardiac remodelling in a Type II diabetic rat model. *Diabetologia* 2002; **45**: 402–415.

28 Twigg SM, Cao Z, McLennan SV, and *et al*. Renal connective tissue growth factor induction in experimental diabetes is prevented by aminoguanidine. *Endocrinology* 2002; **143**: 4907–4915.

29 Varela-Roman A, Grigorian Shamagian L, Barge Caballero E, Mazon Ramos P, Rigueiro Veloso P, Gonzalez-Juanatey JR. Influence of diabetes on the survival of patients hospitalized with heart failure: a 12-year study. *Eur J Heart Fail* 2005; **7**: 859–864.

30 Buyukdevrim AS. Islet pathology in streptozotocin-induced auto-immune diabetic mice: new insights into clinical practice. *Diabete Metab* 1994; **20**: 87–94.

31 Shiomi T, Tsutsui H, Ikeuchi M, and *et al*. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. *J Am Coll Cardiol* 2003; **42**: 165–172.

32 Smith HM, Hamblin M, Hill MF. Greater propensity of diabetic myocardium for oxidative stress after myocardial infarction is associated with the development of heart failure. *J Mol Cell Cardiol* 2005; **39**: 657–665.

33 Molnar J, Yu S, Mzhavia N, Pau C, Chereshnev I, Dansky HM. Diabetes induces endothelial dysfunction but does not increase neointimal formation in high-fat diet fed C57BL/6J mice. *Circ Res* 2005; **96**: 1178–1184.

34 Matsushima S, Kinugawa S, Yokota T, and *et al*. Increased myocardial NAD(P)H oxidase-derived superoxide causes the exacerbation of postinfarct heart failure in type 2 diabetes. *Am J Physiol Heart Circ Physiol* 2009; **297**: H409–H416.

35 Chandler MP, Morgan E, McElfresh TA, and *et al*. Heart failure progression is accelerated following myocardial infarction in type 2 diabetic rats. *Am J Physiol Heart Circ Physiol* 2007; **293**: H1609–H1616.

36 Kinugawa S, Tsutsui H, Hayashidani S, and *et al*. Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. *Circ Res* 2000; **87**: 392–398.

37 Matsushima S, Ide T, Yamato M, and *et al*. Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 2006; **113**: 1779–1786.

38 Asaba K, Tojo A, Onozato ML, and *et al*. Effects of NADPH oxidase inhibitor in diabetic nephropathy. *Kidney Int* 2005; **67**: 1890–1898.

39 Fortuño A, San José, Moreno MU, Beloqui O, Diez J, Zalba G. Phagocytic NADPH oxidase overactivity underlies oxidative stress in metabolic syndrome. *Diabetes* 2006; **55**: 209–215.

40 Guzik TJ, Mussa S, Gastaldi D, and *et al*. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 2002; **105**: 1656–1662.

41 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.

42 Wei M, Gibbons LW, Kampert JB, Nichaman MZ, Blair SN. Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. *Ann Intern Med* 2000; **132**: 605–611.

43 Okita K, Yonezawa K, Nishijima H, and *et al*. Skeletal muscle metabolism limits exercise capacity in patients with chronic heart failure. *Circulation* 1998; **98**: 1886–1891.

44 Mogensen M, Sahlin K, Fernström M, and *et al*. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* 2007;

doi: 10.3164/jcbn.11-012FR

56: 1592–1599.
45 Ramakrishna V, Jailkhani R. Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. *Acta Diabetol* 2008; **45**: 41–46.
46 Bonnard C, Durand A, Peyrol S, and *et al*. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest* 2008; **118**: 789–800.
47 Iossa S, Lionetti L, Mollica MP, and *et al*. Effect of high-fat feeding on metabolic efficiency and mitochondrial oxidative capacity in adult rats. *Br J Nutr* 2003; **90**: 953–960.
48 Lionetti L, Mollica MP, Crescenzo R, and *et al*. Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. *Int J Obes (Lond)* 2007; **31**: 1596–1604.
49 Yokota T, Kinugawa S, Hirabayashi K, and *et al*. Oxidative stress in skeletal muscle impairs mitochondrial respiration and limits exercise capacity in type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 2009; **297**: H1069–H1077.
50 Mootha VK, Lindgren CM, Eriksson KF, and *et al*. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003; **34**: 267–273.
51 Kinugawa S, Wang Z, Kaminski PM, and *et al*. Limited exercise capacity in heterozygous manganese superoxide dismutase gene-knockout mice: roles of superoxide anion and nitric oxide. *Circulation* 2005; **111**: 1480–1486.
52 Gohil K, Packer L, de Lumen B, Brooks GA, Terblanche SE. Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. *J Appl Physiol* 1986; **60**: 1986–1991.
53 Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res* 2008; **102**: 488–496.
54 Ide T, Tsutsui H, Kinugawa S, and *et al*. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res* 1999; **85**: 357–363.
55 Ide T, Tsutsui H, Hayashidani S, and *et al*. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circ Res* 2001; **88**: 529–535.
56 Ide T, Tsutsui H, Kinugawa S, and *et al*. Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. *Circ Res* 2000; **86**: 152–157.
57 Tsutsui H, Kinugawa S, Matsushima S. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. *Cardiovasc Res* 2009; **81**: 449–456.
58 Tsutsui H, Ide T, Hayashidani S, and *et al*. Enhanced generation of reactive oxygen species in the limb skeletal muscles from a murine infarct model of heart failure. *Circulation* 2001; **104**: 134–136.