Reactive oxygen species and insulin resistant cardiomyopathy

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Summary

1. The prevalence of insulin resistance has increased dramatically in the past decade and is known to be associated with cardiovascular risk. Evidence of an insulin resistant cardiomyopathy, independent of pressure or volume loading influences, is now emerging.

2. Cardiac oxidative stress is often observed coincident with insulin resistance, and there is accumulating evidence that reactive oxygen species (ROS) mediate deleterious effects in the insulin resistant heart. It is established that ROS-modification of signalling proteins can adversely modulate cellular processes, leading to cardiac growth remodelling and dysfunction. The mechanisms of ROS-induced damage in insulin resistant cardiomyopathy are yet to be fully elucidated.

3. A number of different animal models have been used to study cardiac insulin resistance including high sugar dietary interventions, genetically modified diabetic mice and streptozotocin-induced diabetes. Mechanistic studies manipulating cardiac antioxidant levels, either endogenously or exogenously in these models, have demonstrated a role for ROS in the cardiac manifestations associated with insulin resistance.

4. This review summarizes the cardiac specific characteristics of insulin resistance, the features of cardiac metabolism relevant to ROS generation and ROS-mediated cardiomyocyte damage pathways. *In vivo* studies where a combination of genetic and environmental variables have been manipulated are considered. These studies provide particular insights into the induction and suppression of insulin resistant cardiomyopathy.

Introduction

The prevalence of insulin resistance has increased dramatically in the past decade and has now reached epidemic levels in many countries.1,2 Cardiovascular disease is known to be closely linked to insulin resistance and evidence of an insulin resistant cardiomyopathy is emerging.3 A specific cardiac pathology has been identified in various animal models of insulin resistance and, in some cases, this has been demonstrated to be independent of pressure or volume loading influences. Numerous cardiac pathologies have been linked with oxidative stress4,5 and there is accumulating evidence that reactive oxygen species (ROS) have an important role in the development of insulin resistant cardiomyopathy.6

Both genetic and environmental factors contribute independently and in combination to the development of insulin resistance.7 In humans, diets high in fat and refined sugar have been associated with the occurrence of insulin resistance.8,9 Experimentally, dietary interventions such as high fat, and/or high sugar have been utilised to investigate the induction of insulin resistance.10,12 In some studies, these dietary interventions have allowed investigation of the cardiac phenotype associated with insulin resistance independent of the loading effects from hypertension or obesity.13,14 Insulin resistance has also been studied in *db/db* and *ob/ob* mice (genetic models of obesity-induced type II diabetes) and in the glucose transporter 4 (GLUT4) knockout mouse.15-18

These studies have been informative in relation to characterising the systemic effects of insulin resistance, and attention is now focussed on developing a more complete understanding of the insulin resistant myocardial phenotype. Clinical studies suggest that the extent of insulin resistance in the myocardium may be less dramatic than that observed in skeletal muscle and adipose tissue, based on the comparative reductions in glucose uptake observed in these tissues.19,20 However, the myocardial metabolic demand is unrelenting, and glucose supply to support excitation-contraction coupling (ECC) is highly insulin reliant. Restricted availability of glycolytic substrate associated with cardiac insulin resistance may confer particular cardiomyocyte functional vulnerability. Impaired myocardial glucose uptake has been shown to provoke major cardiomyocyte metabolic and structural adaptations.21,22

This review focuses on describing the features of the insulin resistant myocardium and the potential role for ROS in the induction of cardiomyopathy in this setting. The cardiac specific characteristics of insulin resistance are detailed, and a discussion of cardiac oxidative metabolism and ROS-mediated damage pathways is presented. Finally, a number of *in vivo* studies where both genetic and environmental variables are manipulated to demonstrate the interaction of these factors in the induction of insulin resistant cardiomyopathy are considered.

Evidence for myocardial insulin resistance

Cardiomyocyte metabolic resistance

Excitation-contraction dysregulation

Excitation-contraction coupling is achieved by calcium entering the myocyte upon depolarisation which triggers a larger amount of calcium to be released from the sarcoplasmic reticulum to initiate myofilament cross-bridge interaction and myocyte contraction. Demand for the energy intermediate ATP, to support ATPases involved in
cross-bridge cycling (force production) and electrochemical homeostasis (Na$^+$-K$^+$ ATPase) is substantial and comprises about two-thirds of the cardiomyocyte ATP usage. Under normal conditions, myocardial production of ATP is predominantly from $\beta$-oxidation of fatty acids (60 - 90%). The remaining 10 - 40% of ATP production is sourced from glucose oxidation. It is well-established that ATP production from glucose is more efficient than $\beta$-oxidation of fatty acids, producing 40% more ATP per oxygen molecule consumed. There is some evidence that cardiomyocyte calcium handling, and consequently contractility, is highly-reliant on cytosolic glycolytic ATP production. The sarcoplasmic reticulum calcium ATPase (SERCA2a) drives the re-uptake of calcium into the sarcoplasmic reticulum, to achieve relaxation (Figure 1). This pump alone has been estimated to require 15% of ATP production by the cardiomyocyte. Thus a small reduction in ATP availability to SERCA2a would be expected to have a marked impact on the removal of calcium from the cytoplasm, contributing to a prolonged relaxation phase and diastolic dysfunction. Indeed, impaired SERCA2a function may play a direct causal role in diastolic dysfunction.

Glucose uptake is predominantly mediated via the ubiquitous glucose transporter GLUT1 (under basal conditions) and by the insulin-stimulated GLUT4 (Figure 1). Glucose is an important substrate supporting both oxidative and non-oxidative metabolism in the cardiomyocyte. In insulin resistant states, where glucose uptake via GLUT4 is impaired, glycolytic activity is limited. Type II diabetic (db/db) mice exhibit decreased myocardial glucose oxidation and increased fatty acid oxidation associated with reduced cardiac efficiency in the isolated working heart. Type I diabetic streptozotocin (STZ)-treated rats, in which glucose uptake is limited by low circulating insulin, exhibit markedly decreased myocardial glucose oxidation. In these STZ-treated rats, the insulin-sensitising agent, vanadyl, increased sarcolemmal GLUT4 availability and restored glucose oxidation demonstrating that glucose uptake and oxidation are closely linked.

Increased glycolysis is observed in association with pressure-overload induction of cardiac hypertrophy. Thus, it could be expected that in insulin resistant hearts, the switch away from glycolytic production of ATP might
Cardiomyocytes isolated from GLUT4-KO mice exhibit reduced extent of shortening and reduced rates of shortening and lengthening (Domenighetti et al., unpublished data). Interestingly, two studies have shown that, despite deleterious systemic effects, the reperfusion injury following ischemia is less marked in fructose-fed rats. The mechanism behind this effect is unclear. It is possible that chronic metabolic adaptations in insulin resistant cardiomyopathy may blunt the acute impact of ischemic insult. This could represent a form of pathology-conferred short-term cardioprotection, albeit in the context of a longer term functional demise. In non-insulin-resistant hearts, the means by which acute ischemic preconditioning blunts the extent of reperfusion injury involves ROS mediation. Taken together, these findings are consistent with the contention that ROS play an important role in shaping the pathophysiology of the insulin resistant myocardium.

### Reactive oxygen species and cardiomyocytes

#### Cardiac-specific oxidative metabolism

Excess ROS are implicated in a number of cardiac pathological processes -- yet ROS are also necessary mediators of normal cellular function. Myocardial ROS act as important second messengers in signalling pathways. In healthy tissue, appropriate ROS levels are maintained by endogenous antioxidant systems which neutralise or scavenge the ROS to minimise oxidative damage. Oxidative stress occurs when production of ROS is elevated and/or the level of antioxidants is decreased and this balance is disturbed.

The most common forms of ROS in the myocardium are superoxide (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)), peroxynitrite (\(ONOO^-\)), and hydroxyl (\(\cdotOH\)). Superoxide is produced by the one-electron reduction of oxygen and is a highly reactive molecule. The rapid dismutation of superoxide to hydrogen peroxide by superoxide dismutase (SOD) maintains the concentration in the micromolar range. Unlike superoxide, \(H_2O_2\) can freely cross lipid membranes and is relatively more stable. \(H_2O_2\) acts as an important signalling molecule and is maintained in the nanomolar range by catalase, an antioxidant that metabolises \(H_2O_2\) to form oxygen and water. \(\cdotOH\) also rapidly reacts with nitric oxide (NO) to form peroxynitrite, an oxidant which is responsible for inflicting further structural damage through lipid peroxidation.

Thus, the capacity to produce and eliminate ROS in subcellular domains by strategic and sufficient enzyme localization allows for relatively short range and effective cell signalling processes. Signalling over-activation, and/or structural oxidative damage, potentially occurs when increased ROS production is not dealt with adequately by endogenous antioxidant systems.

### Regulating ROS in the cardiomyocyte

In the cardiomyocyte, an important source of ROS is the one-electron reduction of \(O_2\) to superoxide by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, using NADPH as the electron donor. This enzyme is located in the surface membrane and makes a...
significant contribution to ROS production, especially in chronic pathologic states. The heterodimeric structure of NADPH oxidase comprises a catalytic subunit, Nox, which has 5 known isoforms (Nox1-5). These differ in their tissue distribution and kinetics of ROS formation. Nox2 is expressed in cardiomyocytes and is often upregulated in states of oxidative stress. Angiotensin II is a potent activator of NADPH oxidase in the heart and can play an important role in mediating oxidative stress in cardiac pathology.

Mitochondria provide another significant source of cardiomyocyte ROS – particularly in situations of acute stress. In the mitochondria, superoxide derives from electrons leaking from the electron transfer chain reacting with O$_2$. This non-enzymatic reaction is known to occur predominantly at Complex III. High concentrations of intra-mitochondrial SOD catalyse the formation of hydrogen peroxide from superoxide and maintain superoxide at low concentrations within the mitochondria. Following myocardial infarction, this source of ROS may be involved in mitochondrial damage and dysfunction. Other sources of ROS in the heart include xanthine/xanthine oxidase, uncoupled nitric oxide synthase, cyclooxygenase isoforms and β-oxidation of fatty acids. Peroxisosmal production of H$_2$O$_2$ serves to detoxify cellular toxins and other peroxidative metabolism, but a small fraction can escape to participate in other oxidative reactions. Peroxisosmal β-oxidation of long chain fatty acids can also release electrons to form ROS (Figure 1).

There is emerging evidence that this pathway is upregulated in insulin resistance, and may play a role in the induction of oxidative stress.

In healthy tissues, antioxidants regulate intracellular ROS concentrations, maintaining a level required for normal cellular signalling yet preventing excess ROS elevation and oxidative damage. Enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase, and thioredoxin reductase. Non-enzymatic antioxidants in the heart include vitamins C, A and E, these are however less potent. The glutathione peroxidase and thioredoxin reductase antioxidant systems are involved in the conversion of H$_2$O$_2$ to O$_2$ and H$_2$O and are the predominant regulators of mitochondrial and cytosolic H$_2$O$_2$ concentrations in the myocardium.

Mechanisms of oxidative damage

There is accumulating evidence that excessive production of ROS perturbs cellular signalling pathways and interferes with calcium handling in cardiomyocytes. ROS can modify key thiol groups of signalling and ion transport proteins interfering with their normal function. The L-type calcium channel is subject to oxidation by H$_2$O$_2$ and channel activity has been shown to be regulated by antioxidant treatment. Key thiols on SERCA2a proteins are also susceptible to oxidation and in vivo antioxidant therapy has been shown to restore SERCA2a function in diabetic cardiomyocytes.

and chronic oxidative stress in cardiac insulin resistance. Oxidative stress in the insulin resistant myocardium is associated with lipid peroxidation of membrane-bound structural components by ROS such as peroxynitrite. Membrane lipid peroxidation can adversely affect operation of key ion transport systems, and modify membrane fluidity in a manner which constrains membrane macromolecular mobility.

A role for ROS in mediating hypertrophic growth signalling has also been established. Exogenous oxidants can activate mitogen-activated protein kinase (MAPK) pathways in vitro leading to promotion of cellular growth. In cultured adult rat cardiomyocytes, direct application of H$_2$O$_2$ has been shown to increase phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, an effect which could be completely abolished by application of a ROS scavenger. This study also demonstrated that ERK1/2 upregulation induced by α$_1$-adrenoreceptor stimulation was attenuated by pretreatment with a ROS scavenger or with NADPH oxidase inhibitors. Similarly, hypertrophic responses in neonatal rat cardiomyocytes are largely attributed to superoxide.

In vivo administration of antioxidants has also been shown to affect the extent of myocardial growth in a number of rodent models of induced cardiac hypertrophy. Role for oxidative stress in insulin resistant cardiomyopathy

A number of studies have sought to directly link the development of cardiac insulin resistance to ROS-mediated signalling. High sugar dietary intervention studies in animal models have demonstrated that myocardial and renal tissues are more susceptible to damage by ROS, due to impaired antioxidant status in states of systemic insulin resistance. In fructose and sucrose-fed rats, the activities of a range of antioxidant enzymes in ventricular tissue, (i.e. superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) are significantly reduced, and increased lipid peroxidation is observed. Delbosc et al., 2005 demonstrated in Sprague Dawley rats that production of ROS in the aorta, cardiomyocytes and polymorphonuclear cells was elevated within 1 week of a 60% fructose dietary intervention, suggestive of fructose-induced oxidative stress. However, this study was not well controlled for diet composition (the high fructose diet was not specifically matched to the control diet for non-carbohydrate components). Myocardial insulin resistance in GLUT4-KO mice was observed to be associated with upregulation of NADPH oxidase subunit isoforms, Nox1 and Nox2. Interestingly, NADPH oxidase-derived superoxide production was similar to controls yet peroxisosmal metabolism was upregulated, potentially elevating peroxisosmal H$_2$O$_2$ production. These studies suggest that an early increase in ROS generation may be involved in the initiation and development of insulin resistance, and further investigation is warranted.
Antioxidant manipulations and insulin resistant cardiomyopathy

In vivo investigations targeted to defining the role of ROS in mediating cardiopathy in insulin resistant states have used several different approaches combining contrasting paradigms of genetic and environmental manipulations (Table 1). Experimental strategies involving overexpression of cardiac antioxidants, or administration of exogenous antioxidants, in the context of insulin resistance have identified mechanistic links between insulin resistance and ROS in the heart. Studies utilising overexpression of the cardiac antioxidants metallothionein and catalase have demonstrated that antioxidants play an essential role in the signalling and contractile dysregulation associated with insulin resistance.46,84

Tissue insulin resistance is characterised by downregulated activity of the insulin/IGF-1 signalling pathway involving diminished stimulation of the phosphoinositide 3-kinase (PI3K). The protein kinase Akt (also termed PKB) is a downstream PI3K phosphorylation target. Insulin-stimulated Akt phosphorylation leads to translocation of GLUT4 to the sarcolemma (Figure 1). Insulin resistance in the myocardium (and in other tissues) is characterised by impaired PI3K/Akt signalling and GLUT4 translocation.84 Depressed insulin-stimulated Akt phosphorylation observed in STZ and sucrose-diet induced cardiomyopathies can be offset by transgenic overexpression of endogenous antioxidants (catalase or metallothionein). In cardiomyocytes of wildtype animals a high sucrose diet was observed to reduce peak shortening and to slow contraction and relaxation kinetics. Impaired calcium handling was evidenced by smaller calcium transients and slower decay of intracellular calcium. Metallothionein transgenic mice on a high sucrose diet did not however exhibit any of these deficits.46 In a related study from the same group, cardiac-specific catalase transgenic mice were used to investigate the effect of this antioxidant on sucrose-induced contractile dysfunction. Transgenic mice fed a high sucrose diet for 12 weeks did not exhibit reduced peak shortening or calcium transients evident in the sucrose-fed wildtype mice. Interestingly, overexpression of catalase did not improve insulin-stimulated cardiomyocyte glucose uptake.85 These studies demonstrate that antioxidants can rescue the contractile dysfunction and signalling abnormalities associated with insulin resistance, providing further evidence of ROS involvement in insulin resistant cardiomyopathy.

Administration of exogenous α-lipoic acid, an antioxidant with glycolytic-enhancing properties, was used to investigate the interaction between antioxidants and cardiac metabolic dysregulation in fructose-fed rats. α-lipoic acid treatment elevated myocardial free fatty acid in a metabolic shift consistent with a switch towards increased reliance on β-oxidation of fatty acids. Protein expression of myocardial antioxidants, which was markedly depressed in fructose-fed rats, was attenuated in part by α-lipoic acid.43 These findings suggest a mechanistic link between glycolytic dysregulation and oxidative stress in the context of insulin resistance, but further investigation is required to fully elucidate this interaction.

In models of insulin resistance where cardiac hypertrophy is apparent, antioxidant intervention studies have been informative in relation to the link between cardiac growth and ROS in insulin resistant states. GLUT4-KO mice exhibit 61% increase in cardiac weight index (heart weight: body weight) relative to controls. This is associated with elevated levels of myocardial Nox1 and Nox2 (NADPH oxidase isoforms). Administration of tempol (a radical scavenger) in drinking water for 4 weeks significantly regressed the cardiac hypertrophy by half (to a cardiac weight index value of 30% greater than control).37 Chess et al., 2008 used a similar intervention to investigate the hypertrophic and metabolic impacts of fructose feeding on pressure overload-induced cardiac hypertrophy.44 Although no increase in heart growth was evident in fructose fed sham mice, the TAC surgery induced a larger

**Table 1. Summary of studies investigating in vivo antioxidant modulation of insulin resistant cardiomyopathies.**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Antioxidant</th>
<th>Insulin resistance induction</th>
<th>Antioxidant modulation</th>
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<td>Transgenic overexpression</td>
<td>Metallothionein</td>
<td>High sucrose diet</td>
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<td>Catalase</td>
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<td>Streptozotocin</td>
<td>α-Lipoic acid</td>
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<td>Tempol</td>
<td>GLUT4-KO</td>
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<td></td>
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<td>High fructose diet</td>
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Abbreviations: GLUT4-KO (glucose transporter 4 knockout mouse); TAC (transverse aortic constriction).

Symbols ‘+’ (increase) and ‘−’ (decrease) indicate the modulating effect of antioxidant influence on the induced insulin resistant state.

Symbol ‘?’ indicates no effect of antioxidant.

Symbol ‘?’ indicates that no information is available (i.e. measurement/experiment not undertaken).
increase in heart weight: tibia length ratio in fructose fed mice relative to starch fed mice (55% and 22% respectively). Administration of tempol attenuated the cardiac hypertrophy observed in both groups to control-diet sham levels. In addition, Delbosc et al., 2005 demonstrated that cardiac weight index was significantly correlated with myocardial superoxide production in fructose-fed rats. These studies provide evidence that cardiac hypertrophic remodelling in insulin resistant states is closely linked with levels of myocardial ROS and may be driven by ROS activation of growth signalling pathways.

**Conclusion**

Clinically, insulin resistance and cardiovascular disease are frequently linked, with cardiac dysfunction and diabetes coincident. A specific cardiac pathology has been identified in various animal models of insulin resistance, and can occur independently of hemodynamic loading influences. There is evidence to suggest that cardiomyocyte excitation-contraction coupling and activator calcium handling may be particularly reliant on insulin-dependent glucose uptake and glycolytic metabolism. Studies with diabetic rodent models (i.e. STZ-treated, db/db, GLUT4-KO, high sugar diet) have provided insight into altered metabolic and functional performance in the insulin resistant myocardium. There is also evidence that an early increase in ROS production in the insulin resistant myocardium may be involved in the initiation and development of cardiomyopathy. ROS-mediated hypertrophy induction and structural modification of key transporter proteins involved in excitation contraction coupling has been observed. In vivo experimental studies demonstrate that increased antioxidant activity can ameliorate the development of insulin resistant cardiomyopathy. More detailed cellular functional and mechanistic investigations are required to fully elucidate the complex role of ROS in mediating insulin resistant cardiomyopathy, and to assist in developing targeted cardioprotective interventions.

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**References**

17. Buchanan J, Mazumder PK, Hu P, et al. Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. Endocrinology 2005; 146:
ROS and myocardial insulin resistance

C772-80.


79. Muller JM, Cahill MA, Rupec RA, et al. Antioxidants as well as oxidants activate c-fos via Ras-dependent

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