Insulin says NO to cardiovascular disease

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Abstract

It is well recognized that insulin resistance found in patients with type 2 diabetes and obesity is a major risk factor for cardiovascular disease. Since its discovery in the 1920s, insulin has been used as an essential therapeutic agent in diabetes for blood glucose management. Recent studies demonstrate that insulin signalling is essential for normal cardiovascular function, and lack of it (i.e. insulin resistance) will result in cardiovascular dysfunction and disease. Moreover, insulin is the key component of glucose–insulin–potassium cocktail and exerts significant cardiovascular protective effect via a phosphatidylinositol 3′-kinase–protein kinase B–endothelial nitric oxide synthase (PI3K-Akt-eNOS)-dependent signalling mechanism in addition to its metabolic modulation, which renders it a potent organ protector in multiple clinical applications. This review focuses on insulin-initiated PI3K-Akt-eNOS survival signalling, with nitric oxide as an ‘end effector’ delivering cardioprotection in health and disease (especially in ischaemic heart disease), and highlights the impairment of this survival signalling as a key link between insulin resistance and cardiovascular disease.

Keywords

Insulin • Nitric oxide • Cardioprotection • Myocardial ischaemia • Insulin resistance

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1. Introduction

When Frederick Banting successfully isolated the internal secretion of canine pancreas by pancreatic duct ligature in the early 1920s, it is unlikely he would have thought that the newly purified extract, ‘insulin’, would be hailed as one of the twentieth century’s greatest medical discoveries. As a life-saving drug for diabetes treatment, insulin was first recognized mainly for its essential roles in glucose homeostasis regulation 85 years ago. In the last two decades, however, great progress has been made in understanding insulin’s signal transduction mechanism and the non-classical cardiovascular actions that play an important role in coupling metabolism with cardiovascular physiology and pathology.

A remarkable plethora of evidence offers a new perspective that insulin elicits metabolism-independent cardioprotection against myocardial ischaemic injury and the development of atherosclerosis, hypertension and heart failure, in which bio-available nitric oxide (NO) plays a significant role as a second messenger of insulin-mediated survival signals. Furthermore, interruption of insulin signalling with the endogenous cardiovascular NO system is participatory in the pathogenesis of insulin resistance and its cardiovascular complications.

2. Insulin as a key ingredient in the glucose–insulin–potassium regimen for cardioprotection

Prevention of coronary heart disease (CHD) and reduction of its mortality and morbidity remains a major public health challenge throughout the world. In 1962, Sodi-Pallares et al.¹ introduced the concept of infusing a ‘polarizing solution’ comprised of glucose, insulin, and potassium (GIK) to improve recovery from acute myocardial infarction (AMI), wherein insulin stimulates K⁺-ATPase activation while it stimulates glucose uptake for glycolytic energy production. Over the following years, numerous clinical trials evaluated GIK as an adjunct to the contemporary management of AMI, recognizing its potential benefit in reducing in-hospital mortality. The newly coined ‘metabolic cocktail’ was believed to economize metabolic efficiency, as well as decrease accumulation of toxic free radicals in the ischaemic myocardium. In 1970s, Opie proposed the ‘glucose hypothesis’ that the essential rationale for GIK therapy revolved around insulin-mediated promotion of glycolysis in cardiomyocytes and diversion of fatty acids to adipocytes with a resultant reduction of cardiac free fatty acid metabolism.² This rationale has
been well accepted since then as the major mechanism of action of GIK. However, some studies showed that GIK did not affect the pattern of myocardial substrate uptake or oxygen consumption during reperfusion, and insulin-mediated cardioprotection was independent of glucose presence, suggesting that metabolic modulation was unlikely to be the major mechanism underlying GIK cardioprotection in AML. By carefully comparing the individual components of GIK in an in vivo model of myocardial ischaemia/reperfusion (MI/R), we found an intriguing fact that removing insulin from GIK almost completely abolished the anti-apoptotic and infarct-limiting effects of GIK, and insulin alone exerted similar cardioprotective effects to GIK. Moreover, the cardioprotective effects of GIK or insulin can be significantly blocked by wortmannin, a phosphatidylinositol 3′-kinase (PI3K) inhibitor. These results strongly suggest that insulin, rather than glucose or potassium, is the key component of GIK in cardioprotection.

Further studies from our laboratory and others have demonstrated that insulin, in addition to its metabolic modulation, directly activates vascular endothelial and myocardial protein kinase B—endothelial nitric oxide synthase (Akt–eNOS) signalling, leading to enhanced endogenous NO production and cardiovascular protection. In the ischaemic heart in particular, the increased NO confers significant cardioprotection by dilating coronary vessels, hence increasing myocardial perfusion as well as reducing myocardial apoptotic cell death and myocardial infarction. This ‘insulin hypothesis’ highlights insulin as the predominant protective component of the GIK regimen, orchestrating cardioprotection through both metabolic and direct cell survival modulations.

3. Insulin–NO signalling in physiology and pathology

3.1. Insulin increases NO production

In addition to the classical actions of insulin in co-ordinating glucose homeostasis, a complete biochemical signalling pathway linking the insulin receptor to activation of eNOS in vascular endothelium has recently been elucidated. Insulin enhances eNOS-derived NO production, which on the one hand has important vascular actions leading to vasodilatation and increased blood perfusion, and on the other hand exerts anti-apoptotic and pro-survival effects for the ischaemic/reperfused heart. Interestingly, the insulin signalling pathway in vascular endothelium that regulates eNOS activation employs a phosphorylation-dependent mechanism that is completely distinct from classical calcium-dependent mechanisms used by G-protein-coupled receptors, such as the acetylcholine (ACh) receptor. Classical vasodilators, such as ACh, stimulate an increase in intracellular calcium that promotes the binding of calcium/calmodulin to eNOS. In the presence of a variety of co-factors, this results in dissociation of eNOS from caveolin-1 with subsequent dimerization and activation of eNOS, which subsequently stimulates production of the potent vasodilator NO from vascular endothelium. However, insulin-mediated NO production requires activation of the insulin receptor tyrosine kinase that phosphorylates insulin receptor substrate-1 (IRS-1), leading to binding and activation of PI3K, and subsequent activation of phosphoinositide-dependent protein kinase-1 (PDK-1). Downstream, serine—threonine kinase Akt is then phosphorylated and activated, which directly causes eNOS S1177 phosphorylation, and subsequently increases NO production within minutes, whereas the insulin—mitogen-activated protein kinase (MAPK) signalling pathway regulates secretion of the vasoconstrictor endothelin-1 from endothelium. These vascular actions of insulin contribute to the coupling of metabolic and haemodynamic homeostasis that occurs mainly in healthy conditions.

3.2 Insulin–NO signalling in ischaemic myocardium

Importantly, this insulin-initiated PI3K–Akt-dependent mechanism of NO production was not only demonstrated in cultured cells, such as human umbilical vein endothelial cells, but was preserved in ischaemic/reperfused myocardium in vivo. Phosphorylation of eNOS by Akt with subsequent increase in NO production is an important downstream effector in the anti-apoptotic signalling by insulin in MI/R. Moreover, the fact that N\(^{\text{4}}\)-nitro-L-arginine methyl ester (L-NAME), a competitive NOS inhibitor, significantly reduced the protective effects of insulin by blocking NO production further illustrates the critical responsibility of the NO pathway for the observed cardioprotective effects of insulin (Figure 1).

Although eNOS-mediated NO generation has been repeatedly confirmed as a key mechanism underlying insulin-elicited cardioprotection, understanding the specific role of NO as a ‘second messenger of insulin’ is of great clinical import. The majority of experimental studies thus far agree that NO-based therapies exert beneficial effects following MI/R injury. Previous studies have demonstrated that eNOS deficiency exacerbates MI/R injury, whereas the overexpression of eNOS, the administration of NO donors, and inhaled NO gas therapy all significantly protect the myocardium. Endogenous NO was reported to contribute to hibernation of the ischaemic myocardium by reducing oxygen consumption and preserving calcium sensitivity and contractile function without energy consumption during ischaemia. Exogenous NO has been verified in vivo to significantly improve microvascular perfusion, reduce leucocyte infiltration, and decrease cardiomyocyte apoptosis and infarct size. NO possesses a number of physiological properties that make it a potent cardioprotective signalling molecule. Proposed cytoprotective mechanisms include improvement of coronary perfusion and subsequent contractile function via a potent vasodilatory effect, regulation of myocardial oxygen consumption and metabolism via mitochondrial respiration control, reduction of mitochondrial membrane potential and \([\text{Ca}^{2+}]\), and inhibition of mitochondria-mediated apoptosis.

Several interactions of NO with apoptotic signalling machinery have been postulated to explain the apoptosis-inhibitory effects of insulin following MI/R. For example, NO has been shown to nitrosate apoptosis-mediating and -executing enzymes, such as caspase family members, and inhibit caspase-dependent B-cell lymphoma 2 cleavage and the release of mitochondrial cytochrome c. In addition, low levels of NO downregulate MAP kinase phosphatase-3 (MKP-3) mRNA levels, thereby preventing the inactivation of extracellular signal-regulated kinase (ERK)1/2, an anti-apoptotic member of the MAPK family, and subsequently reducing apoptotic cell death.

Meanwhile, endothelial apoptosis was reported to occur at an earlier time point than that of cardiomyocytes in isolated perfused hearts subjected to I/R. It is well known that endothelial dysfunction contributes significantly to subsequent cellular and functional injury, and a disturbed balance between vasorelaxation and vasoconstriction may contribute to the well-known ‘no reflow phenomenon’ following
MI/R. Our recent study using an in vivo canine model of MI/R confirmed that endothelium-dependent vasorelaxation in the I/R coronary artery was significantly blunted, and correlated with markedly decreased endothelial NO production. More importantly, consistent with earlier in vitro studies demonstrating that insulin activates eNOS and increases NO production in cultured endothelial cells, our in vivo MI/R study identified increased NO production as one of the mechanisms by which insulin administration preserves endothelium-dependent vasorelaxation and reduces endothelial apoptosis, thus improving the recovery of coronary flow and subsequent cardiac function.

It is widely appreciated that inflammation is closely associated with poorer AMI outcomes. The anti-inflammatory effect of insulin was first reported as a 40% reduction in plasma high-sensitivity C-reactive protein and serum amyloid A levels in AMI patients. We recently investigated the mechanism of this anti-inflammatory effect of insulin in MI/R animal models, and demonstrated that insulin, via the Akt-activated and eNOS–NO-dependent pathway, inhibits I/R-induced production of tumour necrosis factor-α (TNF-α) by cardiomyocytes, and the endothelial expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1).

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/89/3/516/325849)

**Figure 1** Insulin-initiated PI3K–Akt–eNOS–NO survival signalling and cardiovascular protection. Insulin binds to cell membrane insulin receptor, leading to the activation of mainly two signalling pathways: Ras–MAPK, which results in cell proliferation; and PI3K–Akt–eNOS, which results in metabolic modulation and cardiovascular protection. Among the insulin-activated signalling cascades, PI3K–Akt–eNOS–NO represents a special link between insulin and the cardiovascular system with regard to health and pathology. Activation of this signalling cascade, together with other Akt-activated molecules (such as GSK-3β, mTOR and p70S6 kinase), elicits pro-survival and cardiovascular protective effects, including vasodilatation, anti-apoptosis, anti-inflammation, and anti-oxidative/nitrative stress. Abbreviations: Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; GLUT4, glucose transporter 4; GSK, glycogen synthase kinase; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; mTOR, the mammalian target of rapamycin; PI3K, phosphatidylinositol 3′-kinase; PMN, polymorphonuclear neutrophil; and ROS, reactive oxygen species.

3.3 Insulin–NO signalling and MI/R-associated oxidative and nitrative stress

The activity of NOS is reduced during ischaemia, since NOS requires oxygen to produce NO, as well as being inhibited by the low intracellular pH generated during ischaemia. However, during the first seconds to minutes of reperfusion, myocardial NOS produces a burst of NO and superoxide (O_2^-) simultaneously, which react together to form peroxynitrite (ONOO^-). NO itself is not toxic and does not produce significant tissue injury even at a very high
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3.4 Insulin–NO signalling and mitochondrial functions

Mitochondria are centrally involved in determining cardiomyocyte death or survival following ischaemia and reperfusion. A critical early determinant of cell death in the setting of I/R injury is the opening of the mitochondrial permeability transition pore (mPTP), which leads to rapid dissipation of the mitochondrial membrane potential, ATP depletion, and the onset of rigor contracture. In adult rat ventricular cardiomyocytes, insulin-activated PI3K–Akt pro-survival kinases have been reported to protect cardiomyocytes by reducing the probability of mPTP opening upon reperfusion; more importantly, this effect of insulin was prevented by l-NAME, an inhibitor of nitric oxide production, indicating a critical role of NO downstream of the insulin survival signal in regulating mitochondrial-initiated cell death.

Moreover, increasing evidence suggests that eNOS acts as a metabolic sensor in cardiomyocytes, implying that defective NO production might be linked to cardiomyocyte metabolism dysfunction. Deletion of eNOS was sufficient to reduce mitochondrial biogenesis and function in various cell types and tissues, including cardiac muscle. Several mechanisms have been proposed to account for NO as a regulator of mitochondrial functions. Owing to its vasodilatory properties, NO regulates blood flow to tissues, thus indirectly supplying respiratory substrates to mitochondria and redistributing heat generated by respiring mitochondria. In addition, NO directly regulates the binding and release of O_2 from haemoglobin, and therefore the O_2 supply to mitochondria. NO also inhibits cytochrome c oxidase activity, the terminal enzyme in the electron-transport chain, by competing with O_2, and thus negatively regulating mitochondrial oxidative phosphorylation, particularly at low O_2 concentrations, which allows endothelial cells to adapt to acute changes in O_2 concentration. Moreover, the NO-dependent inhibition of cell respiration functions as part of the adaptive response to stress, such as cardiac failure. Together with hypoxia-inducible factor 1 (HIF-1α), the NO–cytochrome c oxidase system helps to fine-tune cell metabolism upon reduced O_2 availability. Such a sensing mechanism might, for example, allow cardiomyocytes to adapt their metabolic function in increasingly hypoxic conditions.

4. Impairment of the PI3K–Akt–eNOS–NO pathway as a manifestation of cardiovascular insulin resistance

Classically defined insulin resistance is a pathological condition wherein insulin-stimulated glucose uptake and disposal in insulin-targeted organs are decreased. Therefore, insulin resistance in skeletal muscle, adipose tissue, and liver is believed to be the main culprit for impaired glucose homeostasis, and is a defining characteristic of type 2 diabetes and metabolic syndrome. Indeed, pathway-specific impairment in PI3K-dependent insulin signalling contributes to reciprocal relationships between insulin resistance and endothelial dysfunction that foster the clustering of metabolic and cardiovascular diseases in insulin-resistant states. The heart is also an insulin-sensitive organ, and its failure to receive normal insulin signals (e.g. in diabetes) may affect cardiac performance and exacerbate the development of heart failure in some pathological conditions, such as myocardial...
ischaemia and hypertension. Although the metabolic modulation of insulin on myocardium has been intensively studied, little is known so far regarding the changes of myocardial responsiveness to insulin in pathological conditions, such as in myocardial ischaemia, hypertension, and ageing.

In addition to the biochemical index of insulin resistance, decreased Akt phosphorylation has been increasingly recognized as a molecular marker of insulin resistance in insulin-targeted organs. In 1997, Botker et al. reported the first evidence of the existence of myocardial insulin resistance in patients with Syndrome X, where insulin signalling via the PI3K–Akt pathway in the heart, especially in cardiomyocytes, was selectively inhibited in obesity-induced insulin-resistant states, whereas the Ras–MEK (MAPK/ERK kinase)–ERK1/2 signalling cascade remained fully responsive (Figure 2).

4.1 Insulin resistance and myocardial ischaemia

In patients suffering AMI, hyperglycaemia upon hospital admission is common, and has been confirmed as a powerful predictor of both mortality and increased inpatient complications, with or without pre-existing diabetes mellitus. The prevalence of hyperglycaemia in AMI could be attributable to stress hormones, such as catecholamines and cortisol, as well as pro-inflammatory cytokines, such as TNF-α, both of which could lead to insulin resistance with further aggravation of glucose dysregulation. Indeed, increasing evidence indicates that myocardial ischaemia, especially in the presence of hyperglycaemia, is closely associated with insulin resistance, which occurs at both the local myocardium and whole-body levels. Insulin resistance may already be present in overt and undiagnosed diabetes mellitus, and is worsened by stress hormones and cytokines. Conversely, the loss of insulin's action could contribute to the poor outcome after myocardial infarction observed in diabetic and insulin-resistant patients. One obvious mechanism accounting for the detrimental effects of insulin resistance in these circumstances is diminished myocardial metabolic flexibility. It has been well documented that in ischaemic conditions the heart preferentially shifts from typical fatty acid to primarily glucose metabolism, for more rapid and simple ATP conversion. Therefore, impaired glucose utilization due to

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**Figure 2** Impairment of insulin–PI3K–Akt–eNOS–NO signalling defines a key characteristic of insulin resistance and cardiovascular dysfunction. Parallel and balanced insulin signalling between PI3K–Akt–eNOS and Ras–MAPK maintains normal cardiovascular growth, metabolism, and functioning in healthy conditions (left). Insulin resistance differentially affects the PI3K and MAPK signalling pathways, i.e. PI3K–Akt–eNOS signalling is impaired while the Ras–MAPK pathway is less affected or even enhanced due to hyperinsulinaemia (right). Decreased Akt activation and eNOS phosphorylation, together with hyperglycaemia-induced eNOS glycosylation by O-GlcNAc modification, leads to reduced eNOS-derived NO generation and mitochondrial dysfunction with resultant enhanced -O₂⁻ production, eNOS uncoupling, and oxidative stress. Excessive NO production due to inflammation-induced iNOS activation leads to nitrative stress, in which ONOO⁻ generated from increased -O₂⁻ and NO contributes directly to endothelial and myocardial injury and dysfunction in insulin resistance and related cardiovascular diseases. Abbreviations: Akt, protein kinase B; BH₄, tetrahydrobiopterin; Cyt C, cytochrome c; eNOS, endothelial nitric oxide synthase; GlcNAc, N-acetylglucosamine; iNOS, inducible NOS; MAPK, mitogen-activated protein kinase; mK⁺ATP, mitochondrial K⁺-ATPase; mPTP, mitochondrial permeability transition pore; PI3K, phosphatidylinositol 3'-kinase; P, phosphorylation; and SNO, nitrosothiol.
insulin resistance handicaps the heart further during its substrate shift, ultimately resulting in structural and functional dysregulation. Another reported mechanism contributing to the adverse effect of insulin resistance on cardiac injury after I/R is through inhibition of vascular endothelial growth factor expression and vascularization in the ischaemic heart, two protective responses that help to improve vascular perfusion and substrate availability.\textsuperscript{57} Accumulating data suggest that improving insulin sensitivity may exert beneficial effects in experimental models of I/R injury. Yue et al. demonstrated that the peroxisome proliferator-activated receptor-\( \gamma \) (PPAR\( \gamma \)) activator rosiglitazone, an insulin sensitizer, reduced the extent of myocardial infarction and improved contractile performance following M/I/R.\textsuperscript{62} Likewise, Wayman et al. reported benefit of using PPAR\( \gamma \) agonists in reducing myocardial infarct size.\textsuperscript{63}

### 4.2 Insulin resistance and atherosclerosis

A common feature of many cardiovascular risk factors, including insulin resistance, obesity, and hypertension, is the development of endothelial dysfunction. As healthy endothelium protects against the major atherosclerotic key processes, such as inflammation, oxidative stress, thrombosis, and smooth muscle cell proliferation, endothelial dysfunction is a crippling final common pathway by which various risk factors increase atherosclerosis. NO, a major mediator of endothelium-dependent vasodilatation, has important anti-inflammatory and anti-thrombotic properties, i.e. inhibiting leucocyte adherence, and limiting platelet adhesion and aggregation, and the expression of plasminogen activator inhibitor-1 (PAI-1), a prothrombotic protein. Therefore, impaired insulin signalling with reduced bioavailable NO may predispose vasculature to hyper-inflammatory and thrombotic states, while insulin-mediated endothelin-1 expression and mitogenic effects are not affected by insulin resistance, further contributing to endothelial dysfunction and the development of atherosclerosis.\textsuperscript{64} Moreover, insulin-mediated eNOS activation and NO production increase blood flow and functional capillary recruitment to myocardium as well as peripheral tissues, which is required for intact insulin signalling.\textsuperscript{55} Thus, when endothelial dysfunction occurs and vascular redistribution effects of insulin are blunted, a vicious cycle occurs, leading to further diminished insulin effects, attributable to compromised delivery of glucose and insulin to the tissues.

### 4.3 Insulin resistance and hypertension

Insulin, besides its metabolic effects, exerts significant actions on the sympathetic nervous system, renin–angiotensin–aldosterone system (RAAS), and endothelial NO release. These activities can be involved in the short- and long-term control of vascular tone and circulating volume influential of blood pressure. Insulin resistance is thereby widely appreciated to contribute to the pathogenesis of hypertension. For instance, insulin and insulin resistance have complex and interrelated effects on components of the neuro-humoral cascade.\textsuperscript{66} Increased sympathetic tone can induce insulin resistance and, conversely, hyperinsulinaemia and insulin resistance promote increased sympathetic discharge.\textsuperscript{67} A clinical example is glucose intolerance and insulin resistance in patients with sleep-disordered breathing. Nocturnal hypoxaemia with subsequent sympathetic over-activity results in significant elevated plasma endothelin-1, diminished endothelial production of NO, as well as sustained vascular inflammation, and a high prevalence of hypertension.\textsuperscript{68} The whole process is closely connected with vascular insulin resistance, which is recognized as intermediate in the putative causal pathway to increased cardiovascular morbidity and mortality.\textsuperscript{69} Likewise, increased RAAS activity impairs insulin signalling, induces inflammation, and reduces NO availability.\textsuperscript{70} Insulin resistance, in turn, upregulates angiotensin II receptor expression.\textsuperscript{71} Therefore, vascular RAAS activation and insulin resistance perpetuate each other and concordantly contribute to endothelial dysfunction, vascular inflammation/remodelling, and hypertension.

Besides its influence on vasculature, insulin resistance may contribute to the development of cardiac systolic/diastolic dysfunction (i.e. hypertensive heart failure) independent of blood pressure effects. Our recent study showed that insulin-stimulated glucose uptake and contractile response are markedly reduced in cardiomyocytes isolated from hypertensive rats, which are accompanied by reduced myocardial Akt activation, and treatment with insulin sensitizer rosiglitazone partly restores the blunted myocyte contractile response to insulin.\textsuperscript{72} Furthermore, we have provided evidence that iNOS expression and ONOO\( ^{-} \) formation are probably a major cause of vascular endothelial as well as smooth muscle cell death occurring in spontaneously hypertensive stroke-prone rats, and may play a significant role in end-organ injury and morbidity/mortality.\textsuperscript{73} Therefore, improving insulin signalling and pharmacological intervention inhibiting ONOO\( ^{-} \) formation in hypertensive patients may reduce cardiovascular complications and decrease hypertension-related death.

### 4.4 Insulin resistance, ageing, and heart failure

The exact mechanisms of myocardial and generalized insulin resistance occurring as a consequence of ageing and heart failure are complex and multifactorial. Possible explanations include sympathetic-adrenal activation, pro-inflammatory cytokines, and endothelial damage.\textsuperscript{67} Metabolically, insulin resistance, once established, adversely affects myocardial metabolism and bioenergetics. As glucose uptake and metabolism are dependent upon insulin, resistance to its effects is particularly detrimental in the ageing/failing heart, which reverts to a more foetal-like substrate utilization characterized by increased reliance on glucose consumption.\textsuperscript{74} More importantly, the metabolic maladaptation during insulin resistant states, along with molecular signalling deficiency, may finally produce adverse functional outcomes. For example, in both cardiomyocytes and isolated perfused hearts from ageing Sprague–Dawley rats, we observed reduced contractile response and glucose uptake to insulin, and blunted post-insulin receptor signalling (Akt—eNOS—NO cascade).\textsuperscript{75,76}

### 4.5 Insulin sensitivity and physical exercise

Epidemiological studies have documented that regular physical exercise promotes cardiovascular health and reduces the risk in patients with established coronary heart disease. The mechanisms mediating cardiovascular protection of exercise are not well defined. Clinical evidence indicated that physical activity improves endothelial function in patients with coronary artery disease, which is related to Akt activation and eNOS phosphorylation.\textsuperscript{77} Experimental study demonstrated that aerobic exercise reduces myocardial infarction and improves cardiac dysfunction in rats subjected to M/I/R via the PI3K–Akt-mediated mechanism. Moreover, exercise was also reported to induce increases in both circulating and myocardial storage of nitrite, a stable NO metabolite, which is associated with cardiac protection during acute myocardial infarction.\textsuperscript{78,79} Interestingly, recent studies have developed the direct connection between...
the benefits of physical exercise and myocardial insulin sensitivity. Our data, as well as others’, showed that swim training significantly potentiates insulin-induced cardiac contractile function and upregulates myocardial insulin sensitivity, manifested by insulin-stimulated glucose uptake and glucose transporter 4 translocation and, most importantly, the Akt–eNOS–NO cascade plays a critical role in the exercise-induced myocardial insulin sensitization.76,78,80 It has been reported recently that ageing-associated hypertension is attributable to vascular insulin resistance, whereas long-term exercise (swimming) ameliorated hypertension by improving vascular insulin sensitivity in an eNOS-dependent manner.74 These results suggest that exercise increases insulin sensitivity and provides cardiovascular protection partly via activating Akt–eNOS–NO signalling. The exact mechanism by which exercise enhances insulin sensitivity, however, remains to be elucidated.

5. Clinical applications and future perspective

Among the pleiotropic cardiovascular actions of insulin, the stimulation of NO production underlies one of the major mechanisms by which insulin exerts anti-apoptotic, anti-inflammatory and oxidative/nitrative-suppressive effects in a variety of cardiovascular diseases. Exercise, diet, cardiovascular drugs, and insulin sensitizers modulate both metabolic and cardiovascular effects of insulin simultaneously by regulating PI3K–Akt–eNOS signalling. GIK as a cardioprotector has been widely used in cardiac surgery, and until the present, 74 of 91 studies provide convincing evidence for the beneficial effects of insulin and/or GIK in cardiac surgery that go far beyond simple metabolic benefits.9 However, GIK therapy as an adjunct to reperfusion in patients with AMI has had mixed results, although experimental data from animal models of MI/R consistently and strongly supported beneficial effects of the GIK cocktail. The 1995 DIGAMI Study demonstrated a 29% reduction in 1-year mortality, possibly owing to cardioprotection and rigorous glycaemic control with insulin in post-AMI patients. However, the follow-up DIGAMI 2 and CREATE-ECLA trials failed to confirm this positive result. The mechanisms underlying the discrepant results have been under active investigation, and the discussions surrounding these large-scale clinical trials garnered insights into the GIK regimen itself as well as insulin signalling pathways. In retrospect, GIK-infusion protocols differed in dosing regimens, initiation time, intravenous volume and duration. The lack of uniform target blood glucose level, especially the failure in controlling hyperglycaemia in acute AMI due to a lack of adjusting the amount of administered glucose in individual subjects, and the delayed GIK application after AMI, have only further fuelled controversial results.81–83

In addition, it is worth mentioning that the activation of the Ras–MAPK transduction pathway, another major downstream branch of insulin signalling in parallel with the PI3K–Akt pathway, is believed to function as a pro-death signal in MI/R.84 Although these two pathways counterbalance each other in mediating vascular tone, as well as metabolic actions and non-metabolic mitogenic and growth effects of insulin, contributing to the overall biological effects of insulin in maintaining cardiovascular homeostasis, the pathway-specific impairment in PI3K signalling in patients with pre-existing insulin resistance may lead to a shifted balance and produce net effects of unilateral MAPK signalling after exogenous insulin supplementation.85 This situation also applies to patients with heart failure or post-myocardial infarction, which may cause neurohormone-mediated shifts towards MAPK signalling.85 Overall, since GIK/insulin administration is a feasible, low-cost, safe, and easily managed technique as well as effective in clinical practice, it has been routinely used as an adjunctive strategy in peri-operative management of cardiac surgery in most of the heart centres in China, although we believe that the entire potential of the GIK cocktail has yet to be fully disclosed.

Moreover, studies from our laboratory as well as others suggest that the Akt–eNOS survival signalling is a promising therapeutic target for diabetic cardiovascular disease. The pathway-specific impairment in PI3K-dependent signalling in insulin-resistant conditions with reduced bioavailability of NO in vascular endothelium may contribute to reciprocal relationships between endothelial dysfunction and insulin resistance that underlie the associations between metabolic and cardiovascular diseases. Pharmacological and lifestyle modifications may improve insulin sensitivity along with endothelial function, in part, by restoring impaired Akt–eNOS signalling. Therapeutics, such as angiotensin-converting enzyme inhibitors, angiotensin receptor inhibitors, and the PPARγ agonists, thiazolidinediones, have been shown to enhance myocardial insulin signalling in both animal and clinical studies. Interestingly, some Chinese medicinal herbs which target Akt–eNOS signalling, such as ginseng, astragulus root, and fenugreek, have shown promising beneficial effects on prevention of diabetes- and insulin resistance-induced cardiovascular disorders. These so-called ‘phyto-insulins’ exert insulin-like actions, including mild blood glucose-lowering, endothelial function-improving, cardio-protective, and insulin-sensitizing effects in diabetic animals, in part, by restoring impaired Akt–eNOS signalling. Future studies searching for and investigating the mechanism of actions of these ‘phyto-insulins’ as diabetic cardiovascular protectors may be an alternative and promising approach in the prevention of diabetic cardiovascular disease.

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