Cardiac hypertrophy, substrate utilisation and metabolic remodelling: cause or effect?

Rebecca H. Ritchie[2] and Lea M.D. Delbridge[1]

[1]Cardiac Phenomics Laboratory, Department of Physiology, University of Melbourne, Victoria 3010, Australia and [2]Baker Heart Research Institute, PO Box 6492 St Kilda Rd, Central Melbourne, Victoria 8008, Australia

Summary

1. Metabolic remodelling in the heart occurs in response to chronically altered workload and substrate availability. Recently the importance of the metabolic remodelling processes inherent in the hypertrophic growth response (whether primary or secondary) have been recognised.

2. Altered energy demand, shifts in substrate utilisation, and increased oxidative stress are observed in the hypertrophic heart. Both a shift away from carbohydrate usage (i.e. insulin resistance), and a shift to carbohydrate usage (i.e. pressure loading) are associated with disturbed cardiomyocyte Ca^{2+} homeostasis and the development of cardiac hypertrophy.

3. A change in the balance of myocardial usage of fatty acid and glucose substrates must entail a degree of cellular oxidative stress. Increased through-put of any substrate will necessarily involve a regional imbalance between reactive oxygen species (ROS) production and breakdown.

4. In addition to a number of enzyme generators of ROS at various intracellular locations, the heart also contains a number of endogenous antioxidants, to restrict steady-state ROS levels. The balance between ROS generation and their elimination by endogenous antioxidant mechanisms plays a critical role in preserving cardiac function; inappropriate levels of myocardial ROS likely precipitate impairment of myocardial function and abnormalities in cardiac structure.

5. While different metabolic adaptations are associated with hypertrophic responses of contrasting aetiology, there is accumulating evidence that the joint insults of increased production of ROS and disturbed Ca^{2+} handling in the cardiomyocyte comprise the primary lesion. These molecular signals operate together in a feed-forward mode, and have the capacity to inflict substantial functional and structural damage on the hypertrophic myocardium.

Introduction - cardiac hypertrophy and metabolic remodelling

Cardiac hypertrophy, to a greater extent than hypertension, is a major independent risk factor predictive of cardiovascular mortality and morbidity. Secondary only to age, hypertrophy is associated with increased occurrence of detrimental cardiovascular incidents indicative of structural, electrical, metabolic and mechanical dysfunction at the level of the cardiomyocyte. Left ventricular hypertrophy exists in about 10% of the general population under the age of 50, with the prevalence increasing to 43% for those 70 years or older.[1] Monogenic disease conditions which give rise to cardiac hypertrophy independent of hypertension are well recognised (familial hypertrophic cardiomyopathies), but the considerable majority of hypertrophic states are not included within this group of genetic disorders. The Framingham study showed that even amongst a human cohort free of any clinically apparent cardiovascular disease, left ventricular hypertrophy alone was associated with a 50% increase in the cardiovascular risk over a four year follow up period.[2] This relationship persisted even after accounting for the association of hypertrophy with hypertension, obesity, myocardial ischaemia and valvular heart disease.

In healthy populations there is considerable variation in left ventricular mass, for which there is no obvious haemodynamic explanation.[1] This increase in mass is ultimately linked with a higher incidence of clinical events. Population studies show that anti-hypertensive therapies associated with enhanced hypertrophic regression achieve more favourable outcomes.[3] Experimental studies have also demonstrated that hypertrophy and hypertension may be dissociated in polygenic disease models. In the rat, analyses of genetic quantitative trait loci have demonstrated that hypertensive and hypertrophic phenotypes are determined by separate genes.[4]

Hypertrophy, in the context of hypertension, is usually interpreted primarily as an adaptive response in an effort to normalise mechanical wall stress. More recently the importance of the metabolic remodelling processes inherent in the hypertrophic growth response (whether primary or secondary) has been recognised. Altered energy demand, shifts in substrate utilisation, and increased oxidative stress are observed in the hypertrophic heart. Where endocrinological disturbances (such as insulin resistance) induce alterations in substrate availability, the extent to which metabolic remodelling in the heart drives the hypertrophic process is not yet clear. Increased production of reactive oxygen species (ROS) and disturbed Ca^{2+} handling are both implicated as causative molecular signals mediating the link between altered substrate utilization and hypertrophic growth responses.

Altered energy demands of the hypertrophic heart

The heart is a ‘promiscuous’ substrate consumer – that is, opportunistic depending on the types of substrate that are available. The myocardial utilisation of energy sources, approximately 30% glucose and 65% fatty acids, reflects usual circulating substrate levels. Normally the

myocardium produces over 90% of its ATP by mitochondrial oxidative metabolism (fatty acid β-oxidation and glucose-derived pyruvate oxidation *via* the citric acid cycle) and 10% by anaerobic glycolysis.[5]

In the early stages of development, the usage of carbohydrate substrates by the heart is more marked, and glycolysis plays a more important role in servicing the cardiomyocyte energy needs. Metabolic remodelling in the mature heart occurs in response to chronically altered workload and substrate availability. In the aortic banded rat, where a pressure afterload is surgically imposed, and in the genetic spontaneously hypertensive rat (SHR) strain, increased cardiac work is associated with a shift to carbohydrate metabolism.[6,7] In pressure overload and in the SHR, reliance on fatty acid metabolism is decreased. Although insulin-stimulated glucose uptake may also be decreased, basal glucose uptake is elevated.[6,8,9]

This shift in metabolic substrate reliance occurring in load-induced hypertrophy is interpreted as an adaptive response, consistent with a 'foetal recapitulation' of phenotype. The reversion to 'foetal' phenotype is considered to be a 'programmed' response in hypertrophy and has been more broadly invoked to describe alterations in cardiomyocyte Ca^{2+} handling and excitation-contraction coupling.[10] Recently, as the subtleties of various hypertrophic states have been more carefully evaluated, this concept of foetal recapitulation is increasingly recognised as an oversimplified analysis.

There is evidence that the augmented glucose uptake associated with aortic banding produces an increase in the rate of glycolysis that is proportionally greater than the increase in glucose oxidation - so-called 'glycolytic uncoupling'.[11] The adaptive advantage of this situation is not apparent, and may not necessarily recapitulate the early developmental state. A more subtle understanding of how Ca^{2+} homeostasis is impacted by shifts in energy production and utilisation is beginning to emerge.

Hypertrophy induction and altered cardiomyocyte Ca^{2+} handling: is there a link with shifts in substrate utilisation?

Cardiomyocyte shortening and force generation is controlled by the availability of free Ca^{2+} to permit contractile myofilament cross-bridge interaction. To initiate contraction, voltage sensitive Ca^{2+} channels gate the influx of extracellular Ca^{2+} (primarily L-type, possibly some contribution from T-type). This Ca^{2+} directly activates myofilaments and is the trigger to release a larger flux of Ca^{2+} from intracellular sarcoplasmic reticulum (SR) stores *via* the SR release channels. The main means of lowering myoplasmic Ca^{2+} to effect relaxation is through active pumping of Ca^{2+} into the SR by the Ca^{2+} ATPase. Some Ca^{2+} is also removed from the cell by the sodium-calcium exchanger generating an inward current. Active extrusion of Ca^{2+} across the sarcolemma membrane and mitochondrial uptake also occurs, but these mechanisms are of minimal quantitative significance in a normal contraction cycle.

In pressure-loaded hypertrophy, the nature of the mechanical and Ca^{2+}-handling deficits vary depending on the model/aetiology, but some common elements can be identified (see Table 1). Basal cardiomyocyte contractile function is depressed in the young SHR[12] and there is reduced capacity to modulate contraction size in response to altered pacing rate.[13] In contrast, basal contractile function is not diminished in myocytes from animals with aortic coarctation or chronic angiotensin II treatment.[14-16] Myocyte load stress often initiates a shift in gene expression to foetal patterns, such as the expression of a slower myosin isoform (from V1 to V3) with lower ATPase activity. However, such changes appear to be model- and species-dependent, and the energy efficiency/conservation gains by this isoform shift may be of minimal value in the human myocardium where this isoform transition is largely absent.[17]

Table 1. *Summary comparison of myocardial metabolic features and cardiomyocyte Ca^{2+} handling characteristics in insulin resistant/diabetic hearts and pressure loaded hearts. Hypertrophic responses are associated with contrasting metabolic and Ca^{2+} handling adaptations (see text for references). Symbols indicate alterations relative to respective controls (↓ decreased, ↑ increased, = not changed). Where observations conflict or are not consistent, these are indicated by a solidus (/).*

Cardiac Parameter	Insulin resistant/ diabetic	Pressure loaded
glucose uptake (basal)	↓	↑
glucose uptake (insulin stim)	↓	↓
glycosis	↓	↑
glycose oxidation	↓	↓
fatty acid metabolism	↑	↓
glut4 level	↓	↓
glut1 level	↑/=	↑/=
SR Ca cycling/load	↓	↓
SR Ca ATPase	↓/=	↓
contraction kinetics	↓/=	↓/=
contraction amplitude	↓/=	↓/=
Na-Ca exchange	↓/=	↑/=

Extracellular Ca^{2+} influx through the voltage activated channels (L-type) has been shown to be increased, decreased and unchanged in various experimental models.[18] Enhanced expression of a foetal type Ca^{2+} channel (T-type) has been reported in some models of hypertrophy[19] but in SHR, nett myocyte Ca^{2+} current density (L & T type channels combined) is not increased.[20] Evidence that intracellular SR cycling of Ca^{2+} is altered in various hypertrophic conditions is relatively consistent. A number of models (but not all) show slowed re-sequestration of Ca^{2+} into the SR by the SR Ca^{2+} ATPase during relaxation.[14,15,21] There are conflicting data regarding Ca^{2+} transfer *via* the sodium-calcium exchanger in hypertrophy.[22]

In the normal myocardium, about 60% of cardiac energy expenditure is directly used in powering the contractile machinery with a relatively large 15% allocated to the cyclic SR Ca^{2+} pumping.[5,23] In cardiomyocytes there is evidence to suggest that glycolysis is a preferential

source of ATP for membrane associated pumps - in particular SR Ca^{2+} ATPase.[24] Operation of the SR pump is also known to be particularly sensitive to cellular ATP levels. This ATPase requires 85-90% of the free energy of ATP hydrolysis per reaction cycle and is allosterically regulated by the cytosolic ATP/ADP ratio. SR Ca^{2+} pumping and recirculating may be especially vulnerable to limiting sarcolemmal glucose transport. The 'glycolytic uncoupling' observed in pressure loaded hypertrophy[11] (see above) may actually be an adaptive process to protect supply for the SR pump's glycolytic ATP needs.

Although the cellular mechanisms which link pressure-loaded hypertrophy with ultimate contractile failure are not clear, impaired Ca^{2+} homeostasis, altered Ca^{2+} channel expression, elevated diastolic Ca^{2+} levels and depressed SR function are implicated in that pathology.[19,25] The transition from compensated to decompensated hypertrophy in the pressure-loaded situation may at least partially depend on a critical relative reduction in the availability of glycolytically-derived ATP supplied to the SR Ca^{2+} ATPase – this hypothesis has yet to be directly evaluated.

Altered substrate availability, Ca^{2+} handling defects and cardiac hypertrophy

In pressure-load induced hypertrophy the substrate usage shift to increased carbohydrate metabolism is observed in response to increased work demand. In contrast, in the insulin-resistant heart, hypertrophic growth is also frequently present, but in this situation is associated with an impaired capacity to deliver carbohydrate substrate to the cardiomyocyte.

Cardiac glucose uptake is mediated by the glucose transporters GLUT1 and GLUT4.[26] GLUT1 is the responsible for basal 'housekeeping' glucose uptake, while GLUT4-facilitated glucose uptake is increased by both insulin and exercise. There is evidence that in both hypertrophic states, demand-driven and substrate-limited, reduced GLUT4 expression plays a pathological role (see Table 1).

Altered expression of cardiomyocyte GLUT transporters are observed in the hypertrophic myocardium. Experimentally, the enhanced myocardial basal glucose uptake induced by acute haemodynamic loading is associated with an elevation of the GLUT1/GLUT4 transporter ratio and diminished insulin stimulated uptake.[27,28] In the human, information about GLUT transporter expression in the compensated hypertrophic state is lacking, but in failure an increased GLUT1/GLUT4 ratio has been reported.[29] Streptozotocin (STZ)-induced diabetic cardiomyopathy in rodents is associated with a 50% reduction in GLUT4 mRNA and protein expression, and a parallel impairment of glucose transport.

Altered Ca^{2+} handling is observed in cardiomyocytes of insulin resistant and diabetic hearts (see Table 1). Most studies have focussed on streptozotocin-induced models of hypertrophic cardiomyopathy, with a number of discrepant findings reported. While the SR Ca^{2+} uptake is consistently found to be functionally impaired, the reports relating to contractile function and operation of the sodium-calcium exchanger are less consistent. We have shown that cardiomyocytes of STZ-treated rats exhibit delayed timing of contraction parameters and altered Ca^{2+}-kinetics. The extent to which this impacts function is frequency dependent, and is most apparent when dynamic pacing protocols are implemented.[30] *In vitro* studies of cultured rodent cardiomyocytes confirm that exposure to high extracellular glucose levels, simulating the hyperglycaemia associated with insulin resistance, results in impaired glucose uptake. Furthermore, these studies have shown that this impaired glucose uptake directly impacts cardiomyocyte excitation contraction-coupling. Myocyte glucose uptake reduction is correlated with reduced SR Ca^{2+}-ATPase activity, diminished capacity to cycle Ca^{2+} back into the SR, and delayed relaxation.[31] Particularly compelling in demonstrating the link between glucose uptake and Ca^{2+} handling was the finding that cardiomyocytes isolated from the myopathic hearts of streptozotocin treated rats retain abnormal excitation contraction-coupling characteristics *in vitro*.[32] Whether the SR Ca^{2+}-ATPase deficit can be directly attributed to a glycolytic shortage of GLUT4-derived ATP is yet to be demonstrated.

GLUT4 deficiency is a sufficient stimulus to induce hypertrophy

To investigate the impact of reduced GLUT4-mediated glucose uptake, several animal models have been produced in which GLUT4 expression is genetically suppressed. Global GLUT4 deletion (whole body) produces a marked cardiac hypertrophy (2.5 fold increase in cardiac weight index). Coincident upregulation of GLUT1 in these mice preserved myocardial glucose uptake, albeit in the context of marked systemic endocrinologic disturbance.[33,34] In a cardiac-specific GLUT4-'knockout' model, a more modest hypertrophy has been described, 1.3-1.4 fold cardiac weight index elevation) with normal plasma glucose and insulin levels.[35]

Our own investigations of a novel genetic model in which the cardiac effects of moderate and marked GLUT4 deletion can be examined have provided important insight into the relationship between GLUT4 expression and the induction of cardiac hypertrophy. In this model, where the CRE-LOX binary gene excision system was used to create either a cardiac muscle specific 'knockdown' (15% wild type GLUT4 level) or 'knockout' (<5% wild type GLUT4level), we demonstrated a 'dose' relationship between level of GLUT4 expression and degree of cardiac hypertrophy[36] (see Figure 1). Both the knockdown and the knockout animals have similar systemic conditions, including hyperinsulinaemia, hyperglycaemia (insulin-stimulated) and normal mean arterial blood pressure. We found that even when myocardial GLUT4 levels were significantly reduced to just 15% of wild type, only a modest cardiac hypertrophy was observed. When cardiac GLUT4 expression levels were reduced below a 'threshold'

level of 5% of that seen in wild type animals, a dramatic hypertrophy (85% increase in cardiac weight index) and fibrosis was evident. This finding is consistent with the hypothesis that a preserved component of GLUT4-mediated glucose uptake is vital for normal myocardial growth and function. The possibility that glycolytic support of the SR Ca^{2+}-ATPase relies on GLUT4-derived glucose is intriguing. Impaired Ca^{2+}-homeostasis with sustained elevation of Ca^{2+} levels is known to constitute a growth induction signal in the cardiomyocyte.[37] Our findings in the knockdown and knockout models provide strong support for the contention that GLUT4 deficiency has a causative role in the induction of cardiac hypertrophy.

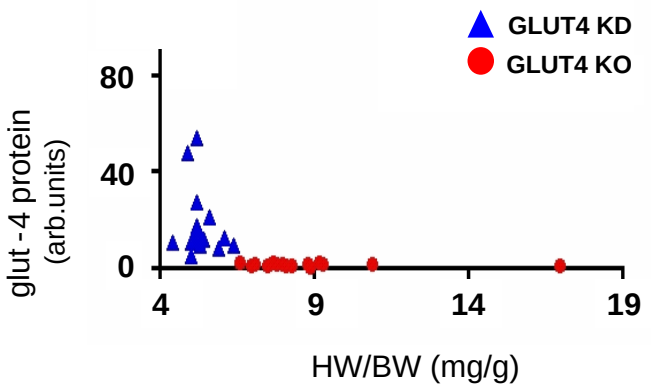

Figure 1. GLUT4 expression levels in GLUT4 'knockout' and 'knockdown' mice ($lox^{+/+}$ and $lox^{+/+}/cre^{+/-}$) inversely related to cardiac weight index (CWI, mg/g). *A threshold effect, where 5% GLUT4 expression associated with 80% increase in CWI. (Adapted from Kaczmarczyk et al.*[36]).

Undoubtedly caution is required in comparing different aetiologies, and the contrasting endocrinological milieux in the various *in vivo* and *in vitro* model systems discussed above need to be taken into account. Notwithstanding, a paradox in relation to substrate shifts in different hypertrophic states is apparent. Both a shift away from carbohydrate usage (i.e. insulin resistance), and a shift to carbohydrate usage (i.e. pressure loading) are associated with disturbed cardiomyocyte Ca^{2+} homeostasis and the development of cardiac hypertrophy.

The resolution of this anomaly involves consideration of the subcellar metabolic adaptations which accompany a shift in the energetic gearing of the cardiac myocyte. A change in the balance of usage of fatty acid and glucose substrates must entail a degree of cellular oxidative stress. Increased through-put of any substrate will necessarily involve a regional imbalance between ROS production and breakdown. The role of elevated levels of ROS as the central lesion in a variety of hypertrophic phenotypes, intimately linked with disturbed cardiomyocyte Ca^{2+} handling, requires elucidation. That these two homeostatic signalling systems have the capacity to feed forward together culminating in progressive hypertrophic myocardial damage is of particular significance.

Role of ROS in the induction of abnormal cardiac growth

ROS have emerged as causative agents in a number of pathological processes in the heart. Elevated ROS are implicated in the development of cardiac hypertrophy, reperfusion injury, remodelling and heart failure.[38] These species include the superoxide anion ($\bullet O_2^-$), nonradical oxidants such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), as well as the hydroxyl radical ($\bullet OH$, primarily derived from $\bullet O_2^-$).[38,39] Superoxide and hydrogen peroxide are the most abundant of these species. Peroxynitrite and H_2O_2 are formed even under normal physiological conditions, *via* the reaction of superoxide with nitric oxide ($NO\bullet$) and the dismutation of superoxide (by superoxide dismutase, SOD), respectively. In sufficient concentrations, these ROS pose a risk of damage to cell membranes (phospholipids), proteins, lipids and DNA. Evidence that ROS mediate the hypertrophic response has been demonstrated both in the intact heart *in vivo* and in cardiac myocytes *in vitro*, whether in response to stretch or other hypertrophic stimuli such as angiotensin II or endothelin-1[40-43]. This increase in ROS triggers cardiomyocyte expression of the proto-oncogene c-*fos*, one of the first indicators of hypertrophy.[41,42] ROS also activate members of the mitogen-activated protein kinase (MAPK) family (ERK1/2, p38MAPK and JNK), protein kinase C, phosphatidyl inositol 3-kinase, NF_κ-B, and calcineurin, which are implicated in the hypertrophic response[44-47]. We have recently demonstrated that the superoxide-degrading actions of tempol are associated with suppression of the cardiomyocyte growth response to angiotensin II and endothelin-1.[42] Taken together, these observations suggest that hypertrophic stimuli, including both cardiac hormones or mechanical stretch, result in increased levels of cardiomyocyte ROS. This increase triggers activation hypertrophic signal transduction (eg MAPK activation and induction of c-*fos*), ultimately leading to increased cardiomyocyte protein synthesis, hypertrophic gene expression and increased cardiomyocyte volume.

Subcellular sites of ROS production

ROS in the heart and vasculature are derived from a number of intracellular enzymatic sources. These sources include NADPH oxidase, mitochondrial oxidative phosphorylation, nitric oxide synthase (NOS), xanthine oxidase, cyclooxygenase, cytochrome P_{450} and β-oxidation of fatty acids.[38,44,48] The sites of these enzymatic sources of ROS within the cell is not however restricted solely to the cytoplasm. At the cell membrane, NADPH oxidase catalyses the one electron reduction of O_2 to produce $\bullet O_2^-$ using NADPH as the electron donor. In the in the cardiovascular system, this enzyme is continuously active at a low level even without stimulation,[39,49] but it is not known whether constitutive activity is different in normal and hypertrophic hearts. NADPH oxidase comprises two sarcolemmal subunits, $gp91^{phox}$ (or Nox2) and $p22^{phox}$, and the cytosolic subunits $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$. On stimulation, $p47^{phox}$ is phosphorylated, and the cytosolic

complex migrates to the cell membrane to interconnect with the membrane subunits and the regulatory G protein Rac1 to become an active enzyme, releasing $\bullet O_2^-$. Nox1 and Nox4 are homologues of gp91^{phox} that can form an active NADPH oxidase with the other subunits in its place.[38,39] Whilst NADPH oxidase is predominantly regarded as a sarcolemmal enzyme, there is some evidence of additional subcellular localisation of both gp91^{phox} and p22^{phox} in the vicinity of the endoplasmic/sarcoplasmic reticulum.[49] Both complex I (also known as NADH:ubiquinone oxidoreductase and NADH:coenzyme Q reductase) and complex III (ubiquinol:cytochrome c oxidoreductase) of the mitochondrial electron transport chain generate $\bullet O_2^-$ as a by-product of energy (ATP) production.[50,51] In certain pathophysiological settings (associated with insufficient L-arginine substrate and/or co-factors such as the pteridine BH_4), uncoupling of oxygen reduction and arginine oxidation by NOS results in $\bullet O_2^-$ generation rather than NO$\bullet$.[44] Isoforms of NOS include those found in the cytoplasm (eNOS, iNOS and nNOS) in addition to a mitochondrial isoform, mtNOS.[44,52]

β-oxidation of fatty acids by both mitochondria (short-medium length) and peroxisomes (long to very long, containing 14-24 carbon chain lengths) also generates ROS as a by-product of respiration, although the process in peroxisomes is relatively inefficient, generating little if any metabolic fuel.[48,53] In the peroxisomes, the first oxidation step is catalysed by acyl CoA oxidase, and in this reaction electrons are passed directly to O_2, forming H_2O_2. ATP is not formed in peroxisomes (in contrast to mitochondria, where acyl CoA dehydrogenase is employed) and the energy is dissipated.[48,53] The predominant enzyme sources (and their subcellular localisation) of ROS in the myocardium are at present regarded to comprise oxidative phosphorylation (in the mitochondria), NADPH oxidase (cytoplasm) and uncoupled NOS (mitochondrial and cytoplasm),[38,44] with a lesser contribution from xanthine oxidase and P450 cytochrome oxidase. Where a substrate usage shift by the heart results in increased peroxisomal β-oxidation, it is likely (but not fully resolved) that increased peroxisomal ROS production may also be of cellular significance.

In addition to a number of enzyme generators of ROS at various intracellular locations, the heart also contains a number of endogenous antioxidants, to restrict steady-state ROS levels, including isoforms of superoxide dismutase (MnSOD, CuZnSOD), catalase and glutathione peroxidase. Localisation of these antioxidants includes the cytoplasm (CuZnSOD, catalase, glutathione peroxidase), mitochondria (MnSOD, glutathione peroxidase) and peroxisomes (which contain catalase, MnSOD, CuZnSOD and glutathione peroxidase.[44,48,53] The balance between ROS generation and their elimination by endogenous antioxidant mechanisms plays a critical role in preserving cardiac function; inappropriate levels of myocardial ROS likely precipitate impairment of myocardial function and abnormalities in cardiac structure (remodelling, fibrosis).[38,44]

Implications in the diabetic heart

As outlined above, the normal adult mammalian heart relies on a balanced combination of fatty acids and glucose utilisation for mitochondrial energy production (generation of ATP).[8,54] In diabetes however, the heart shifts its substrate usage to rely almost entirely on fatty acid oxidation to generate ATP and glucose utilisation is severely limited (see Table 1).[55] Although this predominant reliance of the diabetic heart on fatty acid oxidation does provide myocardial generation of ATP, this comes at significant cost, as fatty acid oxidation is less efficient than oxidation of glucose by the mitochondria.[54] We have recently demonstrated that in animal models of type 1 diabetes, elevated myocardial generation of superoxide is concomitant with molecular and morphological evidence of myocardial hypertrophy and fibrosis (Figure 2).[56,57] Furthermore, both type 1 diabetic and insulin resistant hearts exhibit marked upregulation of gp91^{phox} and Nox1 subunits of NADPH oxidase. Myocardial remodelling (hypertrophy, fibrosis) accompanies this.[57,58] Endogenous antioxidants are also downregulated in the diabetic heart.[59]

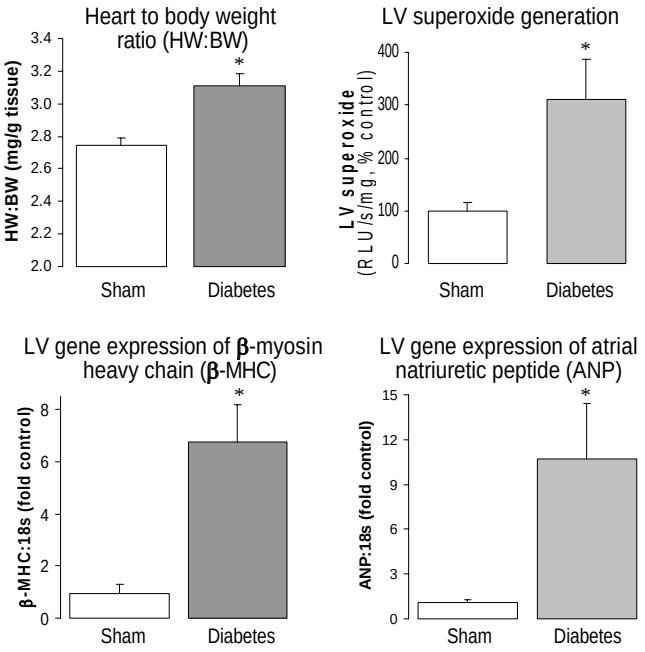

Figure 2. Induction of diabetes with streptozotocin is associated with enhanced myocardial growth and concomitant upregulated ROS signalling.

High levels of fatty acid β-oxidation, both by mitochondria and peroxisomes, have been implicated in the oxidative stress evident in the diabetic heart,[54] and indeed peroxisomal proliferation accompanies the acceleration of peroxisomal fatty acid β-oxidation in the diabetic myocardium.[60] An increase in steady state levels of ROS is an important, early manifestation of diabetes, and is considered to mediate many of the vascular complications of this common, debilitating disease.[61] We have provided interesting preliminary evidence (above) that the cardiac complications specific to the diabetic heart are attributable

to increased steady-state levels of ROS.[57,58] It is timely then to propose the hypothesis that a switch in substrate usage (from a combination of glucose and fatty acids, to almost exclusively fatty acids) such that the means by which the heart generates energy is altered, leads to elevated myocardial levels of ROS and triggers myocardial growth. In support of this hypothesis, we have observed that myocardial growth induced by a high fructose diet is preceded by increased myocardial superoxide generation (Delbridge and Ritchie, unpublished observations).

ROS and altered Ca^{2+} handling – a close relationship between cardiac growth and myocardial function

Alterations in intracellular Ca^{2+} handling and accumulation of ROS are intimately related. Steady-state increases in levels of either molecule in the heart is capable of triggering an increase in the level of the other, raising the possibility that increases in ROS and abnormalities in Ca^{2+} handling may form a deleterious, self-reinforcing signal. Evidence in support of such a feed-forward relationship between the two signalling pathways comes firstly from exogenous administration of oxidants, which increase intracellular Ca^{2+} levels, deplete ATP and impair function in the isolated heart.[62,63] Impaired function following oxidative insult can result from oxidation of Ca^{2+} transport proteins,[64,65] depressed SR Ca^{2+} ATPase activity and expression,[66] reduced sensitivity of the contractile filaments to Ca^{2+} [64] and changes in other myocardial ion channels.[67,68] Conversely, increases in myocardial Ca^{2+} levels, either within mitochondria or in the cytoplasm[64,69] result in upregulation of ROS generation from both mitochondrial and other sources, often consequently activating cell death pathways.[44,46] The mechanism(s) of these deleterious events include overactivation of protein kinase C, Ca^{2+}/calmodulin dependent protein kinase II, phospholipases, protein phosphatases and proteases, many of which are Ca^{2+}-dependent signals.[69] Also in support of the close interaction between intracellular Ca^{2+} and generation of ROS is the attenuation of the reperfusion injury-induced impairment of cardiac function and cardiomyocyte Ca^{2+} handling by antioxidant treatment.[62]

The relationship between Ca^{2+} and ROS not only impacts on cardiac function but also has detrimental consequences on cardiac structure. As discussed above, ROS activate a range of signalling molecules that are key to the development of cardiac hypertrophy (ERK1/2, phosphatidyl inositol 3-kinase, NF_{κ}-B, calcineurin, *etc.*) and many of these are Ca^{2+}-sensitive signals[44-47]. Activation of matrix metalloproteinases by ROS also contributes to extracellular matrix deposition, and hence cardiac fibrosis.[70] Abnormalities in Ca^{2+} handling are clearly a major contributor to dysfunction in the diabetic heart.[32] Our own data provide evidence of increased ROS and abnormalities in cardiac growth in the diabetic (see Figure 2)[56,57] and pre-diabetic heart.[58]

Conclusion

Elevated ROS and defective Ca^{2+} handling are co-conspirators, inflicting both early and late stage damage on the substrate-challenged myocardium. Although hypertrophic responses of contrasting aetiology are associated with different metabolic adaptations, there is accumulating evidence that the joint insults of increased production of ROS and disturbed Ca^{2+} handling in the cardiomyocyte comprise the primary lesion. These molecular signals operate together in a feed-forward mode, and have the capacity to inflict substantial functional and structural damage on the hypertrophic myocardium.

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Author for correspondence:
Lea Delbridge
Department of Physiology
University of Melbourne
Victoria, 3010
Australia

Tel: +61 3 8344 5853
Fax: +61 3 8344 5897
E-mail: lmd@unimelb.edu.au