



How Exercise May Amend Metabolic Disturbances in Diabetic Cardiomyopathy

Anne D. Hafstad, Neoma Boardman, and Ellen Aasum

Abstract

Significance: Over-nutrition and sedentary lifestyle has led to a worldwide increase in obesity, insulin resistance, and type 2 diabetes (T2D) associated with an increased risk of development of cardiovascular disorders. Diabetic cardiomyopathy, independent of hypertension or coronary disease, is induced by a range of systemic changes and may through multiple processes result in functional and structural cardiac derangements. The pathogenesis of this cardiomyopathy is complex and multifactorial, and it will eventually lead to reduced cardiac working capacity and increased susceptibility to ischemic injury. **Recent Advances:** Metabolic disturbances such as altered lipid handling and substrate utilization, decreased mechanical efficiency, mitochondrial dysfunction, disturbances in nonoxidative glucose pathways, and increased oxidative stress are hallmarks of diabetic cardiomyopathy. Interestingly, several of these disturbances are found to precede the development of cardiac dysfunction. **Critical Issues:** Exercise training is effective in the prevention and treatment of obesity and T2D. In addition to its beneficial influence on diabetes/obesity-related systemic changes, it may also amend many of the metabolic disturbances characterizing the diabetic myocardium. These changes are due to both indirect effects, exercise-mediated systemic changes, and direct effects originating from the high contractile activity of the heart during physical training. **Future Directions:** Revealing the molecular mechanisms behind the beneficial effects of exercise training is of considerable scientific value to generate evidence-based therapy and in the development of new treatment strategies. *Antioxid. Redox Signal.* 22, 1587–1605.

Introduction

THE WORLD IS facing an epidemic increase in obesity and insulin resistance due to over-nutrition and sedentary lifestyle. Obesity and insulin resistance are risk factors for type 2 diabetes (T2D), and cardiovascular disease is the leading cause of mortality in patients with T2D. In addition to increased incidence of hypertension and concomitant macro- and microvascular disorders, diabetic patients are also at risk of developing a specific diabetic cardiomyopathy (66). The development of diabetic cardiomyopathy is most likely induced by a range of systemic changes, which through multiple signaling pathways will lead to functional and structural derangements (Fig. 1).

Hallmarks of the diabetic heart include oxidative stress, fibrosis, apoptosis, impaired autophagy, inflammation, and altered calcium handling. In addition, these hearts display

metabolic-related changes, including altered substrate utilization, decreased mechanical efficiency, lipid accumulation, mitochondrial dysfunction, impaired insulin signaling, increased advanced glycation end-products (AGEs), and sustained O-linked N-acetylglucosamine (O-GlcNAc) modification of proteins. The pathogenesis of this cardiomyopathy is, by no doubt, multifactorial and complex, eventually leading to an energetically compromised heart with reduced working capacity and increased susceptibility to ischemia-reperfusion.

Aerobic fitness, measured as maximal oxygen uptake (VO_{2max}), has been shown to be a strong and independent predictor for total and cardiovascular mortality both in healthy individuals and in patients with cardiovascular disease (109, 144). Exercise is a low-cost strategy for prevention and treatment of obesity and T2D (120, 207). It is well known that

Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, UiT—The Arctic University of Norway, Tromsø, Norway.

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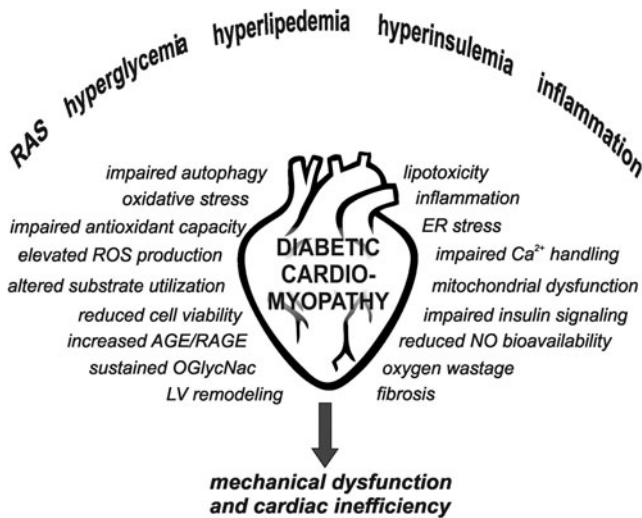


FIG. 1. Hallmarks of diabetic cardiomyopathy. A range of systemic changes contribute to the onset of molecular mechanisms leading to cardiac dysfunction and inefficiency in diabetes. NO, nitric oxide; AGE, advanced glycation-end product; RAGE, AGE receptors; ROS, reactive oxygen species; RAS, renin-angiotensin system; LV, left ventricle; O-GlcNac, O-linked N-acetylglucosamine.

exercise can induce cardioprotection in normal hearts through a range of molecular mechanisms (79, 158). Beneficial cardiac effects after exercise training in diabetes/obesity have been reported in clinical (94, 167, 172, 173) and experimental animal studies (21, 61, 85, 111, 128, 176, 184). This review highlights existing knowledge of how metabolic derangements contribute to the development of diabetic cardiomyopathy, and of how exercise training may influence these changes and the progression of diabetic cardiomyopathy.

Myocardial Substrate Utilization in Diabetes

The requirement to continuously pump blood makes the myocardium the most metabolically active tissue in the body. The majority of its ATP production is derived from mitochondrial oxidative phosphorylation (OxPhos), and to meet the high energetic demand, a constant flow of substrate to the mitochondria is pivotal. The cardiac metabolic network has developed a versatile system, capable of metabolizing all carbon substrates for energy production, where glucose and fatty acids (FA) are the main substrates (Fig. 2). An important feature of the normal heart is metabolic flexibility and the ability to ensure appropriate ATP production rate under diverse physiologic and dietary conditions. Lack of this flexibility has been regarded as fundamental in the development of heart failure, including diabetic cardiomyopathy, but exactly how remains unclear.

Despite the presence of hyperglycemia, the diabetic heart relies predominantly on FA oxidation, with a concomitant reduction in glucose oxidation (1, 17, 155). Obesity and diabetes-induced enhancement of myocardial FA uptake and oxidation is due to the activation of peroxisomal proliferator-activated receptor- α (PPAR- α) and, consequently, transcriptional induction of enzymes involved in FA transport and β -oxidation. This also leads to a concomitant suppression of glucose oxidation, due to increased expression of pyruvate

dehydrogenase kinase 4 (PDK4), which inhibits the pyruvate dehydrogenase complex (PDH) (129). Recent studies in skeletal muscle have also suggested carnitine acetyltransferase (CrAT) to be a key regulator in metabolic flexibility, and that deficits in CrAT activity in diabetes might contribute to a more rigid and inflexible metabolism (140).

Altered cardiac substrate metabolism has been shown in experimental and clinical studies to precede the development of ventricular dysfunction (1, 155). Studies using genetically modified mice in which cardiac FA uptake and/or oxidation resemble that of the diabetic metabolic phenotype also describe development of cardiac dysfunction (48, 49, 67), and interventions that reduce the substrate switch have been shown to ameliorate the development of ventricular dysfunction (17, 81, 96).

Taken together, these studies support the notion that altered myocardial lipid metabolism has a causative role in diabetic cardiomyopathy and that targeting myocardial metabolism is a therapeutic intervention for the maintenance of cardiovascular health in diabetes (129). It should be noted, however, that the presence of FAs or maintenance of FA oxidation has also been reported to have beneficial functional effects in diabetic hearts and cardiomyocytes (64, 88, 191, 203). These discrepancies may suggest that while preventing the switch in substrate utilization may ameliorate the development of diabetic cardiomyopathy, diabetic hearts in an untreated state may undergo adaptive changes that eventually make these hearts dependent on FAs, specifically as an energy substrate.

Endurance training improves myocardial metabolism

An acute exercise session will lead to an altered metabolic state with high levels of plasma lactate (from glycolysis in exercising muscle) and nonesterified FAs (β -adrenergic stimulation and lipolysis). Consequently, FA and lactate become the major substrates for the heart during exercise (76). The myocardial metabolic adaptation in response to endurance training is, however, less clarified. Several studies have reported altered expression of genes and proteins related to myocardial metabolism in normal (83, 126, 185) and diabetic hearts (85, 177) in response to chronic exercise. Studies directly addressing the effects of exercise training on metabolic fluxes in the hearts are, however, few and diverging.

In the normal heart, Broderick *et al.* (37) did not find treadmill running to alter myocardial glucose oxidation and glycolysis, whereas Burelle *et al.* (39) reported an increased rate of both glucose and FA oxidation while glycolysis was decreased. These discrepancies may be related to variations in the intensity of the exercise training protocol and in line with this, Hafstad *et al.* (83) recently reported that only high-intensity training (and not moderate intensity) altered myocardial substrate utilization in normal mice.

In type 1 diabetic (T1D) rats, induced by streptozotocin (STZ), exercise training was reported to increase rates of glucose oxidation and glycolysis (37) whereas FA oxidation was unaltered (153). Similarly, in a recent study using diet-induced insulin-resistant mice, treadmill running increased myocardial glucose oxidation while oxidation rates of FAs were less affected (85). An important finding from the latter study was that in contrast to that reported in healthy mice, both high- and moderate-intensity training induced a similar metabolic response in obese mice.

Evidence from studies on skeletal muscle has shown that exercise training can influence insulin pathways and improve insulin resistance. Insulin signaling seems to have a central role in the exercise-induced hypertrophic response in the healthy heart (164). Exercise has also been shown to increase the ability of insulin to phosphorylate the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), IRS-2, Akt, and Foxo1, as well as to affect other pathways influencing the insulin signaling cascade in cardiac tissue from diet-induced obese rats (134, 157). The metabolic consequence (*i.e.*, substrate utilization) of these changes has, however, not been investigated. It should be noted that compelling experimental evidence has linked excessive cardiac insulin signaling with adverse effects after chronic pressure overload in normal and T1D (180), implying that improved systemic insulin levels by exercise training may provide beneficial cardiac effects under certain types of stress, such as hypertension.

Diabetes-Induced Hyperglycemia Stimulates Nonoxidative Glucose Pathways in Diabetic Cardiomyopathy

Hyperglycemia is an important risk factor for developing cardiovascular disease. In addition to generating pyruvate for oxidation, elevated plasma levels of glucose may also affect nonoxidative glucose pathways (Fig. 3). Both intracellular and extracellular lipids and protein exposed to high levels of sugars may become irreversibly glycosylated and form AGEs. AGE modifications of the extracellular matrix may render the tissue less compliant and induce myocardial stiffness (43). AGEs can also bind to AGE receptors (RAGEs), which may activate redox-sensitive transcription factors, such as nuclear factor- κ B (NF- κ B), and, consequently, affect intracellular signaling pathways leading to remodeling of the extracellular matrix, cell growth, cytokine formation, increased production of reactive oxygen species (ROS), and reduced nitric oxide (NO) bioavailability (80).

There is substantial evidence that AGEs are key mediators in the development of vascular injury in diabetic patients (80), and recent studies also support the role of AGEs and RAGEs in the development of diabetic cardiomyopathy (43, 114). In STZ-induced diabetes, treatment with the AGE cross-link breaker ALT-711 was shown to reduce cardiac AGEs, accompanied by a normalization of collagen III deposition, improvement of calcium handling, and attenuation of diabetes-associated myocardial structural changes (43, 114). The soluble form of RAGE (sRAGE), lacking an intracellular domain, is believed to work as a scavenger for AGE (205). In diabetic patients, an inverse correlation has been reported between plasma levels of sRAGE and HbA_{1c}, insulin resistance index, and C-reactive protein (13).

The polyol pathway, which branches off the glycolytic pathway, is activated after increased concentrations of intracellular glucose. Aldose reductase (AR, the rate-limiting step of this pathway) oxidizes NADPH to NADP and can thus impair myocardial reductive/antioxidant capacity. Introducing hyperglycemia to isolated hearts has been shown to increase activation of the polyol pathway, accompanied by increased oxidative stress and impaired left ventricular diastolic function, while inhibition of AR attenuated these changes (188). Increased AR activity has been demonstrated in T1D hearts (105), while reports from T2D models are lacking.

Hyperglycemia can also increase the synthesis of diacylglycerol (DAG), an activator of the protein kinase C (PKC) signaling pathway in the diabetic heart (104). Increased activity of this pathway is associated with cardiac hypertrophy, fibrosis, adverse Ca²⁺ handling, and reduced cardiac performance (197). Treatment with a PKC β inhibitor has been found to reduce collagen deposition, increase SERCA2a expression, and preserve diastolic function in T1D hearts (54).

Elevated intracellular glucose may also increase flux of carbons from glucose through the hexosamine biosynthetic pathway (HBP). This produces uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is used as a substrate by O-GlcNAc transferase to modify serine/threonine residues in proteins (206). O-GlcNAcylation is reported to be upregulated in obese and diabetic hearts (19, 56, 97, 135). Although O-GlcNAcylation of proteins integrates glucose metabolism with posttranslational modifications, this process is not simply regulated by substrate availability, as multiple stressors have also been shown to induce O-GlcNAcylation in cardiac tissue (47, 198, 199).

Altered O-GlcNAcylation may influence many of the processes known to be affected in diabetic cardiomyopathy, including mitochondrial respiratory capacity (98), insulin signaling (89), FA utilization (118), Ca²⁺ handling (63, 97), autophagy (131), ER stress (147, 198), and fibrosis (3). Protein O-GlcNAcylation seems, however, to be paradoxical in nature; while a reduction in the overall O-GlcNAcylation in diabetic hearts is beneficial in terms of Ca²⁺ handling and ventricular function (97, 133), and O-GlcNAcylation has also been reported to be fundamental in cardiac protection (47, 125, 147, 199). This has led to the suggestion that acute elevation of cardiac protein O-GlcNAcylation is protective, while sustained protein O-GlcNAcylation is detrimental (103).

Exercise training reduces nonoxidative glucose pathways

Given the beneficial effects of exercise on glycemic control (85, 190, 200), one would expect that this should amend the adverse effects of the increased nonoxidative glucose pathways in the diabetic heart (Fig. 3). Interestingly, studies addressing these pathways often report exercise-induced effects that are independent of systemic effects (19, 29, 136). As exercise training also increases myocardial glucose oxidation (85), this may be a mechanism contributing to the shunting of carbons away from nonoxidative glucose pathways.

Although reports on cardiac effects of exercise on the RAGE/AGE system are sparse, exercise has been found to reduce fibrosis together with reduced AGEs in the ventricles of aged rats (202). In T2D patients, exercise training increased plasma levels of sRAGE, associated with reduction of several cardiometabolic risk factors (50). However, in obese Zucker rats, exercise lowered plasma AGEs without affecting circulating glucose, insulin, lipid profile, oxidative status, or inflammatory markers (29). Exercise training has also been shown to ameliorate the obesity-induced activation of both the NF- κ B and JNK pathways in the heart (134), supporting an anti-inflammatory effect of exercise in cardiac tissue (30), which could potentially work through AGE pathways.

The role of the polyol pathway or PKC pathway in exercise-attenuated insulin resistance and cardiac dysfunction in

diabetes remains unclear. The exercise-induced reduction in myocardial DAG levels has been associated with improved left ventricular function in T1D rats, although this was not found to be associated with altered PKC β II protein expression or activity (128).

Long-term intensive swim-training has been reported to reduce total cardiac protein O-GlcNAcylation in both lean mice (15) and STZ mice (19). In *db/db* mice, however, treadmill running was found to increase cardiac protein O-GlcNAcylation (56). A plausible explanation for these findings may be the use of different experimental models, training modes, and intensities. Recently, it has been demonstrated that acute exercise training altered cardiac protein O-GlcNAcylation in a very time- and spatial-restricted manner without any change in overall O-GlcNAc protein levels (136). This suggests a complex regulation of cardiac O-GlcNAcylation in response to exercise and may also partially clarify the discrepancies in the studies addressing cardiac protein O-GlcNAcylation after exercise interventions.

Lipotoxicity in Diabetic Cardiomyopathy

As a consequence of excess cardiac FA uptake relative to oxidation, long-chain FAs are incorporated into triacylglycerols (TAG), leading to cardiac steatosis in diabetes/obesity (85, 165). Elevated intramyocellular TAG content in skeletal muscle, in the absence of exercise, is a strong predictor of global insulin resistance (139), and intramyocardial TAG accumulation has been found to be an independent predictor of cardiac dysfunction in T2D patients (165).

Although TAG is not regarded as a toxic molecule in itself, shunting of FAs into nonoxidative pathways can lead to accumulation of intermediate metabolites, including DAG and ceramides at high concentrations (Fig. 2), and may increase fibrosis through the activation of protein kinases (PKC β or PKA) (10), induce apoptosis (151), and disrupt insulin signaling (208). In addition, long-chain acylcarnitines and acyl-CoAs accumulate in skeletal muscle in diabetes due to incomplete β -oxidation and impaired CrAT activity (175). This has fostered support for a lipid-induced insulin resistance associated with obesity (113, 140).

Accumulation of mitochondrial long-chain acylcarnitine was also shown to impair glucose metabolism in the heart (130). Furthermore, shunting of lipid intermediates into TAG in lipid droplets has been shown to ameliorate ventricular dysfunction in models of lipotoxicity by maintaining a low concentration of lipotoxic intermediates (126, 127). In this regard, Perilipin-5 (Plin-5) is believed to play an important role in the stabilization and facilitation of lipolysis in the cardiomyocyte and in the direct transfer of FA between lipid droplets and mitochondria (Fig. 3) (115). Interestingly, although Plin-5 knockout mice showed a decline in cardiac function with age, they were resistant to STZ-induced cardiac dysfunction (116). These findings suggest that the diabetic heart may undergo adaptations, enabling them to cope with elevated lipid supply.

The effects of exercise on myocardial lipotoxicity

In healthy individuals, an acute bout of exercise can increase cardiac lipid content through exercise-induced elevation of FA concentrations in the blood (22). In normal mice, increased cardiac TAG storage after short-term intensive

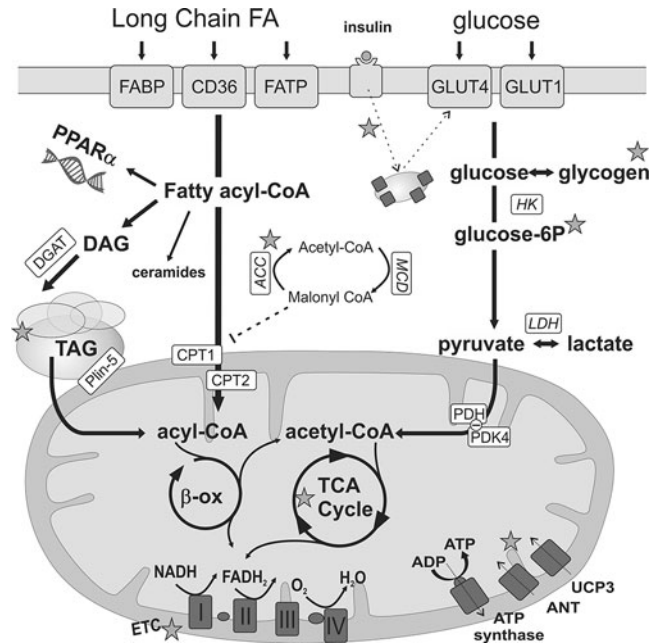


FIG. 2. Glucose and FA uptake and oxidation in the diabetic cardiomyocyte. A continuous elevation in FAs supply leads to increased long-chain FA (LCFA) utilization due to activation of the peroxisome proliferator-activated receptor (PPAR)- α and, consequently, transcriptional induction of enzymes involved in FA transport (FAs transport protein [FATP], FA binding protein [FABP] and FA translocase [FAT/CD36]) and β -oxidation (β -ox). The entry of Acyl-CoA into the mitochondria is mediated by the carnitine-palmitoyltransferase (CPT1 and 2) system. After β -oxidation, acetyl Co-A enters the tricarboxylic acid (TCA) cycle to form reducing equivalents (FADH₂/NADH) that drive the electron transport chain (ETC) and generate the proton motive force that powers ATP synthesis. Glucose uptake (facilitated diffusion by the glucose transporters 1 (GLUT1) and the insulin-sensitive GLUT4) are decreased in diabetes due to impaired insulin signaling. Pyruvate formed from glycolysis is taken up by the mitochondria, and acetyl-CoA is generated by the pyruvate dehydrogenase complex (PDH). Myocardial glucose oxidation is reduced in diabetes due to increased pyruvate dehydrogenase kinase 4 (PDK4), which inhibits the PDH. Excess acyl-CoA is stored as triacylglycerol (TAG) in lipid droplets and may result in the accumulation of toxic intermediates such as diacylglycerol (DAG) and ceramides. Impaired mitochondrial respiration may be due to reduced respiration capacity and/or efficiency, increased uncoupling *via* uncoupling proteins (UCP), and/or the adenine nucleotide translocase (ANT). Exercise-induced changes reported in the literature are indicated by stars and described in detail in the text. See “Myocardial Substrate Utilization in Diabetes” and “Lipotoxicity in Diabetic Cardiomyopathy” sections for references. DGAT, diglyceride acyltransferase; MCD, malonyl-CoA decarboxylase; ACC, acetyl-CoA carboxylase; Plin-5, perilipin-5.

swim training was found to be accompanied by a marked upregulation of the TAG storage enzyme diacylglycerol transferase 1 (DGAT1), whereas the level of ceramide and DAG was reported to be reduced (126) (Fig. 2).

In obese subjects, endurance/strength training was shown to reduce myocardial TAG content in combination with improved ejection fraction (172). This finding, however, was

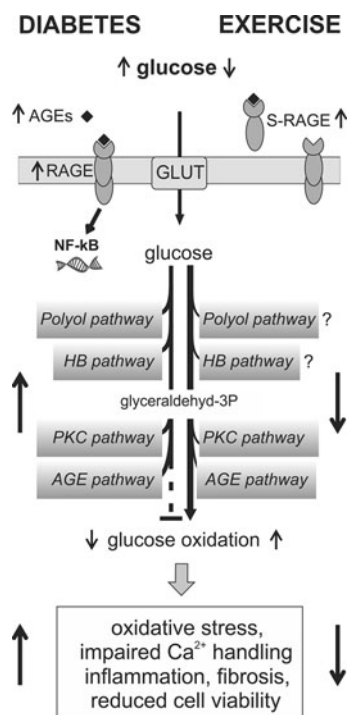


FIG. 3. Nonoxidative pathways in diabetic cardiomyopathy and proposed effects of exercise training. Hyperglycemia and reduced shunting of carbons from glycolysis into glucose oxidation may lead to the induction of nonoxidative glucose pathways, including the polyol, hexosamine biosynthetic (HBP), protein kinase C (PKC), and advanced glycation end-products (AGE) pathway in the diabetic heart. Increased plasma levels of AGEs can also bind to AGE receptors (RAGEs), which together with PKC and oxidative stress are known activators of nuclear factor- κ B (NF- κ B). Increased NF- κ B activity together with these pathways may mediate adverse effects in the diabetic heart due to impaired myocardial reductive capacity, formation of AGEs, and sustained O-GlcNAcylation of proteins. Exercise training may, through improved glucose homeostasis and increased myocardial glucose oxidation, reverse the induction of several nonoxidative glucose pathways in the diabetic heart. In addition, exercise can increase plasma levels of the soluble form of RAGE (sRAGE), which may work as a scavenger for AGEs and therefore reduce the intracellular effects of AGE/RAGE signaling. See “Diabetes-Induced Hyperglycemia Stimulates Nonoxidative Glucose Pathways in Diabetic Cardiomyopathy” section for references.

not reproduced in obese T2D patients where myocardial TAG contents were found to be unaltered (107, 173). Exercise-induced reduction in myocardial TAG content has been reported in diet-induced obese mice (85), while the effects on myocardial DAG and ceramides levels in obese/diabetic models are not known. Although increased Plin-5 expression has been reported after acute exercise in human skeletal muscle (112), the effects of exercise on Plin-5 in the heart remain to be elucidated.

Mitochondrial Disturbances in Diabetic Cardiomyopathy

The heart is highly dependent on mitochondrial OxPhos, which must be closely linked to the rate of ATP hydrolysis.

Mitochondrial dysfunction has been suggested to play a central role in the pathogenesis of diabetic cardiomyopathy, as an imbalance between energy availability and demand will lead to an energetically compromised heart with reduced working capacity. In human diabetic atria fibers, Anderson *et al.* (5) found that ADP-stimulated respiration (state 3 respiration) was decreased when using both palmitate and glutamate as substrates. In experimental studies from obese/diabetic models, decreased and unchanged mitochondrial respiratory capacity has been reported using carbohydrate-based substrates (2, 14, 32, 33, 53, 68, 85), while there is less agreement when FAs are used as substrates (2, 14, 31–33, 53, 68).

Mitochondrial uncoupling is regarded as a key mediator of mitochondrial dysfunction in the diabetic heart. The underlying mechanisms mediating diabetes-induced uncoupling are unclear, but both uncoupling proteins (UCPs) (31, 33, 142) and adenine nucleotide translocator (33) have been proposed as candidates. Although there are inconsistent findings regarding the gene and protein expression of myocardial UCPs in models of T2D (32, 142), UCP activity is known to be increased by FAs, ROS, and lipid peroxidation products (60). Recent studies have demonstrated that both inhibition (53, 141) and genetic deletion (31) of UCP3 improve P/O ratios in models of diet-induced obesity.

The coupling efficiency of diabetic mitochondria evaluated by respiratory control ratio (*i.e.*, RCR or state 3/state 4) or P/O ratio (a measure independent of the oxygen consumption) is decreased or unchanged in diabetic conditions (31–33, 53, 85, 142). Finally, mitochondrial respiration after administration of oligomycin (an inhibitor of ATP synthase) has also in some cases been reported to be increased (32, 33). Although the role of this proton leak is unclear, it has been suggested to lower mitochondrial membrane potential and, subsequently, reduce mitochondrial ROS production. Further research is, however, required to better elucidate the precise physiological role and/or the pathophysiological consequence of mitochondrial uncoupling in the heart.

Increased mitochondrial mass, area, and number have been reported in diabetic hearts (178, 179), possibly as an adaptive mechanism to overcome impaired mitochondrial respiratory capacity. In line with this notion, Shen *et al.* (178) found in hearts from T1D mice that improving the antioxidant capacity amended mitochondrial respiration, which was accompanied by normalization of mitochondrial density and morphometry (178).

Calcium is an important activator of key metabolic enzymes in the mitochondria (168) and accelerates the production of NADH (34). Mitochondrial Ca^{2+} cycles *via* the uniporter entry and export system (12, 168) and will be influenced by changes in cytosolic Ca^{2+} homeostasis. Depression of mitochondrial Ca^{2+} uptake and handling has been reported in diabetic cardiomyocytes (65, 156), as a consequence of impaired Ca^{2+} handling (as will be discussed later), and is therefore a plausible contributing factor to impaired energy metabolism in diabetic cardiomyopathy. Mitochondria can also induce cell necrosis *via* opening of the Ca^{2+} -sensitive permeability transition pore (mPTP). Several studies have shown that mitochondria from diabetic hearts are sensitized to mPTP opening (6, 182), which may be partly responsible for the increased susceptibility to ischemic injury described in diabetic hearts.

The effect of exercise on mitochondrial function

Increased palmitoyl-carnitine and glutamate supported respiration capacity has been demonstrated after exercise training in cardiac mitochondria from normal (83, 148) and obese/diabetic rodent models (68, 85). Exercise-mediated improvement in mitochondrial capacity may be linked to mitochondrial Ca^{2+} handling. Although this has not been specifically studied, there could be an obvious link between enhanced mitochondrial calcium handling in diabetes and the beneficial effects on mitochondrial respiratory capacity and efficiency observed in hearts from endurance trained diabetic models (68, 85, 148).

Hafstad *et al.* (85) did not find exercise training to improve RCR, but to normalize P/O ratios, which was accompanied by increased proton leak after inhibition with oligomycin. This may suggest that the mitochondrial adaptations induced by endurance training compensate for mild mitochondrial proton leak and preserve the efficiency of ATP synthesis. Interestingly, similar mitochondrial changes were recently reported in skinned cardiac fibers from high-fat-fed UCP3KO mice (31).

The impact of exercise training on mPTP opening remains largely unknown. Targeted treatment to reduce mitochondrial ROS was shown to improve resistance to mPTP opening in diabetic hearts (182). One could therefore speculate that exercise-induced effects on mitochondrial redox environment (as will be discussed later) may potentially increase resistance to mPTP opening in the diabetic heart and, consequently, increase cell survival in response to insults such as ischemia or Ca^{2+} overload.

Although exercise training has been shown to induce mitochondrial biogenesis in skeletal muscle (83, 123), its effect on mitochondrial biogenesis in the heart is less clear. In a study using lean mice, it was demonstrated that exercise induced skeletal muscle but not cardiac mitochondrial biogenesis (123). In contrast, high-intensity interval training was reported to increase cardiac mitochondrial content both in lean (83) and in obese (85) animal models. Interestingly, the same studies reported that this was not found after moderate-intensity continuous training (83, 85), suggesting a role of intensity in exercise-induced cardiac mitochondrial biogenesis.

Sources of ROS and Their Roles in the Progression of Diabetic Cardiomyopathy

Apart from being the final electron acceptor in electron transport chain (ETC), oxygen plays a role in cellular redox signaling, and may become toxic through formation of high concentrations of ROS. Thus, redox modulation of cellular proteins comes in “different flavors”; while moderate reversible oxidations may increase protein activity, and a more sustained ROS may induce pathological modifications through stable interactions with lipids, proteins, and DNA (40).

Several systemic alterations (Fig. 4) are believed to contribute to increased ROS formation in diabetes and obesity. In particular, the cardiovascular system is characterized by increased mitochondrial and extra-mitochondrial ROS generation as well as by impaired antioxidant defense systems (5, 33, 77, 162). Clinical and experimental studies support increased oxidative damage in diabetes (5, 33, 191, 195), and oxidative stress is likely to be a key mediator in the pathogenesis of diabetic cardiomyopathy and to be causative for the increased ischemic susceptibility in the diabetic heart (42, 57).

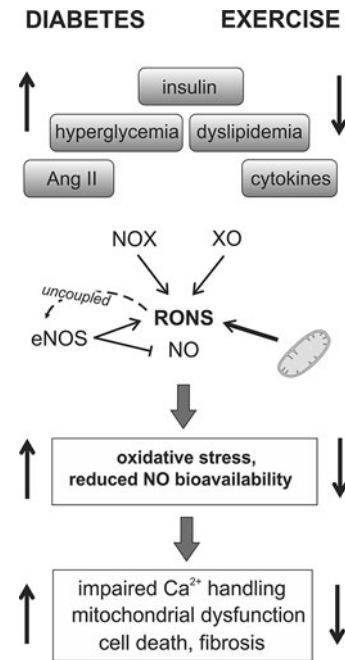


FIG. 4. Myocardial ROS producing systems in diabetes and the effect of exercise training. Systemic changes in diabetes such as increased levels of angiotensin II (Ang II), insulin, cytokines, glucose, and dyslipidemia may induce the production of reactive oxygen and nitrogen species (RONS) from both mitochondrial and nonmitochondrial sources, including NADPH oxidases (NOX), xanthine oxidase (XO), and nitric oxide synthase (NOS). Reactive oxygen species may also lead to the uncoupling of endothelial NOS (eNOS), which will decrease NO production, limiting its bioavailability. These changes can lead to several adverse effects in the diabetic myocardium, which can be ameliorated through the systemic effects of exercise. Direct effects of exercise on ROS-producing enzymes are also reported and described in the text.

Despite substantial evidence from experimental studies showing beneficial cardiac effects of different pharmacological and genetic antioxidant therapies (124, 178, 189, 204), clinical trials have failed to show beneficial effects of conventional antioxidant therapies (24). A plausible explanation for these discrepancies is that general exogenous antioxidant therapies target all sources of ROS, not only pathophysiological ROS but also physiological ROS signaling in the heart.

In hearts from both humans (5) and animal models (33, 64, 191), a high rate of mitochondrial ROS generation is believed to contribute to diabetes-induced mitochondrial dysfunction. In accordance, Shen *et al.* (178) demonstrated that cardiac overexpression of the mitochondrial antioxidant manganese superoxide dismutase (MnSOD) was able to reduce ROS and normalize mitochondrial function.

Mitochondrial ROS production in diabetes is suggested to be due to an increased supply of reducing equivalents from myocardial FA oxidation to the ETC, without a parallel increase in the OxPhos capacity. The subsequent loss of electrons from the ETC leads to the generation of superoxide ($\text{O}_2^{\bullet-}$) at complex I and II. This notion has been challenged by Fauconnier *et al.* (64), who found that although palmitate profoundly increased mitochondrial ROS production in wild-type cardiomyocytes, this was not observed in cardiomyocytes

from *ob/ob* mice. In addition, Tocchetti *et al.* (191) recently found that palmitate restored redox balance and counteracted the exaggerated ROS emission induced by hyperglycemia and β -adrenergic stimulation in isolated cardiomyocytes from *db/db* mice. Again, this suggests a complex role for FAs in the diabetic heart and the possibility that an adaptive response in diabetic mitochondria allows them to maintain homeostasis in the presence of high FAs.

A major nonmitochondrial source of ROS in cardiomyocytes are NADPH oxidases (NOXs) that produce ROS by using reducing equivalents from NADPH to molecular oxygen. NOX2 and NOX4 are the main isoforms in the cardiomyocyte, and their activation may modulate multiple signaling pathways and redox-sensitive proteins (7, 8). NOX signaling has been shown to influence hypertrophic pathways, fibrosis, cell viability, calcium handling, ER stress, and antioxidant capacity (40, 86, 145, 170). Potent activators of NOXs are angiotensin II, hyperglycemia, cytokines, oxidized low-density lipoprotein, mechanical forces, and hypoxia (8, 145). Interestingly, the two isoforms differ in mode of activation, subcellular location and they mediate distinct redox responses to agonist stimulation (8).

NOXs have been shown to play key roles in the pathogenesis of vascular disease in diabetes (74), and in the myocardium increased expression of NOXs has been found in type 1 and T2D models (72, 195). Both genetic and pharmacological inhibition of NOX2 has been found in T1D mice to reduce myocardial fibrosis, improve ER stress markers and cardiac function (122). Furthermore, treatment of T2D rodents with an angiotensin receptor blocker was found to reduce the cardiac expression of NOX2 together with reduced cardiac ROS and fibrosis (72, 195).

NO produced by different NO synthase (NOS) isoforms, working in discrete subcellular domains to induce S-nitrosylation, may alter the activity of proteins involved in physiological processes such as excitation-contraction (EC) coupling (174), β -adrenergic inotropic response (78), and also the Frank-Starling mechanism (159). However, under conditions of increased ROS, endothelial NOS (eNOS) may become uncoupled (45) and produce $O_2^{\bullet-}$, which can react with inducible NOS (iNOS)-produced NO to form highly oxidant reactive nitric species.

Hyperglycemia favors iNOS expression in normal hearts (46), and in the diabetic myocardium an increased monomer-to-dimer ratio of eNOS leads to production of $O_2^{\bullet-}$ (209). Reduced NO bioavailability, increased nitrosative stress, and peroxynitrite formation are believed to contribute to the pathogenesis of vascular disease in diabetes (4, 150, 209), and nitrosative stress has also been shown to be associated with the progression of diabetic cardiomyopathy and increase of myocardial cell death (70, 108).

Another extra-mitochondrial source of ROS in the diabetic myocardium is the enzyme xanthine oxidase (XO) (161). Treatment with an inhibitor of XO (allopurinol) was shown to reduce the development of T1D-induced cardiac dysfunction together with decreased oxidative/nitrosative stress and fibrosis (75, 161).

Acute and long-term effects of exercise on ROS-producing enzymes

An acute bout of exercise will induce a cardiac stress (acute exercise stress, [AES]) and lead to a transient increase

in reactive oxygen and nitrogen species (RONS) (26, 143, 160) (Fig. 4). This burst of RONS seems to be of utmost importance for the cardiac response to exercise since antioxidant therapies have been shown to impair health-promoting exercise effects in healthy young humans (166) and to impair beneficial cardiac responses in rodents (169).

Although the source of this transient increase in RONS is not clear, rigorous cardiac contractions during intense exercise have been suggested to increase the flow of electrons through the ETC and favor increased $O_2^{\bullet-}$ generation. In line with this, Bo *et al.* reported an elevated ROS production in isolated mitochondria from healthy rat hearts after prolonged acute exercise. This was associated with a transient increase in mitochondrial membrane potential, a marked increase in the expression of UCP2, increased uncoupled respiration, and reduced P/O ratio (26). Interestingly, mitochondrial ROS production was decreased and returned to resting levels as the exercise continued. The mitochondrial response was therefore regarded as "a first line of defense against ROS" where (at the expense of reduced mitochondrial efficiency) alleviation of mitochondrial membrane potential reduced $O_2^{\bullet-}$ generation (26).

It should be noted, however, that in the recently introduced "Redox-Optimized ROS Balance" (R-ORB) hypothesis (9), where the redox environment is taken into account as an important intermediary between mitochondrial respiration and ROS production, increased mitochondrial respiration is not accompanied by increased ROS, but rather by reduced ROS (55). This questions whether the mitochondria could be the main source of ROS during an acute exercise bout. Accordingly, several reports have suggested nonmitochondrial ROS-producing enzymes to be activated after AES.

An acute bout of exercise was shown to induce recruitment of catalytic subunits ($p47^{phox}$ and rac_1) to NOX2 and increase its activity in ventricular tissue from dogs (169) as well as to induce a robust expression of NOX4 in mouse hearts (143). Furthermore, exhaustive exercise was reported to acutely increase XO activity in the myocardium of aging rats (99). Hence, there is evidence to support that several ROS-producing systems are activated in the heart after an AES; however, whether these systems work together or in discrete subcellular locations to induce specific cellular redox signaling remains to be elucidated.

Long-term endurance exercise may influence ROS-producing enzymes through very different mechanisms. First of all, several of the exercise-mediated systemic changes (improved insulin signaling, reduced inflammatory status, alteration of plasma lipids, and reduced renin-angiotensin system activity) most likely participate in decreasing ROS in the diabetic cardiomyocyte by dampening the activators of ROS-producing enzymes (Fig. 4). Bo *et al.* reported that the exercise-induced elevation of mitochondrial ROS production was attenuated in cardiac mitochondria from endurance trained rats (26), suggesting an adaptive response to better tolerate AES in the myocardium.

In the diabetic myocardium, where chronic NOX2 activation is believed to be detrimental, long-term endurance training significantly reduced NOX2 activity in T2D rats (82). Although the acute effects of exercise on cardiac NOS enzymes remain largely unexplored, long-term exercise was shown to increase NOS expression, NO levels, and NOS activity in healthy rat hearts (100). Such adaptations would be

of particular benefit in the diabetic myocardium where NO bioavailability is reduced. In accordance with this, low-intensity endurance training of the T2D Goto-Kakizaki rat increased myocardial eNOS protein expression and eNOS dimer/monomer ratio, which facilitates NO generation compared with ROS production (82).

Impaired Endogenous Antioxidant Capacity in the Diabetic Myocardium

ROS formed is usually efficiently removed by endogenous antioxidants in the cardiomyocyte. Diabetic hearts, however, exhibit reduced expression and/or activity of several endogenous antioxidant enzymes and systems, including heme oxygenase 1 (HO-1) (117), superoxide dismutase (SOD) (4), catalase (195), glutathione peroxidase (GPx) (4), and the thioredoxin (Trx) system (149). The diabetic myocardium is also associated with a reduced ratio between reduced and oxidized glutathione (GSH/GSSG) (5, 68, 191). Genetic approaches aimed at increasing the endogenous antioxidants have been shown to reduce oxidative stress and rescue cardiomyocyte contractility, cardiac morphology, and/or mitochondrial function in diabetic models (124, 178, 192).

An important regulator of intracellular defense against ROS is the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), which regulates the antioxidant response elements (ARE) mediated expression of detoxifying and antioxidant enzymes (106). Nrf2 stability and activity is regulated through redox modifications of the Kelch-like ECH-associated protein 1 (Keap1), where elevated H_2O_2 maintains the activation of Nrf2 (36). While acute hyperglycemia and short-term diabetes is associated with increased Nrf2 activation (91), sustained ROS and long-term diabetes seem to impair Nrf2 activity, most likely through the ERK pathway (187).

Exercise increases myocardial antioxidant capacity

In the study of an AES, Bo *et al.* (26) demonstrated a time-dependent increase in myocardial MnSOD protein and activity, which is likely an adaptive response to minimize exercise-induced increase in cardiac oxidative stress. Muthusamy *et al.* (143) also demonstrated that AES induced antioxidant defense pathways in the myocardium through activation of Nrf2. These changes were lost in Nrf2^{-/-} mice, where AES resulted in increased oxidative stress and blunted the antioxidant responses in the myocardium (Fig. 5). Although there is no absolute consensus as to how endurance exercise influences antioxidant systems in the healthy heart, most studies have reported increased or unchanged gene expressions of antioxidant enzymes and systems after both long and short-term endurance training (11).

As the diabetic myocardium is associated with impaired antioxidant capacity, one would expect that exercise training could be particularly beneficial to the diabetic heart through enhancement of the myocardial antioxidant systems. Despite few studies on this issue, increased gene and protein expression of myocardial antioxidant systems have been reported in endurance trained diabetic and obese models (68, 85). Increased exercise-induced insulin sensitivity could potentially increase Nrf2 activity, as insulin signaling has been shown to increase myocardial Nrf2 activity *via* the PI3K pathway (187) (Fig. 5).

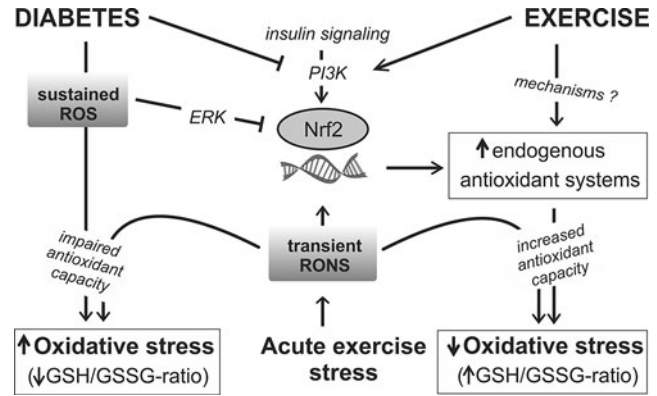


FIG. 5. Proposed effects of exercise on endogenous antioxidant responses in the diabetic myocardium. In long-term diabetes, sustained reactive oxygen species (ROS) and impaired insulin signaling may lead to impaired nuclear factor erythroid 2-related factor 2 (Nrf2) activity due to exaggerated phosphorylation of the extracellular signal-related kinase (ERK) and reduced activity of phosphoinositol 3-kinase (PI3K). This may lead to reduced endogenous antioxidant capacity and oxidative stress, here illustrated by the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). Acute exercise leads to a transient increase in reactive oxygen and nitrogen species (RONS) that stabilize and activate Nrf2, leading to transcriptional increase in endogenous antioxidant systems. Endurance training with repetitive bouts of RONS and improved insulin signaling can increase Nrf2 activity, which increases the antioxidant capacity of the myocardium. In conditions of impaired Nrf2 signaling and blunted antioxidant responses, exercise stress may lead to increased oxidative stress. See “Impaired Endogenous Antioxidant Capacity in the Diabetic Myocardium” section for references.

Finally, the progression and the severity of diabetes may be an important determination in how the diabetic heart tolerates and adapts to an AES and endurance training. Short-term (2 weeks) intense exercise of old and severely diabetic *db/db* mice using motorized wheels failed to induce the same antioxidant responses as seen in the myocardium of their control littermates (119). These hearts also exhibited reduced GSH/GSSG ratio, increased 4HNE content, and elevated fibrosis compared with untrained mice (119). Although not addressed or discussed in this article, this observation could be related to an impaired Nrf2 induction after the long-term diabetes (187). It could also be speculated that the use of motorized running wheels induced a considerable greater stress on the animals as compared with the use of treadmills or nonmotorized wheels where the animals can engage in more normal running behavior.

Autophagy in Diabetic Cardiomyopathy and the Potential Effects of Exercise

Elevated levels of nitrosative and oxidative stress are also believed to contribute to the disruption of autophagy that has been reported in diabetic cardiomyopathy and in hearts from rodents fed obesogenic diets (42, 194). Autophagy is viewed as a housekeeping process to maintain cellular homeostasis and is essential for the removal of protein and lipid aggregates, damaged and unwanted organelles. As the diabetic heart is more prone to adverse molecular modifications of

proteins and lipids, impaired autophagy may be particularly harmful and thus a potential contributor to the development of diabetic cardiomyopathy (194). Autophagy has been shown to be an important adaptive response that counteracts the development of insulin resistance in response to a high-fat diet in mice, and loss of autophagy was shown to impair insulin action in obese mice (59).

Acute exercise has been demonstrated to induce autophagy both in skeletal and in cardiac muscle (90). Interestingly, both short- and long-term exercise has been shown to improve insulin sensitivity through an autophagy-dependent mechanism (90). Whether endurance exercise can amend blunted autophagy in diabetic myocardium is yet to be explored.

Altered Calcium Handling in the Diabetic Cardiomyocyte

Changes in intracellular Ca^{2+} handling are believed to be an important factor leading to ventricular dysfunction in diabetes. At the cellular level, diabetic cardiomyocytes are characterized by Ca^{2+} transients with lower amplitude and slower rate of decay, which is linked to impaired Ca^{2+} handling by the sarcoplasmic reticulum (SR) (Fig. 6). A reduced SERCA2a activity (18, 176, 184) most likely contributes to both diastolic and systolic dysfunction in diabetes, as it may lead to impaired SR Ca^{2+} release and reuptake and, subsequently, reduced SR Ca^{2+} load. Although the exact mechanisms behind these alterations are not fully elucidated,

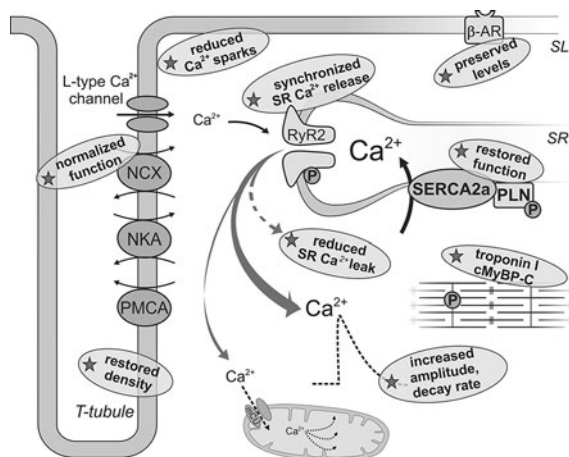


FIG. 6. Impaired calcium homeostasis in diabetic cardiomyocyte is improved by exercise training. In diabetes, Ca^{2+} transients are altered due to impaired SERCA2a function and increased RyR2 Ca^{2+} leak. These cardiomyocytes are also more dependent on SL Ca^{2+} transport, have increased spontaneous Ca^{2+} spark frequency, dyssynchronous Ca^{2+} release, and reduced T-tubule density, as well as reduced β -adrenergic response. Mitochondrial Ca^{2+} cycling via the uniporter entry system (mCU) is essential in the activation of key metabolic enzymes. Indicated by stars are exercise-induced changes after endurance training in models of obesity/diabetes that may contribute to restoring myocardial Ca^{2+} homeostasis (see text for details and references). NKA, Na^+/K^+ ATPase; PLN, phospholamban; β -AR, β -adrenergic receptor; TCA, tricarboxylic acid; PDH, pyruvate dehydrogenase complex; cMyBP, cardiac myosin binding protein C. SL, sarcolemmal; SR, sarcoplasmic reticulum; RyR2, ryanodine receptor 2; SERCA2a, SR Ca^{2+} ATPase; NCX, $\text{Na}^+/\text{Ca}^{2+}$ exchange; PMCA, SL Ca^{2+} ATPase.

studies have reported a decrease in SERCA2a expression and/or changes in its regulator phospholamban (PLN) (18, 61, 114, 121, 152).

Due to its slow turnover rate, SERCA2a is susceptible to post-translational modifications. An extensive SERCA2a glycation has been reported in the myocardium of STZ-induced diabetic rats (20), and treatment with the AGE cross-link breaker was found to partially normalize SR Ca^{2+} handling and cardiac function in an STZ model (114). Increased O-GlcNAcylation of PLN, SERCA2a and its transcription factor Sp1 have also been shown to be involved in myocardial SERCA2 downregulation and dysfunction after exposure to high glucose and in diabetes (52, 69). Although not extensively studied in diabetes, redox regulation of SERCA2a may also play a role, as ROS has been shown to reduce SERCA2a pump activity in heart failure (51).

It has also been shown that the decreased expression and function of SERCA2a is accompanied by an increased NCX expression and function in the diabetic myocardium (121, 184), which most likely serve as a compensatory mechanism to lower cytoplasmic Ca^{2+} levels. Furthermore, diabetic cardiomyocytes display increased SR Ca^{2+} leak through ryanodine receptors (RyR2) (16, 184), which may contribute toward reducing SR Ca^{2+} content. This does not seem to be associated with increased RyR2 protein expression (61, 152, 163, 184), but rather to altered RyR phosphorylation status due to changes in the activity of upstream kinases (149, 163, 176). O-GlcNAc-modified CaMKII was recently demonstrated in the heart of diabetic humans and rats by Erickson *et al.* (63), linking diabetic hyperglycemia to increased SR Ca^{2+} release events. In accordance with this, SR Ca^{2+} leak was normalized after inhibition of CaMKII in *db/db* cardiomyocytes (184).

Another plausible mediator of this SR Ca^{2+} leak is ROS, as it can increase the activity of upstream kinases such as PKA and CaMKII (35, 62). Oxidation of RyR2 can also promote increased opening probability of the channel and, consequently, promote Ca^{2+} leak (58). Increased spontaneous Ca^{2+} spark frequency and dyssynchronous Ca^{2+} release, in addition to reduced transverse (T)-tubule density, has also been reported in cardiomyocytes from *db/db* mice (184).

The diabetic myocardium is associated with an altered adrenergic contractile response due to reduced density of β -adrenoceptors (β -AR) (93), impaired β -adrenergic G-protein-adenylat cyclase system, and PLN phosphorylation (21, 73). Recent studies also suggest a cross-talk between IR activation and the β -AR2, where the contractile response to cardiac β -adrenergic stimuli was blunted through a β 2AR-dependent mechanism (71).

It should be stressed that a common limitation in studies addressing calcium transport in cardiomyocytes is the lack of FAs as energy substrates. The diabetic cardiomyocyte most likely is adapted to and potentially dependent on FAs as an energy substrate. This notion is supported by studies showing that the addition of FA as a substrate significantly increases Ca^{2+} transients in diabetic cardiomyocytes (64, 191).

Exercise improves calcium homeostasis

Exercise training has been shown to improve cardiomyocyte contractility in obese/diabetic models, changes that have been associated with improved Ca^{2+} homeostasis (61, 137,

184). Exercise effectively restored myocardial expression and activity of SERCA2a (19, 61, 176, 184), associated with an increase in PLN phosphorylation at the Serine 16 (61, 184). In addition, exercise normalized the diabetic-induced Ca^{2+} SR leak in cardiomyocytes from *db/db* mice (184) and STZ rats (176). The reduced leak does not seem to be linked to altered RyR2 expression (61, 152, 176, 184) but rather to its phosphorylation status due to a normalization of CaMKII activity (176, 184). Stabilization of the RyR was also found to restore the synchronicity of SR Ca^{2+} release (184). In addition, Stolen *et al.* (184) reported that high-intensity interval training restored T-tubule density, which by improving the spacing between L-type Ca^{2+} channels and RyR2 may improve the efficiency of EC coupling.

Clinical studies have demonstrated that exercise interventions after the onset of diabetes can improve the responsiveness of the heart to β -adrenergic stimulation (196). In experimental models, this has been shown to be associated with preserved levels of β -adrenoceptors (21, 137). Finally, exercise-induced improvement of function in diabetic hearts may also be related to PKA-induced phosphorylation of the myofibrillar proteins troponin I and myosin-binding protein-C (111).

Cardiac Inefficiency and Oxygen Wasting in Diabetes

Despite its high metabolic activity, the heart has a relatively low content of high energy compounds. Consequently, the heart depends on optimal substrate metabolism and efficient ATP hydrolysis at energy consuming sites. Any imbalance between energy demand and availability can ultimately lead to an energetically compromised heart with reduced working capacity. In support of this notion, decreased myocardial energetics, measured as a reduced PCr:ATP ratio, has been shown to correlate with the severity of heart failure in patients with idiopathic dilated cardiomyopathy (146). Decreased PCr:ATP ratio has also been reported in hearts from obese and/or T2D subjects (154, 171), and impaired cardiac energetics due to cardiac inefficiency has been suggested to be an important mediator in the progression of obesity/diabetes-induced cardiac dysfunction.

Reduced cardiac mechanical efficiency, defined as the ratio between external work (stroke work) and MVO_2 (23), has become a hallmark of the obese/diabetic heart, as it has been reported in a range of experimental studies (1, 32, 38, 53, 68, 85, 201) as well as in obese young women (155). Impaired mechanical efficiency also precedes the development of obesity/diabetes-related ventricular dysfunction (85, 155, 201).

A range of factors are known to determine MVO_2 ; apart from heart rate, this includes factors related to the energy used for generating force/tension by the contractile machinery (*i.e.*, pre- and after-load) and factors related to nonmechanical processes. It should be noted that mechanical efficiency is a load-dependent parameter and that afterload-dependent peak efficiency was not found to be affected in STZ-induced diabetes (87).

The underlying mechanisms leading to decreased efficiency in diabetic cardiomyopathy are far from clear. It is acknowledged that an acute elevation of FAs in the normal heart induces oxygen wasting (28, 53, 95, 138), and as FA is a less efficient substrate when compared with glucose (lower

P/O ratio), increased FA oxidation is generally suggested to be causative for this increase in MVO_2 . Likewise, elevated FA oxidation in the diabetic heart is also regarded to be an important factor leading to decreased mechanical efficiency. It should be noted, however, that the heart should only use 11–12% more MVO_2 when shifted from exclusively glucose to FA oxidation, which is too low to explain the reported increase in MVO_2 under both increased FA load and diabetes where the change in substrate utilization is far less (95, 138).

Supporting the notion that additional oxygen consuming processes are activated are experimental data indicating that it is not the high myocardial FA oxidation rate (accompanying elevated FA concentration) that leads to the increased MVO_2 in normal hearts (28, 102), but that FAs increase the nonwork-related oxygen demand for processes associated with EC coupling (28, 41). In contrast to what is observed in normal hearts, exposing hearts from T2D mice to high FA levels does not further increase MVO_2 (despite elevation of FA oxidation rates) (28, 53, 95). Taken together, it is clear that the oxygen wasting effects of acute and a more long-term elevation of FAs levels are not necessarily *via* the same mechanisms and, importantly, FA oxidation rates *per se* do not seem to be the sole mechanism for the increased MVO_2 .

Assessment of left ventricular mechanoenergetic properties [by using the PVA- MVO_2 framework (186)] has demonstrated that the diabetic heart uses more oxygen for nonmechanical processes (84, 85, 95), including processes associated with basal metabolism and EC coupling (27, 28, 85). The energy cost for EC coupling is primarily related to calcium transport, and changes in myocardial Ca^{2+} handling in the diabetic heart may therefore contribute to the observed increase in the oxygen cost of EC coupling. Oxygen wasting processes may include SR Ca^{2+} leak (16, 184) and subsequent futile cycling of Ca^{2+} , increased use of the less energetically favorable NCX instead of SERCA2a for cytoplasmic Ca^{2+} clearance, as well as dyssynchronous SR Ca^{2+} release (181).

In addition, increased ventricular stiffness (and thus wall stress) can occur due to diabetes-induced fibrosis (101) and excessive accumulation of AGEs (114) may also contribute to a decreased mechanical efficiency in the diabetic heart. Myocardial oxygen consumption has been found to be correlated with levels of ROS in cardiac tissue (85), which suggests that oxidative stress may play a role in diabetes-induced oxygen wasting. In support of this, although not addressed in the diabetic myocardium, allopurinol (a XO inhibitor) infusion to heart failure patients significantly increased cardiac mechanical efficiency (44).

Exercise restores cardiac efficiency through oxygen sparing effects

Improved cardiac efficiency after endurance training is supported by studies done on patients with dilated cardiomyopathy (183, 193) and in a swine model of heart failure with a preserved ejection fraction (132). Recently, exercise training was also shown to improve mechanical efficiency due to reduced oxygen consumption for both basal metabolism and EC coupling in hearts from diet-induced obese mice (85). The exercise-induced reduction in oxygen wasting associated with EC coupling can be related to reduced RyR2 Ca^{2+} leak, improved SERCA2a function and normalization

of SL Ca^{2+} transport, and synchronization of SR Ca^{2+} release. It is known that calcium-handling proteins are sensitive to the intracellular redox environment (35, 51, 58, 62), and it is, therefore, tempting to suggest that the reported positive correlation between myocardial ROS and MVO_2 (85) reflects ROS-mediated changes in Ca^{2+} handling.

In addition, as exercise has been found to counteract obesity-induced left ventricular remodeling and to reduce ventricular fibrosis (85, 119, 128, 167); a reduced wall stress can also positively affect cardiac efficiency. Finally, as decreased efficiency will be particularly disadvantageous under conditions of reduced oxygen availability, one could also speculate that exercise-induced amendments of cardiac efficiency could also reduce ischemic susceptibility in the diabetic heart.

Concluding Remarks

Cardiovascular disease is the leading cause of mortality in people with diabetes, and there is no doubt that exercise training is efficient in the prevention and treatment of obesity and T2D. Exercise influences several of the systemic changes associated with diabetes/obesity such as insulin resistance, glycemic control, plasma lipid levels, and inflammatory status, and beneficial cardiac effects after exercise training in this patient group are reported.

Due to the complex nature of the development of T2D, the diversity of exercise training regimens, and wide range of physiological adaptations to exercise, it is not unexpected that reports regarding the mechanisms that may lead to the cardiac response to exercise training are diverging. In addition, studying molecular adaptations to exercise in the diabetic heart advocates the use of animal models where the severity and the progression of diabetes may vary, which may influence the tolerance and the adaptive response to acute and chronic exercise. It is essential that the models used in experimental studies of T2D closely resemble those of the human disease, and that changes in STZ-induced T1D models do not necessarily reflect the changes in obese T2D models. It is also likely that variations in animal diets can influence the adaptive response to exercise and the restitution between exercise sessions.

In many exercise studies, it is challenging to discriminate between the indirect effects linked to exercise-mediated systemic changes and the direct cardiac effect originating from the high contractile activity of the heart during bouts of physical training. In addition, there are clearly different molecular pathways involved in the cardiac response to an AES as opposed to the adaptive changes after repetitive bouts of high contractile activity during physical training. Moreover, there are most likely transient time-dependent changes during the cardiac adaptive responses to exercise.

The different training regimens with respect to exercise mode, duration, and intensity between studies may also cause discrepancies between findings. For instance, experimental and clinical studies have suggested that exercise interventions with higher intensities produce greater effects in terms of improving glycemic control and insulin sensitivity (85, 190, 200). High-intensity training has also been shown to produce greater aerobic capacity (92) and to produce greater cardiac adaptations than isocaloric low-moderate intensity training (83, 110).

Few experimental studies actually report how fitness and aerobic capacity are affected by the exercise protocols, and the role of exercise intensity in the adaptive responses in the diabetic heart is not well elucidated. As low aerobic capacity is a strong predictor of cardiovascular disease (25, 109), it would be a great advantage if the effect of the exercise regime used on $\text{VO}_{2\text{max}}$ was reported in experimental studies.

The beneficial cardiac effects of exercise in diabetic cardiomyopathy is supported by studies reporting exercise training to amend many of the metabolic disturbances characterizing the diabetic myocardium, including mitochondrial capacity and efficiency, carbohydrate oxidation, lipotoxicity, and disturbances in nonoxidative glucose pathways. In addition, direct and systemic effects of exercise training may influence ROS production and enhance endogenous antioxidant capacity, making exercise intervention a potent tool in amending the pro-oxidant milieu of the diabetic heart. Exercise-induced improvements of myocardial calcium handling are also of great therapeutic potential in the diabetic heart, as they may improve ventricular function, cardiac efficiency, and potentially also mitochondrial function. Revealing the molecular mechanisms behind the beneficial effects of exercise training is of considerable scientific value to generate evidence-based therapy and in the development of new therapeutic strategies.

Acknowledgments

ADH is supported by a fellowship funded by the Norwegian Health Association. NTB is supported by a fellowship funded by UNIKARD (Norwegian Research Council and the Northern Norway Regional Health Authority).

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Address correspondence to:
 Prof. Ellen Aasum
 Cardiovascular Research Group
 Department of Medical Biology
 Faculty of Health Sciences
 UiT-The Arctic University of Norway
 Tromsø N-9037
 Norway

E-mail: ellen.aasum@uit.no

Date of first submission to ARS Central, February 20, 2015;
 date of acceptance, March 3, 2015.

Abbreviations Used

β -AR = β -adrenoceptors
 4HNE = 4 hydroxynonenal
 ACC = acetyl-CoA carboxylase
 AES = acute exercise stress
 AGEs = advanced glycation end-products
 ANT = adenine nucleotide translocator
 AR = aldose reductase
 ARE = antioxidant response elements
 CaMKII = Ca²⁺-calmodulin-dependent protein kinase II
 cMyBp = cardiac myosin binding protein C
 CPT1 and 2 = carnitine-palmitoyltransferases system 1 and 2
 CrAT = carnitine acetyltransferase
 DAG = diacylglycerol
 DGAT1 = diacylglycerol transferase 1
 EC = excitation-contraction
 eNOS = endothelial NOS
 ERK = extracellular signal regulated kinase
 ETC = electron transport chain
 FA = fatty acids
 FABP = fatty acid binding protein
 FAT/CD36 = fatty acid translocase
 FATP = fatty acid transport protein
 Foxo1 = Forkhead box protein O1

GLUT1 = glucose transporter1
 GLUT4 = glucose transporter4
 GPx = glutathione peroxidase
 GSSG = oxidized glutathione
 HbA1c = glycated hemoglobin
 HBP = hexosamine biosynthetic pathway
 HK = hexokinase
 HO-1 = includes heme oxygenase 1
 iNOS = inducible NOS
 IR = insulin receptor
 IRS-1 = insulin receptor substrate-1
 IRS-2 = insulin receptor substrate-2
 JNK = c-Jun N-terminal kinase
 LCFA = long chain fatty acids
 LDH = lactate dehydrogenase
 LV = left ventricle
 MCD = malonyl-CoA decarboxylase
 mCU = mitochondrial Ca²⁺ uniporter
 MnSOD = manganese superoxide dismutase 0
 mPTP = mitochondrial permeability transition pore
 MVO2 = myocardial oxygen consumption
 NADP = nicotinamide adenine dinucleotide phosphate
 NCX = sodium-calcium exchange
 NF- κ B = nuclear factor- κ B
 NO = nitric oxide
 NOS = NO synthase
 NOX = NADPH oxidase
 Nrf2 = nuclear factor erythroid 2-related factor 2
 O-GlcNAc = O-linked N-acetylglucosamine
 OxPhos = oxidative phosphorylation
 PCr:ATP = phosphocreatinine:adenosine triphosphate
 PDH = pyruvate dehydrogenase complex
 PDK4 = pyruvate dehydrogenase kinase 4
 PI3K = phosphatidylinositol-4,5-bisphosphate 3-kinase
 PKC = protein kinase C
 Plin-5 = perilipin-5
 PLN = phospholamban
 PMCA = sarcolemmal Ca²⁺ ATPase
 PPAR- α = peroxisomal proliferator activated receptor- α
 PVA = pressure volume area
 RAGEs = AGE receptors
 RAS = renin-angiotensin system
 RCR = respiratory control ratio
 RONS = reactive oxygen and nitrogen species
 ROS = reactive oxygen species
 RyR2 = ryanodine receptors
 Serca = sarcoplasmic reticulum Ca²⁺ ATPase
 SERCA2a = sarcoplasmic reticulum Ca²⁺ ATPase
 SL = sarcolemmal
 SOD = superoxide dismutase
 SR = sarcoplasmic reticulum
 sRAGE = soluble form of RAGE
 STZ = streptozotocin
 T1D = type 1 diabetes
 T2D = type 2 diabetes
 TAG = triglyceride
 UCP = uncoupling proteins
 UDP-GlcNAc = uridine diphosphate N-acetylglucosamine
 VO_{2max} = maximal oxygen uptake
 XO = xanthine oxidase