Cardiomyocyte Adaptation to Exercise: K^+ Channels, Contractility and Ischemic Injury

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ABSTRACT

Cardiovascular disease is a leading cause of morbidity and mortality, and exercise-training (TRN) is known to reduce risk factors and protect the heart from ischemia and reperfusion injury. Though the cardioprotective effects of exercise are well-documented, underlying mechanisms are not well understood. This review highlights recent findings and focuses on cardiac factors with emphasis on K^+ channel control of the action potential duration (APD), β-adrenergic and adenosine regulation of cardiomyocyte function, and mitochondrial Ca^{2+} regulation. TRN-induced prolongation and shortening of the APD at low and high activation rates, respectively, is discussed in the context of a reduced response of the sarcolemma delayed rectifier potassium channel (IK) and increased content and activation of the sarcolemma K_{ATP} channel. A proposed mechanism underlying the latter is presented, including the phosphatidylinositol-3kinase/protein kinase B pathway. TRN induced increases in cardiomyocyte contractility and the response to adrenergic agonists are discussed. The TRN-induced protection from reperfusion injury is highlighted by the increased content and activation of the sarcolemma K_{ATP} channel and the increased phosphorylated glycogen synthase kinase-3β, which aid in preventing mitochondrial Ca^{2+} overload and mitochondria-triggered apoptosis. Finally, a brief section is presented on the increased incidences of atrial fibrillation associated with age and in life-long exercisers.

Introduction

Cardiovascular disease is a leading cause of morbidity and mortality worldwide, and exercise-training (TRN) is known to be effective in countering cardiovascular risk factors, reducing the incidences and severity of ischemic heart disease, and protecting against heart failure [1–3]. The beneficial effects of TRN are mediated by both organ and system adaptations that reduce the relative workload of daily living, such as increased cardiac output, improved tissue blood flow regulation, and increased maximal oxygen uptake, and direct cellular adaptations that improve heart function and reduce diseases of the heart. These include improved coronary circulation, enhanced contractility, increased left ventricular end diastolic di-

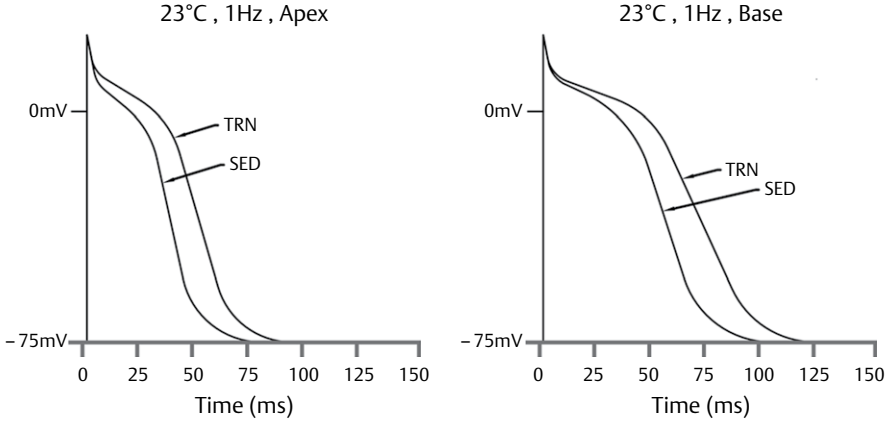
mension, elevated myofibrillar Ca^{2+} sensitivity, improved metabolic efficiency, and altered electrical properties of the heart [4–10]. This review will focus on cardiac factors considering how TRN affects surface membrane receptors, membrane channels, excitation-contraction coupling, and mechanical properties of cardiomyocytes. We will also consider 1) a comparison of moderate and high intensity TRN; and 2) TRN effects on diseases of the heart with special emphasis on ischemic heart disease and arrhythmias.

Importance of moderate versus intense exercise

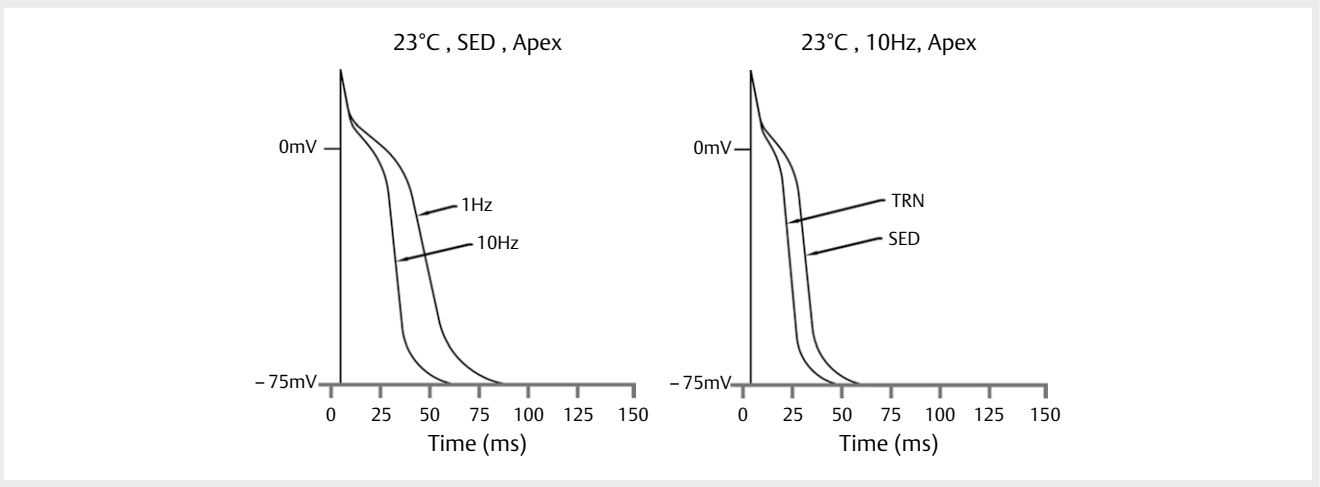
A question of considerable interest to the public is how much exercise is enough to generate cardiovascular protection, and is moderate exercise enough or do exercise programs require some degree of intense exercise. This topic has been recently reviewed in detail [11, 12] and is reviewed only briefly here. TRN programs can be defined as those primarily employing moderate-intensity continuous exercise-training (MICT) utilizing work loads of approximately 60 to 75 % of heart rate (HR) peak or high-intensity interval training (HIIT) generally at 85 to 95 % of HR peak [13, 14]. Arguments favoring a component of HIIT stem in part from the observation that it generates a greater increase in aerobic power than MICT in both healthy and cardiovascular patients. This is particularly important to the latter group as low aerobic power has been reported to be the best predictor of cardiac and all caused death among these patient groups [12, 14]. Additionally, in cardiac patients HIIT increases left ventricular ejection fraction and isovolumetric relaxation time factors unaffected by MICT [11, 12, 14–16]. HIIT is also more effective in reducing exercise blood pressure and norepinephrine levels, which would contribute to a reduction in blood pressure [11, 17]. At the cell level, HIIT has been shown to cause a greater increase in shortening rate than MICT an effect that would contribute to the TRN-induced increase in stroke volume [18]. Additionally, HIIT requires less exercise time than MICT for similar health benefits [11, 12]. Nonetheless, for many cardiovascular risk factors, such as resting blood pressure and heart rate, and blood glucose and lipid control, HIIT does not outperform MICT [12]. Adherence to TRN is also a consideration and this appears higher with MICT than HIIT [11]. Another concern is that HIIT is associated with a greater number of life-threatening events [12, 15]. For healthy individuals of all ages, incorporating a HIIT component into a TRN program is important for optimal health gains [13, 14]. The published literature suggests that HIIT offers advantages over MICT for cardiovascular patients as well, but the optimal protocol for improving cardiovascular function and the risk to benefit ratio needs further investigation for this population group [11, 12].

TRN induced adaptations in cardiac action potential

Regulation of the cardiac action potential duration (APD) during rest and exercise is important to ensure adequate Ca^{2+} influx while preventing Ca^{2+} overload, excess ionic pump activity (Na^+-K^+ and SR Ca^{2+} pumps) and/or inadequate time for relaxation [7]. It is well known that transmural differences exist in the AP shape with cardiomyocytes (CMs) in the base region of the heart (dominated by endocardial CMs) showing longer durations compared to the apex region (dominated by epicardial CMs) (▶ **Fig. 1**) [6, 19–22]. Regulation of the various sarcolemma K^+ channels seems particularly important in controlling APD [7, 23]. The most relevant K^+ channels are as follows: the rapidly activating, transient I_{to} channel that generates the early repolarization preceding the AP plateau; the delayed rectifier channel IK, which consists of two Kv (voltage-gated) channel isoforms; a rapidly activating IK_r and a slowly activating IK_s, and the K_{ATP} channel as they regulate the duration of the action potential plateau and thus Ca^{2+} influx, and in animals all three have been shown to be altered by programs of exercise-training



▶ **Fig. 1** Overlapping representative action potential traces from apex and base myocytes in sedentary (SED) and exercise-trained (TRN) female rats. Action potential durations (APD) measured at 90 % repolarization of the action potential (APD90) in apex and base myocytes show a regional difference, with APD90 of base myocytes (representative of endocardial cells) significantly longer than apex myocytes (representative of epicardial cells). The APD90 values for both are significantly prolonged by exercise training. Measurements were obtained at room temperature with 1-Hz stimulation.



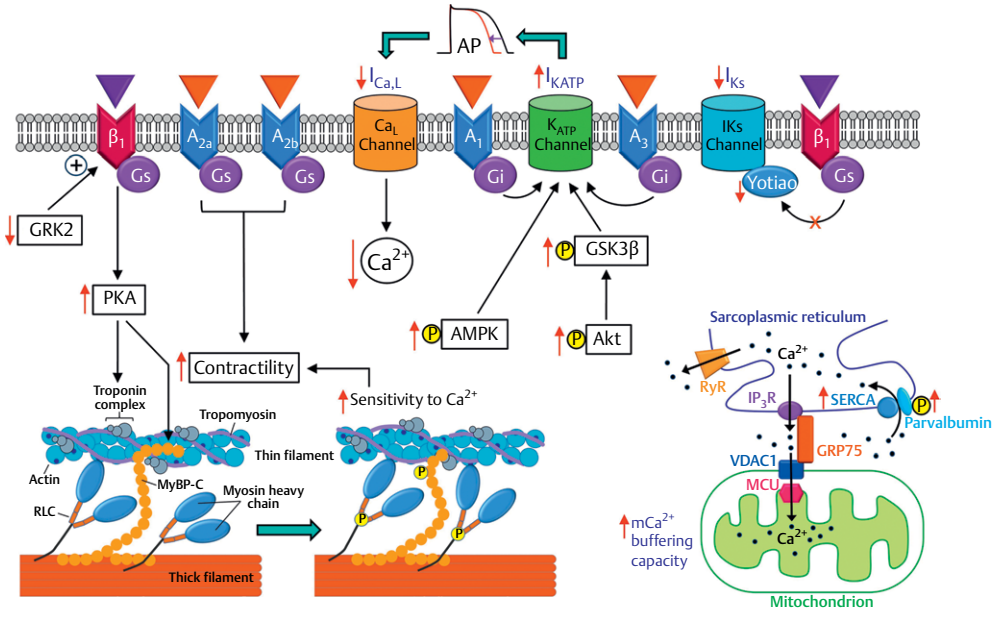
▶ **Fig. 2** Representative action potential traces highlighting high stimulation rate (10 Hz) induced shortening of action potential duration (APD) measured at 90 % repolarization of the action potential (APD90) compared to 1 Hz stimulation (*left*) and exercise-training effect at 10 Hz (*right*). At 10 Hz, APD90 was shortened more in myocytes from exercise-trained (TRN) than sedentary (SED) rats.

[6, 7, 19, 24, 25]. With low activation rates (1 Hz) up to rates observed in resting rat hearts (5 Hz), we showed that a type of MICT (wheel running in rats) prolonged the CM APD while regional differences base > apex were maintained (▶ **Fig. 1**). However, at CM stimulation rates simulating heavy exercise (10 Hz), APD is reduced with greater reductions in CMs isolated from TRN compared to sedentary (SED) animals (▶ **Fig. 2**) [19]. These adaptations in the APD are important for improving the efficiency of the heart at rest (i. e. lower heart rate) and during exercise (reduced energy requirement for ion homeostasis), and for preserving time for cardiac relaxation and venous return during exercise. The TRN-induced prolongation of the APD at low CM stimulation rates (1 to 5 Hz) is in part mediated by a downregulation in the response to β-adrenergic receptor (β-AR) agonist causing inhibition of the I_{to} and reduced activation of the delayed rectifier (Ik) [6, 25, 26]. The observation that this effect was observed in isolated CM suggests that TRN also directly inhibited K^+ channel function. In unstressed CM, the primary repolarizing current is carried by the Ik, where the relative importance of the two major isoforms is species dependent [27–29]. In rat CMs, the Ik_s is relatively more important than the Ik_r[25], and recently we showed TRN to reduce channel protein (KCNQ1 and KCNE1) content and current density of the Ik_s and the kinase anchoring protein Yotiao, a protein required for the PKA phosphorylation of IK_s [25]. This TRN-induced down regulation of the Ik_s channel and Yotiao provides a mechanism for the reduced responsiveness of the action potential to β-AR agonists despite no change in adrenergic receptor content (▶ **Fig. 3**) [25, 26]. The reduced Yotiao content would reduce PKA phosphorylation of the pore forming Ik_s subunit KCNQ1, which accounts for most of the functional modulation of Ik_s by the sympathetic nervous system [30]. It is not known whether TRN downregulates the delayed rectifier channel in human cardiac muscle, but the known exercise-training induced reduction in resting heart rate in humans is consistent with a delayed onset of action potential repolarization and outward K^+ current. Human ventricles have higher Ik_r current than Ik_s so a TRN downregulation in the latter may be less important [31]. However, at heart rates higher than resting, the rapidly activating Ik_r channel may become inactivated, increasing the importance of the Ik_s channel to repolarization and the APD even in human CM [31]. The higher content of the pore forming subunit KCNQ1 of the Ik_s in the apex region compared to the base region of the heart explains the greater APD observed in CM base (▶ **Fig. 1**). This regional difference in Ik_s content is important as it allows the endocardium while depolarizing first to repolarize after the epicardium facilitating ventricular ejection and reducing the likelihood of arrhythmias caused by premature excitation of the endocardium [32].

TRN-induced upregulation and activation of the sarcolemma K_{ATP} channel

Recently, we observed TRN to increase sarcolemma K_{ATP} (sK_{ATP}) channel content in CM isolated from both the apex and base regions of the heart; however, regional differences existed with TRN upregulating the Kir6.2 and SUR2A subunits in apex and base CM, respectively [22]. The greater reduction in CM APD at high stimulation rates in TRN compared to SED was associated with an increased sK_{ATP} repolarization current, an effect at least in part caused by a TRN-induced increase in sK_{ATP} channel content [7, 19, 22, 33]. In support of this conclusion, the greater decrease in the APD with high CM stimulation (10 Hz) following TRN was blocked by glibenclamide, a sK_{ATP} channel blocker [7, 19]. Additionally, pinacidil, a sK_{ATP} channel activator, shortened the APD more in CM from TRN than SED rats and in the presence of pinacidil, 10 Hz stimulation had no additional effect on the APD than pinacidil plus 1 Hz stimulation [22].

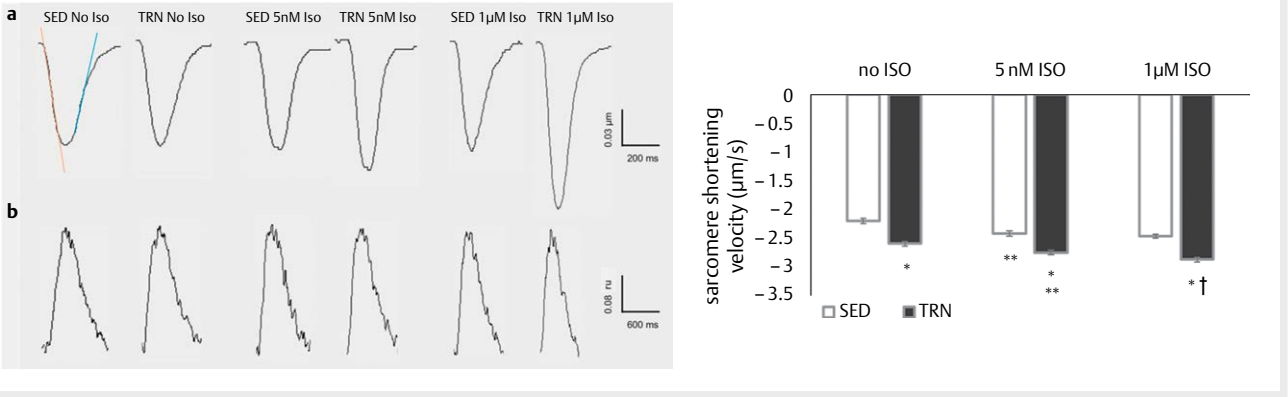
TRN increases in the sK_{ATP} channel content and shifts the primary control of action potential repolarization during high activation rates (i. e. exercise) from the Ik channel to the metabolically controlled sK_{ATP} channel (▶ **Fig. 3**). At rest, the channel is inhibited by



▶ **Fig. 3** Schematic of key cardiomyocyte components, including the sarcolemma, myofibrils, sarcoplasmic reticulum, and mitochondria, and adaptations with exercise-training (TRN). Downward and upward arrows indicate a TRN-induced decrease and increase, respectively, in proteins or physiological conditions. The downward arrows for $I_{Ca,L}$ and cytosolic Ca^{2+} refer to less of an increase in TRN compared to sedentary (SED) during stress. Abbreviations: AP, action potential; I_{KATP}, outward repolarizing potassium current through the KATP channel; I_{KS}, outward repolarizing potassium current through the slowly activating, delayed rectifier potassium channel; $I_{Ca,L}$, current thru the L-type Ca^{2+} channel; A_1, A_{2a}, A_{2b}, A_3; adenosine receptor isoforms; β_1, beta adrenergic receptor; G_s, stimulatory G protein; G_i, inhibitory G protein; GRK2, G-protein-coupled receptor kinase 2; PKA, protein kinase A; AMPK, AMP-activated protein kinase; pGSK3β, phosphorylated glycogen synthase kinase-3β; pAkt, phosphorylated protein kinase B; VDAC1, voltage dependent anion channel 1; GRP75, glucose-regulated protein 75; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase pump; MyBP-C, myosin binding protein C; RLC, regulatory light chain; IP_3R, IP_3 receptor. Figure created using Microsoft PowerPoint.

nonhydrolytic binding of ATP an effect relieved during exercise by the elevated metabolic demand causing reduced ATP/ADP and ATP/AMP ratios [34–36]. The TRN-induced increase in the sK_{ATP} channel allows for rapid beat to beat control of heart APD in response to changes in cell metabolism, which should result in a more optimal match of cell activation (i. e. intracellular Ca^{2+} influx), contractility, and HR leading to a more efficient heart. While it is known that the sK_{ATP} channel is activated with exercise and with ischemia and reperfusion (IR), the mechanism of activation is not well understood [37]. A decline in the ATP/ADP and ATP/AMP ratios likely contribute to sK_{ATP} channel activation with exercise; however, that is unlikely to be the only mechanism or explain the elevated activity following TRN. A prime candidate for the observed TRN-induced activation of the channel is the phosphatidylinositol-3kinase (PI3K)/protein kinase B (Akt) pathway. It is well established that TRN increases growth hormone and insulin-like growth factor (IGF-1) leading to an activation of phosphatidylinositol-3-kinase (PI3K), which phosphorylates phosphatidylinositol bisphosphate (PIP2) to triphosphate (PIP3). The PIP3 in turn phosphorylates Akt [38]. The ATP inhibition of the sK_{ATP} channel is partially blocked by PIP3 [36, 38]. Importantly, PIP3 is known to activate 3-phosphoinositide-dependent kinase 1 (PDKI), which phosphorylates itself and Akt. Phosphorylated Akt (pAkt), the active form of the protein, can detach from PIP3 and activates multiple cytosolic proteins, including glycogen synthase kinase-3β (pGSK3β) [39], and the mammalian target of rapamycin (mTOR), which has been implicated in the TRN-induced physiological cardiac hypertrophy [9, 38]. While there is no evidence that the mTOR pathway has any effect on the sK_{ATP} channel, it has been reported that GSK3β promotes sK_{ATP} channel closing, an activity inhibited by pGSK3β [40, 41]. Importantly, TRN has been shown to cause a 2.5-fold increase in pGSK3β [39]. Consequently, the TRN-induced activation of the sK_{ATP} channel with exercise may be in part caused by activation of the PI3K-Akt-GSK3β pathway (▶ **Fig. 3**). Activation of this pathway might be facilitated by brain-derived neurotrophic factor (BDNF) as this protein has been shown to increase with swim exercise-training in mice and activate PI3K-Akt [42, 43].

Due to its importance, activation of the sK_{ATP} channel with stress (cardiac ischemia or exercise) is likely controlled by multiple factors. Besides the PI3K-Akt-GSK3β pathway, there is evidence that activation of AMP-activate protein kinase (AMPK) may play a role [37, 44]. AMPK is known to physically interact with the sK_{ATP} channel, and with stress AMPK activation via phosphorylation (pAMPK) by upstream kinases (AMPKKs) promotes channel opening [44, 45]. With exercise, AMP increases and binds to AMPK, which reduces ATP inhibition of AMPK and makes it a better substrate for AMPKKs, which increases pAMPK [46]. Importantly, TRN is known to increase pAMPK, which might contribute to the TRN-induced activation of



▶ **Fig. 4** Exercise training and isoproterenol (ISO) effect on sarcomere shortening rate. *Left*: representative cardiomyocyte sarcomere shortening (**a**) and Ca^{2+} transient traces (**b**) at 37 °C with 1-Hz stimulation. Best-fit lines are shown to demonstrate the measurement of sarcomere shortening and relaxation rates. *Right*: in the absence and presence of β-agonist, exercise training (TRN) increased sarcomere shortening velocity compared to the sedentary (SED) group. The addition of 5 nM ISO increased sarcomere shortening velocity in both SED and TRN groups. One micromolar ISO had a larger effect than 5 nM ISO on shortening velocity in the TRN group but not in SED. $^{*}P = 0.05$, TRN group vs. SED group; $^{**}P = 0.05$, 5 nM ISO vs. no ISO; †$P = 0.05$, 1 μM ISO vs. 5 nM ISO. Source: *Am J Physiol Heart Circ Physiol* 2018; 315: H885-H896.

the sK_{ATP} channel (▶ **Fig. 3**) [46, 47]. While not proven, it is possible that the TRN upregulation of the sK_{ATP} channel in the heart could play an important metabolic role in addition to its regulation of the APD and Ca^{2+} influx by upregulating glucose uptake and mitochondrial biogenesis, and increasing glucose metabolism during stress a role that might be enhanced by pAMPK [37, 46, 47]. The idea that the sK_{ATP} channel plays an important metabolic role is supported by the observation that channel activation increases PGC-1α expression, a transcriptional coactivator known to stimulate mitochondrial biogenesis [48].

Adenosine may be important in TRN-induced increases in the sK_{ATP} channel. Adenosine has been shown to decrease channel sensitivity to the inhibitory effects of ATP and, acting via A_1 and A_3 adenosine receptors (AR), activate the channel via a G_i protein (▶ **Fig. 3**) [49–51]. Adenosine may also mobilize PKC_ξ a kinase linked to increased incorporation of K_{ATP} channel subunits into the sarcolemma [52, 53]. However, adenosine may be more effective in regulating atrial than ventricular tissue, as it appears to have little effect on the ventricular AP [54]. Additionally, the effect of TRN on AR is unknown. Clearly, the mechanisms by which TRN increases the sK_{ATP} channel content and activation are important topics for future investigation.

TRN-induced increase in CM contractility

The TRN-induced increase in biomechanical function and cardiac efficiency are mediated by a combination of factors, including the increase in CM length, AP regulation of Ca^{2+} influx, the Ca^{2+} transient rate of rise and duration, β-AR regulation, increased myofilament Ca^{2+} sensitivity, and an elevated rate of CM shortening [5, 13, 19, 21, 22, 55]. TRN-induced cardiac hypertrophy and, in resting CM, a prolonged APD and Ca^{2+} transient facilitates an increased stroke volume and reduced HR at a given CO producing a more efficient heart [9, 10, 19, 38, 56]. Cardiac hypertrophy appears to be primarily due to a TRN-induced increase in CM cell length [19, 57], but Natali et al. [21]. did observe TRN to increase CM width and peak tension. This adaptation was associated with an increased steepness in the tension-length relationship which would contribute to an increased SV. In agreement with Moore et al. [58] and Wisløff and colleagues [56, 59], we found wheel running in rats to increase CM shortening velocity and the rate of rise of the intracellular Ca^{2+} transient (▶ **Fig. 4**) [22]. Our results extended the previous findings to show that these adaptations occurred in CM isolated from both the apical (primarily epicardial CM) and basal (primarily endocardial CM) regions of the heart in both sexes [22]. The mechanism for the TRN-induced increase in shortening velocity is unknown. A possibility may be that it could reflect an increased myofibril ATPase as this enzyme has been shown to regulate shortening velocity [60]. However, Baldwin et al. [61, 62] found only a transient increase in this enzyme with endurance treadmill running in rats. It is unknown how TRN increases the rate of rise in the CM Ca^{2+} transient. An untested possibility is that TRN might increase the number, open probability, or activation rate of the SR ryanodine receptor [63]. Alternatively, SR Ca^{2+} release is known to be dependent on SR Ca^{2+} content; so, TRN might increase the rate of Ca^{2+} release by shifting the SR Ca^{2+} content-SR Ca^{2+} release relationship to favor release at a given SR Ca^{2+} content [63, 64]. In the resting state, the TRN-induced prolongation of the APD would allow the sarcolemma L-type Ca^{2+} channel to remain open longer, thus facilitating Ca^{2+} influx and activation of Ca^{2+} induced SR Ca^{2+} release. This factor could contribute to a faster onset of the Ca^{2+} transient in resting, unstressed CM, but not during exercise, as TRN shortens the APD, which would close the L-type Ca^{2+} channel sooner reducing Ca^{2+} influx. Additionally, TRN has been shown to have no effect on L-type Ca^{2+} channel number or current, which decreases the likelihood that it mediates TRN-induced changes in the Ca^{2+} transient [65].

TRN not only increased CM shortening velocity but also the extent of shortening, and this occurred in the face of no change or even a decrease in the amplitude of the Ca^{2+} transient (▶ **Fig. 4**)

[18, 19, 56, 58]. A logical explanation for the latter is that TRN increases the Ca^{2+} sensitivity of the myofilaments [5, 18]. The mechanism of this effect is unknown, but it could involve molecular adaptations in filament proteins (▶ **Fig. 3**). There are multiple studies assessing the importance of phosphorylation of cardiac myosin binding protein C (cMyBP-C), regulatory light chain (RLC), Troponin-I, and phospholamban (PLB) in regulating Ca^{2+} sensitivity, rate of tension development (K_{tr}), and the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) pump [66–70]. For example, phosphorylation of RLC and cMyBP-C are known to move the myosin head toward the actin binding site, increasing Ca^{2+} sensitivity and cross-bridge kinetics (▶ **Fig. 3**) with phosphorylation of RLC and cMyBP-C having the greatest effect on Ca^{2+} sensitivity and K_{tr}, respectively [68, 71–74]. It is unknown whether TRN increases K_{tr}, the content or phosphorylation of RLC or cMyBP-C. However, Diffee and Nagle [4] showed that TRN increased CM Ca^{2+} sensitivity at long but not short sarcomere lengths, which suggests that the effect is most noticeable when filament spacing is reduced. Phosphorylation of RLC is known to move the myosin filament closer to the actin filament increasing Ca^{2+} sensitivity, and this may be the mechanism by which TRN increases Ca^{2+} sensitivity. We observed TRN to have no effect on CM relaxation under basal conditions but accelerate relaxation in response to β-AR agonist [22]. In contrast, Kemi et al. [18] and Wisløff et al. [56] observed a TRN-induced acceleration of relaxation at all activation frequencies from 2 to 10 Hz, with HIIT having a greater effect than MICT. The accelerated relaxation was likely caused by a 25 % upregulation of SERCA2 and PLB (▶ **Fig. 3**) [56]. For the most part, the effects of TRN on key myofilament proteins (▶ **Fig. 3**) and how they alter biomechanical properties of the CMs is unknown.

Role of β-adrenergic agonist and adenosine in mediating TRN-induced adaptations in CM function

The heart is controlled by the autonomic nervous system with HR and contractility regulated by the degree of parasympathetic versus sympathetic tone. It is well established that TRN increases parasympathetic and decreases sympathetic tone in the resting individual, which contributes to the reduced resting HR [75, 76]. With the onset of exercise, sympathetic tone increases and acting primarily through β_1-AR increases PKA, which accelerates contraction and relaxation by phosphorylating cMyBP-C, troponin I, the ryanodine receptor and PLB (▶ **Fig. 3**) [68, 73, 75]. As reviewed above, sympathetic activation acting via the β_1-AR is important in the activation of the Ik_s channel and this response is down regulated by TRN [19]. This effect was not caused by a decline in β_1-AR content but rather to a reduced Ik channel protein and the kinase anchoring the protein Yotiao [25]. In the face of a maintained β_1-AR number, TRN upregulated the contractile response of CMs to adrenergic agonist [22]. As little as 5 nM of isoproterenol increased the amount of sarcomere shortening and relaxation rate, and the response was greater than that observed in the sedentary group (▶ **Fig. 4**) [22]. A possible explanation for the TRN-induced increase in the contractile response to adrenergic agonist is TRN downregulated β-adrenergic receptor kinase 2 (GRK2), which would reduce inactivation of the β-AR, thus maintaining higher PKA and contractility (▶ **Fig. 3**) [25]. The down regulation of GRK2 may be particularly important in heart failure, as lowering this protein has been shown to reverse dysfunction of both the β_1-AR and β_2-AR, and decrease the high sympathetic nervous system activity associated with heart failure [77].

Regulation of mammalian cardiac muscle contractility is complex and depends on multiple factors including the ratio of parasympathetic/sympathetic tone, and the content and activation of adrenergic and adenosinergic receptors [75, 76, 78]. Besides the three β-receptor subtypes (β_1-R, β_2-R, and β_3-R), mammalian CMs express four adenosine receptors A_1AR, A_{2a}AR, A_{2b}AR, and A_3AR [79]. The primary inotropic receptors are the β_1-R and the A_{2a}AR, and while both increase the extent and rate of CM shortening, only β_1-R increases the rate of CMs relaxation [8, 78]. Importantly, the extent of contractile enhancement resulting from β_1-R activation can be altered by adenosine receptor activation. For example, activation of A_1AR reduces CM contractility via an anti-adrenergic effect, while A_{2a} and A_{2b} both increase contractility (▶ **Fig.3**) [8, 78, 79]. The A_{2b}AR is thought to have a direct effect on the myofilaments while A_{2a}AR acts indirectly by modulating the A_1AR effects [79]. To our knowledge, there is no information on whether TRN alters the CMs response to adenosine, the interplay between adrenergic and adenosinergic stimulation or the AR content for any of the four receptor subtypes. These are important factors to consider, as TRN-induced changes in AR content or adenosine interaction with β_1-R activation would have direct effects on the K_{ATP} channel (A_1 and A_3) and contractility (A_1, A_{2a}, and A_{2b}) (▶ **Fig. 3**).

Mechanisms by which TRN protects the heart from ischemic injury and heart failure

Cellular effects of reperfusion injury and TRN-induced protection

It is well known that TRN protects the heart form ischemia and reperfusion (IR) injury, and while there are multiple theories on potential mechanisms, to date no unifying concept has emerged [3, 80, 81]. The degree of protection is related to the amount of activity, and while not definitively tested, HIIT seems to protect better than MICT [11, 12, 82]. The prognosis following an acute myocardial infarct (AMI) and the likelihood of progressing to heart failure is directly related to the extent of CM cell death [83]. Heart injury with AMI is exacerbated by IR, and *in vivo* and *ex vivo* animal models of IR show that TRN results in a 30 to 40 % reduction in IR-induced CMs cell death [81, 84, 85]. The observation that protection due to TRN exists in *ex vivo* hearts suggests that it is at least, in part, mediated by adaptations in cell/molecular factors innate to the heart. The cellular effects of IR have been extensively reviewed [3, 80, 81] and they include reduced ATP, increased glycolysis, low pH, increased production of reactive oxygen species (ROS), activation of Ca^{2+} activated proteases, impaired energy dependent ionic pumps, and increased intracellular Ca^{2+} (iCa^{2+}) and mitochondrial Ca^{2+} (mCa^{2+}); all of which could contribute to reduced cardiac function. IR injury has been attributed to increased ROS production, and the protective effects of TRN to increased production of

antioxidants that limit oxidative stress and damage to proteins, such as myofilaments and SR [3, 80, 86]. Clearly, ROS production can be an important mediator of IR injury, but disruption in iCa^{2+} regulation resulting in mCa^{2+} overload and apoptosis seems likely to be a key factor in orchestrating IR injury. Support for this idea comes from the demonstration that activation of the sK_{ATP} channel is critical to protecting the heart from IR injury and that the beneficial effects of TRN are in part due to the upregulation and activation of the sK_{ATP} channel [7, 33]. Knock-out or pharmacological inhibition of the sK_{ATP} channel eliminates the TRN-induced protection form IR injury [7, 33]. Presumably, protection from IR injury in hearts of trained individuals is initiated by the early and rapid repolarization of the sarcolemma that results from outward K^+ current through the sK_{ATP} channel closing the L-type Ca^{2+} channel and limiting Ca^{2+} influx (▶ **Fig. 3**) [25, 82]. Rat data suggests that TRN may induce greater protection from IR-injury in females than males, and that this relates to a greater incorporation of the sK_{ATP} channel subunits into the sarcolemma [87]. It has been suggested that this sex effect is mediated by PKC_ξ and that it can be blocked by ovariectomy and PKC_ξ blockers [53, 88].

Comparison of TRN with preconditioning/ postconditioning

Besides TRN, brief periods of ischemia and reperfusion, preceding deleterious IR (ischemic preconditioning, IPC) and at the onset of reperfusion (postconditioning, POC), have been shown to reduce IR-induced CM cell death [7, 87, 89, 90]. In 1986, Murry et al. [89] were the first to discover IPC, while Zhao et al. [90] discovered POC and compared it to IPC. All except TRN, have a limited period of protection; so while IPC and POC have clinical value, they cannot be used to limit heart damage due to AMI. The mechanisms by which these modalities protect the heart are not completely defined, but it seems likely that some of the same signaling pathways, substrates and enzymes induced by TRN may also be triggered by IPC and POC. Frasier et al. [81] suggests that while TRN and IPC may utilize some common features, the mechanisms are not the same, as IPC seems to involve the PI3K-Akt-GSK3β pathway, while increases in pAkt and pGSK3β are not involved in the TRN-induced protection from IR-injury. However, that conclusion may not be appropriate, as we noted above whereby TRN does increase pAkt and pGSK3β [39]. Thus protection could result from pGSK3β increasing the open probability of the sK_{ATP} channel, and by inhibiting ER protein inositol 1,4,5-trisphosphate receptor (IP_3R) release of ER Ca^{2+}, thus preventing mCa^{2+} overload [39–41]. The increased sK_{ATP} open probability could be aided by a TRN-induced increase in BDNF [42]. Support for this comes from the observation that BDNF knock-out mice showed increased CM cell death and left ventricular dysfunction following IR compared to the wild type control [91]. It is established that IPC and TRN have some overlapping signaling pathways, and evidence that they may converge on the same target in protecting against IR injury is based on the finding that IPC plus TRN did not produce greater protection than either treatment alone (unpublished data).

Importance of mitochondria associated membrane and its role in mediating TRN-induced cardioprotection from IR injury

While the causative events in IR- induced CM injury and death are not well established, mitochondria-triggered apoptosis is thought to be a final event leading to CM death [92, 93]. Important to the structural and functional integrity of the CM is the mitochondria-endoplasmic reticulum (SR/ER) network, also known as the mitochondria associated membrane (MAM). The MAM constitutes a complex of molecular tethers that associates the outer mitochondrial membrane (OMM) with the ER (▶ **Fig. 3**), which mediate interorganelle communications [93, 94]. Functionally, the MAM facilitates lipid and Ca^{2+} exchange between mitochondria and ER. This anatomical and functional coupling is the hub for the integration of mitochondrial function during normal cell physiology and the preservation of life during stress [95]. Mitochondria, are a major hub for Ca^{2+} handling and are central in energy metabolism, and the MAM domain provides a crucial link between ER Ca^{2+} signaling and the control of cellular energy demand by regulating mitochondrial bioenergetics [92, 94]. Dysregulation of ER-mitochondria (MAM) crosstalk is known to alter cardiac physiology and is implicated in cardiac IR injury. As noted above, and also well reported, one of the salient hallmarks of cellular damage by IR is iCa^{2+} and mCa^{2+} overload. Indeed, mCa^{2+} overload due to disruption of ER-mitochondria crosstalk in the MAM domain is implicated in the formation of the deleterious and permanent mitochondrial permeability transition pore (mPTP) opening and CM death [92, 93, 96, 97]. Deleterious mPTP opening following oxidative stress, mCa^{2+} overload or combination of both, has been reported as a trigger for IR injury or AMI [92, 98–101]. As alluded to previously, it is well known that the benefits of TRN encompass adaptations in heart and CM function as well as other systems that have secondary beneficial effects on cardiac efficiency [3, 10, 102, 103]. TRN is known to be a powerful way to protect the heart from ischemic stress [3] and while untested, our hypothesis is that TRN preserves MAM Ca^{2+} homeostasis and protects against mCa^{2+} overload to preserve mitochondrial function during IR. Therefore, TRN regulating ER/-mitochondrial Ca^{2+} homeostasis in the MAM domain could represent a novel feature, biochemically and biophysically, in mediating cardioprotection against injury by ischemic stress.

The MAM domain contains numerous transport proteins and signaling molecules that act as a platform for multiple physiological functions, as well as the regulation of cytosolic Ca^{2+} homeostasis. In the CM MAM domain, the chaperone protein glucose-regulated protein 75 (GRP75), the ER protein IP3R for Ca2 + release, and the voltage-dependent anion channel 1 (VDAC1) in the OMM form the IP3R-GRP75-VDAC1 complex that regulates the direct transfer of Ca^{2+} from the ER to mitochondria (▶ **Fig. 3**) and regulates cytosolic Ca^{2+} [96, 104–107]. Under normal physiological conditions, the ER-mitochondria interaction via domain $[Ca^{2+}]$ provides the necessary physiological coupling between muscle contraction/relaxation and the required mitochondrial ATP necessary for muscle function [108]. The MAM complex is also modulated biochemically by hexokinase II (HKII), and the signaling molecules Akt and the serine/threonine kinase GSK3β further regulate domain Ca^{2+} during IR injury or cytoprotection against IR stress [109–111]. Regarding cardioprotection, a crucial cell survival strategy involves HKII

association with VDAC1 [110, 112, 113]. For example, in cancer cells, HKII translocates at the MAM and its displacement from MAM triggers mCa^{2+} overload following IP3R opening [114, 115]. We also reported that hypothermic cardioprotection against acute IR led to increased Akt and phosphorylation of Akt, and HKII association with VDAC1 [112]. During cardiac ischemic stress, the increase in GSK3β activity leads to the phosphorylation of IP3R, and to the transfer of excess Ca^{2+} from the ER to mitochondria via the IP3R-GRP75-VDAC1 complex [116]. Furthermore, during ischemic stress, it is reported that GSK3β phosphorylation of VDAC1 reduces HKII binding to VDAC1 and abrogates its protection [92, 110, 117]. Thus, disruption of the ER-mitochondria interaction in the MAM region or inhibition of GSK3β during reperfusion has been shown to protect and preserve CMs from IR injury [107, 116, 118, 119]. Importantly, TRN has been shown to increase Akt activity and inhibit GSK3β activity by phosphorylation (pGSK3β), leading to stimulation of cardiac hypertrophy [10, 39]. As discussed above, TRN is known to activate the PI3K/Akt signaling pathway and increase pAkt [120–122]. Consistent with these findings, we have shown in pilot studies (unpublished data) that TRN increased total Akt (tAkt), phospho-Akt (pAkt), and pGSK3β after IR, suggesting that the TRN-induced inhibition of GSK3β may decrease the IP3R-induced aberrant MAM Ca^{2+} release. These observations strongly implicate the role of MAM proteins in modulating TRN-induced cardioprotection against IR-mediated dysregulation of Ca^{2+} dynamics and homeostasis. Whether TRN-induced protection against IR injury involves adaptation of MAM Ca^{2+} handling, and whether this adaptation leads to decreased mCa^{2+} overload by the activation of the PI3K/Akt/GSK3β signaling axis during ischemic stress remains to be fully explored. A better understanding of the underlying molecular mechanisms of how TRN regulates MAM Ca^{2+} handling, minimizes mCa^{2+} overload, and preserves mitochondrial function during IR stress represents an innovative approach that may contribute to the development of optimal TRN protocols that lead to long-term protection of cardiac viability and function.

Impact of life-long exercise on incidence of atrial fibrillation

Programs of regular exercise (TRN) are known to promote cardiovascular health, reduce conditions associated with heart disease, such as obesity and type 2 diabetes, and increase longevity [3, 10, 123]. Despite these known benefits, recently there has been considerable discussion on whether chronic life-long TRN can lead to detrimental cardiovascular effects and, in particular, to an increased incidence of atrial fibrillation (AF) [124–126]. The majority of AF patients are over the age of 65 [127], and interestingly, the incidence of AF has been reported to be 2 to 10-fold higher in life-long exercisers, with the risk increasing based on the total hours and number of years of TRN [123, 124]. This observation has led to the hypothesis that TRN beyond a certain threshold may not produce additional benefits and could be detrimental to cardiovascular health such that the benefits of life-long TRN may show a reversed j-shaped dose-response curve, where chronic moderate-intensity/duration TRN reduces the risk of AF and chronic high-intensity/duration TRN increases this risk [125, 128]. The causes for the age-related increase in AF are poorly understood, but a portion of the increase occurs in concert with other health issues, such as diabetes and coronary artery disease, that place the heart in a condition susceptible to arrythmias [129]. There are also changes in sarcolemma channel function with aging, such as a reduced L-type Ca^{2+} current and slowed conduction velocity, that might facilitate AF [129]. TRN reduces heart disease and diabetes, and has no effect on L-type Ca^{2+} channel [10, 65]. So the question is why does AF show a higher incidence with life-long TRN? This question remains unanswered, but its etiology is likely heterogeneous, i. e. relating to structural and electrophysiological changes [123, 126, 130]. TRN increases the ratio of parasympathetic/sympathetic tone and the size of all four chambers, and it has been suggested that either or both increase the risk of AF [123]. However, this cannot be the only explanation, as increases in vagal tone and heart hypertrophy occur early with the onset of TRN when there are no electrical changes in the heart or an increased risk for AF [126, 131]. TRN has been reported to increase left atrial (LA) volume more than left ventricular (LV) volume [126], and this disproportionate increase in LA volume was shown to be an independent predictor of AF [127]. Life-long TRN has also been shown to increase myocardial fibrosis and coronary artery calcification (CAC), factors linked to AF [1, 128]. Athletes with high life-long TRN volumes had higher CAC scores than those who TRN with low volume; however, the high volume group showed mostly calcified atherosclerotic plaques that were benign, with lower risk for cardiovascular disease, including AF [132]. While a TRN-induced increase in myocardial fibrosis seems to increase with the amount of TRN and could contribute to a slower conduction velocity, the incidence is low and its relationship to AF is unknown [128]. It seems reasonable to suggest that the combination of age-related changes in heart function coupled with the TRN-induced increase in vagal tone, LA volume, and myocardial fibrosis could contribute to the increased AF in individuals who maintain a high degree of TRN for multiple years. Support for this notion comes from the study of Wilhelm et al. [133], who stratified athletes according to training hours as low (< 1,500 hrs), medium (1,500 to 4,500 hrs), high (> 4,500 hrs), and very high (> 20,000 hrs) and observed a progressive increase with TRN hours in P-wave duration, LA volume, vagal tone, and premature atrial contractions.

The question remains, what is the primary driver for the increased incidence of AF with life-long high intensity TRN? We hypothesize that the TRN-induced changes in K^+ channel function, specifically a downregulation in the Ik_s and upregulation of the sK_{ATP} channels that allow the heart to adjust the APD to meet the metabolic demand and provide protection from ischemia (reviewed above), also contribute and may in fact be the primary drivers for the increased incidence of AF in older life-long exercisers. AF is characterized by a shortening of the APD and the atrial refractory period (ARP). Gonzalez et al. [134] showed that the Ik_s was markedly increased in chronic AF patients due to upregulation of β-AR, which contributed to the abbreviated APD and ARP and to the maintenance of AF. This mechanism cannot explain the increased incidence of AF in chronically TRN older adults compared to their sedentary counterparts, as TRN downregulates the Ik_s channel and its regulation by β-AR [25]. However, the increased AF in chronically TRN individuals could be mediated by the TRN-in-

duced increase in the sK_{ATP} channel. Balana et al. [135] hypothesized that an increase in the sK_{ATP} channel content or activation would reduce the APD and contribute to chronic AF. However, what they observed was the opposite as myocytes from AF patients showed markedly reduced sK_{ATP} channel density. The authors concluded that the downregulation of the sK_{ATP} channel was a secondary compensation mechanism to prolong the APD and ARP to help reduce AF. This protective mechanism would be muted in chronically TRN individuals where the sK_{ATP} channel is upregulated [22]. While the sK_{ATP} channel is not open during resting, non-stressed conditions in young individuals, this may not be true of older adults, where stress maybe increased and activate the channel even in a resting, non-exercising individual [124]. Additionally, TRN not only increases sK_{ATP} content but it may also increase the likelihood of sK_{ATP} activation (▶ **Fig. 3**).

It is important that life-long exercisers are aware of their increased susceptibility to AF so that they along with their family physician can track their heart health. It is also important to realize that despite an increased incidence of AF, life-long TRN reduces the risk of stroke and heart failure likely because of other beneficial effects of TRN, such as reduced diabetes, increased coronary circulation, and improved contractility [129]. The increased risk of AF is not a reason to become less active as data shows that for every MET (metabolic equivalent of task where one MET is the amount of energy used while sitting quietly) of exercise > 4 METS there is a 12–20 % reduction in cardiovascular mortality [123]. Simply put, life-long exercisers live longer than those with a sedentary life style.

Limitations in our current knowledge/future studies

While the value of TRN in promoting cardiovascular health is well documented, and major progress has been made in understanding the cellular and molecular mechanisms, considerable gaps in our knowledge still exist [10]. Relating to this review, the mechanism of the TRN-induced increased expression, sarcolemma incorporation, and activation of the sK_{ATP} channel is not well understood. The extent to which it involves the PI3K-Akt-GSK3β pathway producing an increase in pAkt and pGSK3β, increases in AMPK or BDNF, and/or adenosine regulation of the channel needs to be explored (▶ **Fig. 3**).

As reviewed above, TRN increases CM contractility, and this is, in part, due to an increased response to adrenergic agonist [22]. The regulation of CM contractility is complex, and almost nothing is known about how TRN alters adenosine's effect on contractility or its interaction with adrenergic activation. Down field from these events, it is unclear how TRN increases fiber ATPase and Ca^{2+} sensitivity or whether it alters the functional states of the cross-bridge or kinetics of tension development (k_{tr}) [136, 137]. Resolving these questions will require a detailed analysis of TRN-induced changes in content and phosphorylation level of the key contractile proteins, such as RLC, cMyBP-C, troponin, titin, etc. (▶ **Fig. 3**), and the ability to separate out how a change in each contractile protein alters function.

Another important unresolved question is understanding what role TRN plays in regulating MAM Ca^{2+} handling and minimizing mCa^{2+} during IR, and elucidating the mechanism of these effects. Finally, working out the mechanisms of how life-long TRN increases AF is an important yet difficult problem. Longitudinal studies assessing cardiac function are problematic and unlikely to provide definitive answers, and obtaining atrial tissue from sedentary and life-long exercisers in the appropriate numbers to study sarcolemma channel function will be difficult. A novel approach would be to produce iPSC-CM from skin, blood, or urine of sedentary and life-long exercisers. This approach would allow a detailed assessment of channel function and the role of the sK_{ATP} channel in the induction of arrythmias.

Conclusion

The beneficial effects of regular exercise (TRN) are well known and include both systemic and cellular adaptations. While controversy exists regarding the importance of HIIT versus MICT, the preponderance of evidence suggests that the former provides certain advantages to cardiac patients by inducing increases in left ventricular ejection fraction and isovolumetric relaxation, and by stimulating a greater increase in aerobic power. An important sarcolemma adaptation with TRN is the downregulation of the Ik_s and upregulation of the sK_{ATP} channels, which results in a prolonged APD at rest due to reduced Ik_s activation and current and a shortened APD with exercise/stress due to increased sK_{ATP} channel content and current. Important TRN-induced adaptations in contractility include a faster rise in the Ca^{2+} transient, and faster and greater CM shortening. The greater CM shortening occurred with no change in the amplitude of the Ca^{2+} transient suggesting that TRN increased Ca^{2+} sensitivity.

IR injury has been attributed to ROS production and the protective effects of TRN to increased production of antioxidants. While ROS production seems to be involved, disruption in iCa^{2+} regulation resulting in mCa^{2+} overload and apoptosis seems likely to be a key factor in orchestrating IR injury. Support for this notion comes from the well-established observation that the TRN-induced increase in the sK_{ATP} activation is critical in protecting the heart from IR injury and that blockage of this channel removes the protection. Opening of the sK_{ATP} channel depolarizes the CM and closes the L-type Ca^{2+} channel, reducing iCa^{2+} and mCa^{2+}. The TRN-induced increase in pGSK3β not only participates in the activation of the sK_{ATP} channel but may also decrease the IP3R-induced aberrant MAM Ca^{2+} release during IR and protect the mitochondria from Ca^{2+} overload and cell death. Finally, chronic life-long TRN is linked to a ~5-fold increase in AF compared to sedentary older adults. Importantly, despite this, life-long TRN is known to improve cardiovascular function, reduce IR injury, and promote longevity.

Conflict of Interest

The authors declare that they have no conflict of interest.

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