ATP-Sensitive K^+ Channel Knockout Compromises the Metabolic Benefit of Exercise Training, Resulting in Cardiac Deficits

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Exercise training elicits a metabolic and cardiovascular response that underlies fitness. The molecular mechanisms that orchestrate this adaptive response and secure the wide-ranging gains of a regimented exercise program are poorly understood. Formed through association of the Kir6.2 pore and the sulfonylurea receptor, the stress-responsive ATP-sensitive K^+ channels (K_{ATP} channels), with their metabolic-sensing capability and broad tissue expression, are potential candidates for integrating the systemic adaptive response to repetitive exercise. Here, the responses of mice lacking functional Kir6.2-containing K_{ATP} channels (Kir6.2-KO) were compared with wild-type controls following a 28-day endurance swimming protocol. While chronic aquatic training resulted in lighter, leaner, and fitter wild-type animals, the Kir6.2-KO manifested less augmentation in exercise capacity and lacked metabolic improvement in body fat composition and glycemic handling with myocellular defects. Moreover, the repetitive stress of swimming unmasked a survival disadvantage in the Kir6.2-KO, associated with pathologic calcium-dependent structural damage in the heart and impaired cardiac performance. Thus, Kir6.2-containing K_{ATP} channel activity is required for attainment of the physiologic benefits of exercise training without injury. *Diabetes* 53 (Suppl. 3): S169–S175, 2004

Physical exertion elicits an array of metabolic and cardiovascular responses that allow the body to adapt to the demands of exercise, thereby achieving fitness, a state of enhanced aerobic capacity, and generalized well-being (1–4). Reinforced by frequent repetition of the exercise regimen, the specific systemic benefits of regular activity range from reductions in body weight and adiposity to improved glucose and insulin homeostasis (1–7). Moreover, the inverse relation of physical exercise with both cardiovascular disease and mortality has long been recognized (1,4,8,9). Yet, the molecular determinants that orchestrate the adaptive response to exercise and that assure the wide-ranging gains of a regimented training program are poorly understood.

Unique metabolic-sensing capabilities and broad tissue expression point to the ATP-sensitive K^+ channel (K_{ATP} channel) as a potential candidate for integrating the systemic adaptive response and thereby securing the benefits of exercise training. K_{ATP} channels are evolutionarily conserved plasma-membrane protein complexes, widely represented in tissue beds with high metabolic activity (10–14). There, they are formed through the physical association of the inwardly rectifying potassium channel pore, most typically Kir6.2, and the regulatory sulfonylurea receptor subunit, an ATP-binding cassette protein (15–17). Energetic signals, received via tight integration with cellular metabolic pathways, are processed by the sulfonylurea receptor subunit that in turn gates the nucleotide sensitivity of the channel pore, thereby controlling membrane potential–dependent cellular functions (18–21). Recent findings, elicited from genetic disruption of channel proteins, have established in vivo the requirement of intact K_{ATP} channels in the proper function of the pancreatic β-cell, brain, and skeletal and/or cardiac muscle (22–30). Indeed, in the heart, where K_{ATP} channels were originally discovered (10), the traditional role for the channel complex as cardioprotective under ischemic insult has been confirmed (29,30), while new data further implicate the requirement of intact K_{ATP} channels for the cardiac adaptive response to acute adrenergic stress (31). Yet, in the absence of stress, mice lacking K_{ATP} channel activity do not appear appreciably impaired (22,32), underscoring the general role of this ion channel as an endogenous mediator of stress tolerance.

Here, the responses of mice lacking functional Kir6.2-containing K_{ATP} channels (Kir6.2-KO) were compared with wild-type controls following a 28-day endurance swimming protocol. While chronic aquatic training resulted in lighter, leaner, and fitter wild-type animals, the Kir6.2-KO manifested less augmentation in exercise capacity and lacked metabolic improvement. Moreover, the repetitive stress of swimming unmasked a survival disadvantage in the Kir6.2-KO, associated with pathologic calcium-depen-

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DCIP, 2,6-dichloroindophenol; K_{ATP} channel, ATP-sensitive K^+ channel; MEF, myocyte-enhancing factor; SDH, succinate dehydrogenase.

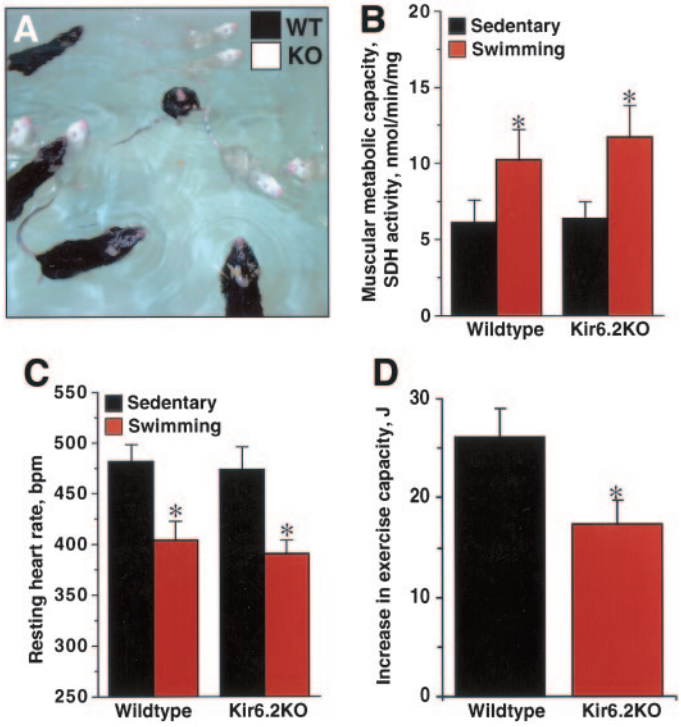

FIG. 1. Impaired exercise capacity in aquatic endurance-trained Kir6.2-KO. In wild-type (WT) and Kir6.2-KO (KO) mice, a 28-day swimming protocol (*A*) produced enhanced skeletal muscle metabolic capacity as measured by SDH activity (*B*) (P* < 0.05), a reduction in basal heart rate (*C*) (**P* < 0.01), and better performance on treadmill stress testing (*D*) (*P* < 0.05 from control; **P* < 0.05 vs. WT swim). Yet, Kir6.2-KO improved significantly less than WT (*D*) (*P* < 0.05).**

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RESEARCH DESIGN AND METHODS

Kir6.2-KO and swimming protocol. K_{ATP} channel–deficient mice (Kir6.2-KO) were generated by targeted disruption of the *KCNJ11* gene encoding the pore-forming Kir6.2 subunit and were backcrossed for five generations to a C57BL/6 background (22). Due to the proximity of the mutated *KCNJ11* gene with the gene encoding for albino hair color in the SV129 embryonic stem cells used to create the knockout, the Kir6.2-KO mice remain white upon back-breeding into the black C57BL/6 line (Fig. 1*A*). With approval of the Mayo Foundation Institutional Animal Care and Use Committee, 20-week-old male Kir6.2-KO or matched C57BL/6 wild-type mice underwent collective chronic swimming endurance training (33). Mice swam together in 20-cm-deep water (at 33–36°C) for 90 min twice daily for 28 days. Sedentary control mice were not swum. All mice were given standard rodent food ad libitum, housed 2–4 per cage with a 12-h day/night cycle, and observed daily throughout.

Succinate dehydrogenase. The activity of succinate dehydrogenase (SDH), an index of metabolic capacity, was determined by spectrophotometry in whole-tissue homogenates of hamstring muscle groups (34). The assay mixture contained 66.7 mmol/l Tris · HCl buffer (pH 8), 6.67 mmol/l KCN, 420 μmol/l phenazine methosulfonate, and 86 μmol/l 2,6-dichloroindophenol (DCIP), 1 μmol/l rotenone, and 10 mg/ml skeletal muscle extract. The reaction was initiated by 20 mmol/l succinate, and the rate of DCIP reduction was followed at 600 nm. SDH activity is expressed as nmol/l of reduced DCIP · min^{-1} · mg^{-1} of extract protein, with protein content determined by assay (Bio-Rad).

Treadmill. To assess the impact of swimming training on exercise capacity, a comparison of performance was made on a graded treadmill exercise test before and after the swimming protocol. Workload (J) was calculated as the sum of kinetic [$E_k = m \cdot v^2/2$] and potential ($E_p = m \cdot g \cdot v \cdot t \cdot \sin\theta$) energy, where m represents animal mass, v treadmill velocity, g acceleration due to gravity, t elapsed time at a protocol level, and θ is the angle of incline (31).

Histopathology. Cross-sectional area of individual adipocytes was measured on hematoxylin-eosin–stained, paraffin-embedded, formalin-fixed samples of gluteal subcutaneous white or intrascapular brown fat. Typically, 100 cells were measured from each of five mice from all groups. Skeletal muscle morphology was examined in hematoxylin-eosin–stained hamstring muscle sections by light microscopy. For measurement of cardiac contraction band necrosis, left ventricular hematoxylin-eosin–stained sections were examined at low and high magnification from five to six mice in each group (31). Cardiac and skeletal muscle ultrastructure was assessed in thin (90-nm) sections, cut on an ultramicrotome (Reichert Ultracut E), placed on 200-μm mesh copper grids, and stained with lead citrate. Transmitted electron microscopy was performed with a JEOL 1200 EXII electron microscope operating at 60 kV (31,35,36). All quantification was performed by an observer blinded to sample origin. For immunohistochemistry, formalin-fixed, paraffin-embedded left ventricular sections were deparaffinized with xylene and rehydrated in serial alcohol washes. To optimize antigen retrieval, sections were incubated in 0.5 mol/l NH_4Cl with 0.25% Triton X-100 for 30 min and then for an additional 30 min in 1 mmol/l EDTA in a pressure cooker. Primary rabbit and mouse polyclonal antibodies to the cardiac transcription factor myocyte-enhancing factor (MEF2C 1:300; Cell Signaling Technologies) and the cardiac sarcomeric protein (α-actinin 1:500; Sigma), respectively, were applied overnight. Accordingly, Alexa 563-labeled anti-mouse (1:200) and Alexa 488-labeled anti-rabbit (1:200) secondary antibodies (Molecular Probes) were applied for 30 min, along with nuclear counter-staining achieved by a 3-min application of 300 nmol/l 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) (Molecular Probes). Images were acquired by laser confocal microscopy (Zeiss LSM 510 Axiovert) as described (37).

Blood sampling. Plasma leptin levels were quantified by enzyme-linked immunosorbent assay (Crystal Chem). Blood glucose levels were measured in awake mice by tail sampling (OneTouch Ultra; Lifescan) at 8:00 A.M. ("fed") and the following day after a 16-h overnight fast ("fasting"). A glucose tolerance test was performed by the intraperitoneal administration of 1.5 mg/g glucose in fasting mice. In the insulin tolerance test, 0.2 units/kg human insulin was administered intraperitoneally in fed mice. All blood sampling was performed after completion of the 28-day swimming/sedentary protocol.

In vivo hemodynamics. Echocardiography with heart rate measurement (c256 and 15L8; Acuson) was performed in lightly sedated (1.25% isoflurane) mice at the end of the 28-day swimming/sedentary protocol. Images were digitally acquired and stored for offline blinded analysis. Echocardiographic measurements of left ventricular dimensions were recorded at end diastole (EDD) and end systole (ESD) from three consecutive cardiac cycles using the leading edge method (36,37). Left ventricular fractional shortening (%FS) was calculated as %FS = [(EDD − ESD)/EDD] × 100. Stroke volume was determined by the sum of aortic root cross-sectional area and the velocity time index integral, taken from peak Doppler tracings from flow across the aortic valve. The product of stroke volume and heart rate, expressed as ml · min^{-1} · 10 g^{-1} body wt, was used to calculate cardiac output.

Real-time quantitative PCR. Total RNA was extracted from left ventricles using the RNEasy Mini Kit (Qiagen). A MEF2C primer was used in real-time PCR analysis with forward 5′-AGATACCCACAACACACCACGCGCC-3′ and reverse 5′-ATCCTTCAGAGAGTCGCATGCGCTT-3′ sequences. Reverse transcription and quantitative PCR (qPCR) were preformed as described (37).

Statistical analysis. Comparisons within or between groups were performed by ANOVA, Student's t tests, or nonparametric tests as appropriate (JMP version 5.1; SAS, Carey, NC). Survival was determined by Kaplan-Meier analysis and the log-rank test. Data are presented as means ± SE; n refers to the sample size. $P < 0.05$ was predetermined.

RESULTS

After a 28-day swimming exercise protocol with equal participation of wild-type and Kir6.2-KO mice (Fig. 1*A*), both groups displayed physiologic changes that are typical indicators of training. All swum mice displayed enhanced skeletal muscle aerobic capacity, measured by SDH activity (Fig. 1*B*), lower resting heart rates (Fig. 1*C*), and superior performance on treadmill stress testing (Fig. 1*D*). Yet, Kir6.2-KO mice manifested only 66% of the improvement in exercise workload seen in wild-type mice ($P < 0.05$) (Fig. 1*D*).

While there was no difference in baseline body weight between groups ($P = 0.15$), in response to swimming, the wild-type mice became 14.1 ± 1.8% lighter while Kir6.2-KO mice demonstrated no loss in body weight (Fig. 2*A*). Over

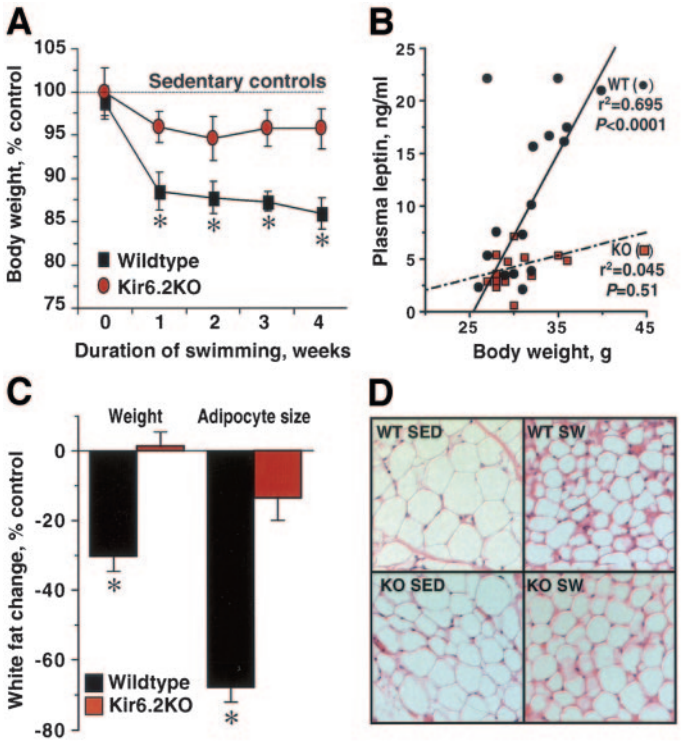

FIG. 2. Training-induced improvement in body weight and fat distribution absent in Kir6.2-KO. In contrast to wild-type mice (WT), the Kir6.2-KO mice (KO) failed to lose body weight with swimming training (*A*) (*$P < 0.001$) and displayed less leptin/body weight correlation (*B*). Only WT demonstrated measurable reductions in epididymal white fat deposits (*C*) and a decline in white adipocyte size (*C* and *D*) ($P < 0.01$). SED, sedentary; SW, swimming.

this range of body weights, the level of leptin, the metabolic hormone linked to K_{ATP} channel activity (38,39), correlated with body mass in the wild-type mice, whereas there was less correlation in mice lacking K_{ATP} channels (Fig. 2*B*). This differential response to training was mirrored by changes in body fat distribution. Wild-type mice demonstrated measurable reductions in white fat stores (Fig. 2*C*), a decline in white adipocyte size (Fig. 2*C* and *D*), and a reciprocal increase in brown adipocyte size (37.9 ± 14%, $P < 0.05$). Kir6.2-KO mice had no significant changes in white fat weight (Fig. 2*C*) or in the size of either white (Fig. 2*C* and *D*) or brown adipocytes (−7 ± 13%, $P = 0.63$).

Swimming training elicited a significant reduction in fasting blood glucose in wild-type mice (−29 ± 7% from wild-type sedentary controls, $P < 0.02$) (Fig. 3*A*), an effect not experienced in mice lacking K_{ATP} channels (12 ± 12% from Kir6.2-KO sedentary controls, $P = 0.35$) (Fig. 3*A*). Furthermore, all wild-type mice had a normal response to glucose tolerance test (Fig. 3*B*), while the glucose intolerance of sedentary Kir6.2-KO mice (22) was not improved by exercise training (Fig. 3*B*). Swimming training in wild-type mice did not affect the glucose response to insulin tolerance test (Fig. 3*C*), whereas the abnormal exaggerated glucose-lowering effect of insulin challenge that occurs in Kir6.2-KO (22) was further magnified in the swum Kir6.2-KO (Fig. 3*C*).

In addition to the lack of these wide-ranging metabolic benefits, participation in the swimming protocol by Kir6.2-KO was achieved only at the substantial cost of myocellular damage (Fig. 3*D*). In contrast to wild-type skeletal muscle that did not display signs of injury following completion of the swimming regimen (not illustrated), hematoxylin-eosin–stained hamstring muscles of the swum Kir6.2-KO compared with sendetary counterparts showed areas of myocyte degeneration with vacuolar destruction and scattered necrosis on light microscopy (Fig. 3*D–F*). Typical for areas of damage, muscle fibers from the swum Kir6.2-KO had evidence of early regenerative changes characterized by cells with basophilic cytoplasm, large nuclei, and prominent nucleoli (Fig. *3D* and *E*), findings that were confirmed by the presence of abnormal internalized nuclei on electron microscopy (Fig. 3*G*).

Kir6.2-KO mice completing the swimming protocol demonstrated impaired cardiac contractile function with a significant reduction in left ventricular fractional shortening (Fig. 4*A*) and an impaired cardiac output on echocardiography (Fig. 4*B*). While in the absence of stress mice lacking K_{ATP} channel activity had a normal survival, even the relatively modest stress imposed by the repetitive physical exertion of the swimming program induced a significant mortality in the Kir6.2-KO mice (Fig. 4*C*) with death occurring during exercise or suddenly in the immediate postexercise period. This was not seen in wild-type mice (not illustrated). Underlying the poor cardiac contractility, Kir6.2-KO hearts following swimming were larger, as measured by heart and left ventricular mass (Fig. 5*A*), and had pathologic evidence of myocyte damage (Fig. 5*B*). Unlike the wild-type mice (zero of five; $P < 0.002$), five of six Kir6.2-KO hearts displayed focal areas of contraction band necrosis, consistent with cytosolic calcium loading (31,40) seen on both light and electron microscopy (Fig. 5*B*). Left ventricular tissue extracts taken from swum Kir6.2-KO demonstrated an increased expression of MEF2C (Fig. 5*C*), a critical calcium-dependent transcription factor that when activated translocates to the nucleus, where it initiates embryonic gene reprogramming and pathologic cardiac hypertrophy (33,41,42). Here, nuclear localization of MEF2C was tested for in left ventricular tissue by in situ immunostaining. Unlike those in wild-type mice, hearts lacking Kir6.2-containing K_{ATP} channels exhibited nuclear localization of MEF2C (Fig. 5*D*). Thus, K_{ATP} channel activity is required not only for both the adaptive response to exercise and the attainment of the physiologic benefits of exercise training, but also for the ability to execute these necessary responses without acquiring myocellular deficits and cardiac dysfunction.

DISCUSSION

While it is well recognized that the modest stress imposed by an exercise program elicits an adaptive metabolic and cardiovascular response that underlies fitness (1–4), the mandatory molecular components required for the execution of this process are less established. Here, genetic deletion of Kir6.2 hindered the attainment of the wide-ranging metabolic benefits of chronic aquatic endurance training and rendered the mice vulnerable, unmasking a survival disadvantage in the Kir6.2-KO that was associated with pathologic calcium-dependant structural damage in the heart and an overall impaired cardiac performance. Thus, intact K_{ATP} channels are required elements in the hierarchy of systemic adaptation to exercise training.

Regular physical activity results in reduced body weight

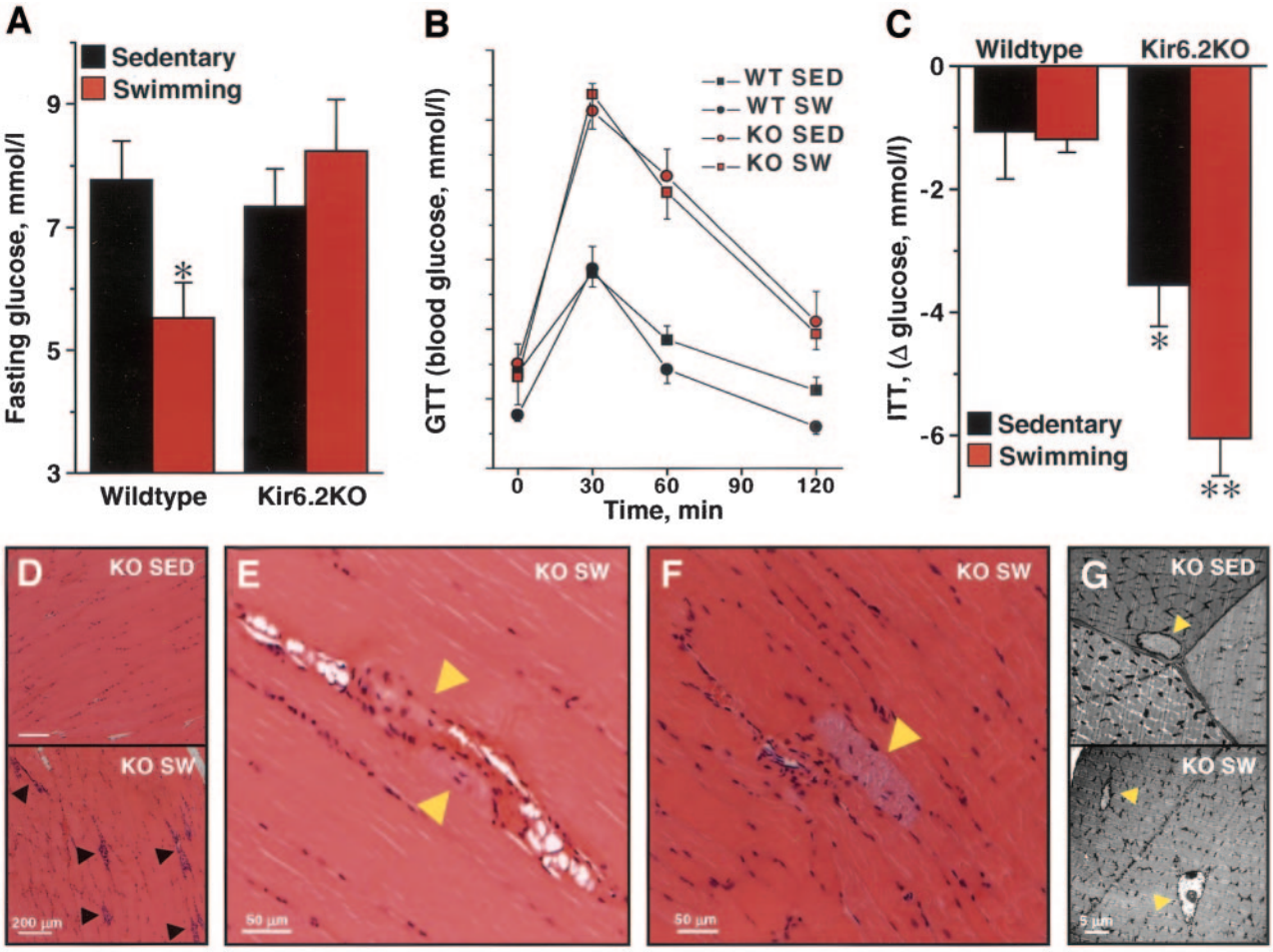

FIG. 3. Kir6.2-KO lack metabolic benefits of physical activity and develop skeletal muscle damage. The swimming protocol elicits a significant reduction in fasting blood glucose in wild-type mice (WT) ($P < 0.05$), an effect not experienced in the Kir6.2-KO mice (KO) (*A*). All WT have a normal response to a glucose tolerance test (GTT), while the glucose intolerance of Kir6.2-KO is not improved by exercise training (*B*). SED, sedentary; SW, swimming. Training exaggerated the abnormal drop in glucose following an insulin tolerance test (ITT) in Kir6.2-KO, without affecting WT (*C*) (*$P < 0.05$ from baseline, **$P < 0.05$ between Kir6.2-KO groups). Participation in swimming resulted in myocellular damage to the Kir6.2-KO (KO SW), with focal areas of myocyte degeneration and vacuolar destruction not observed in the sedentary (KO SED) on histology (arrows in *D–F*). Kir6.2-KO had cells with basophilic cytoplasm, large nuclei, and prominent nucleoli (*D* and *E*), findings typical of early regenerative changes confirmed by abnormal central internalized nuclei on electron microscopy (*G*, *lower panel*) compared with the normal peripheral location in the KO SED (*G*, *upper panel*). In *G*, arrows indicate nuclei.

and fat (4,7,43). Indeed, repetitive aerobic exercise leads to an increased aerobic capacity that brings an enhanced energetic demand and, in response, increased energy production in part through a breakdown of fat stores. A central systemic mechanism controlling body weight is the hormone leptin, produced by adipocytes, that acts in the hypothalamus, where K_{ATP} channel activity has been implicated as the molecular end point of leptin signaling (38,39). In this study, weight loss, due predominantly to reductions in adipocyte size, was a demonstrable feature of a chronic exercise regimen in wild-type mice. In contrast, Kir6.2-KO mice apparently lacked a leptin/body weight association and did not have a loss of body weight or fat following the swimming protocol. Thus, absence of K_{ATP} channel activity may affect appropriate weight control under a stress regimen.

The beneficial effects of physical activity on metabolism are well described, with measurable reductions in insulin resistance, glucose intolerance, and postprandial hyperglycemia (1–7). Wild-type mice with normal glucose homeostasis demonstrated an improvement in fasting glycemia upon completion of the swimming program. Adult Kir6.2-KO mice, which lack K_{ATP} channels in the pancreatic β-cell, hypothalamus, and skeletal muscle, have a disrupted glucose-insulin axis with normoglycemia at baseline but glucose intolerance in response to glucose challenge and aggravated hypoglycemia due to altered insulin sensitivity (22,27,32,44). Here, despite participation in the exercise program, Kir6.2-KO mice lacked the capacity to improve glucose control under the challenge of glucose or insulin, emphasizing the critical role played by K_{ATP} channel activity in the systemic regulation of glucose homeostasis in physiological stress.

Overuse of skeletal muscle, especially with repetitive exercise, may lead to damage, reflected here by focal myocyte necrosis and disorganized myofibrils followed by fiber regeneration. The source of this regeneration is believed to be the satellite cells, which become activated upon injury or overuse, proliferating and fusing to form new muscle fibers (45). In the current experiments, unlike either sedentary Kir6.2-KO or sedentary and swum wild-type mice, Kir6.2-KO mice showed signs of muscle injury and regeneration following the swimming program, supporting a cytoprotective role for K_{ATP} channels in skeletal

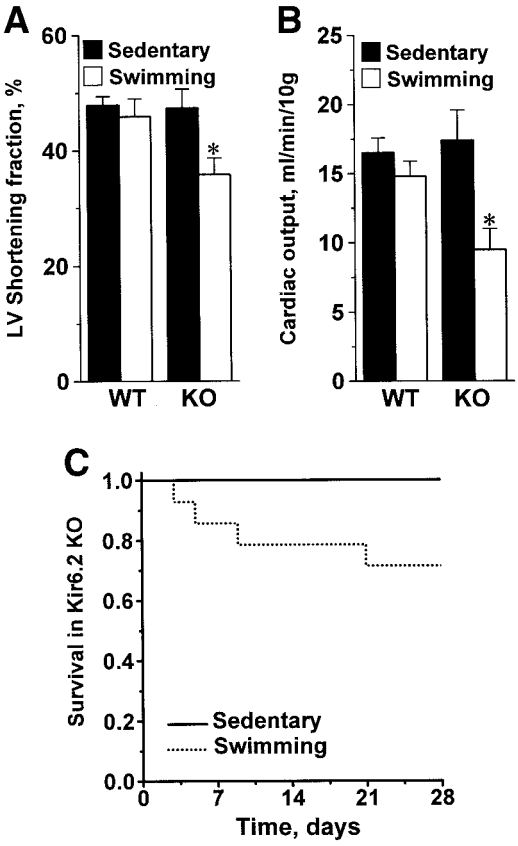

FIG. 4. Cardiac dysfunction and survival disadvantage in exercised Kir6.2-KO. After 28 days of the swimming protocol, Kir6.2-KO had impairment in cardiac function with reduced left ventricular shortening fraction (*A*) ($P < 0.02$) and cardiac output (*B*) ($P < 0.01$) compared with wild-type or unstressed Kir6.2-KO mice. Indeed, swimming induced a significant survival disadvantage in Kir6.2-KO over sedentary controls (*C*) ($P < 0.02$).

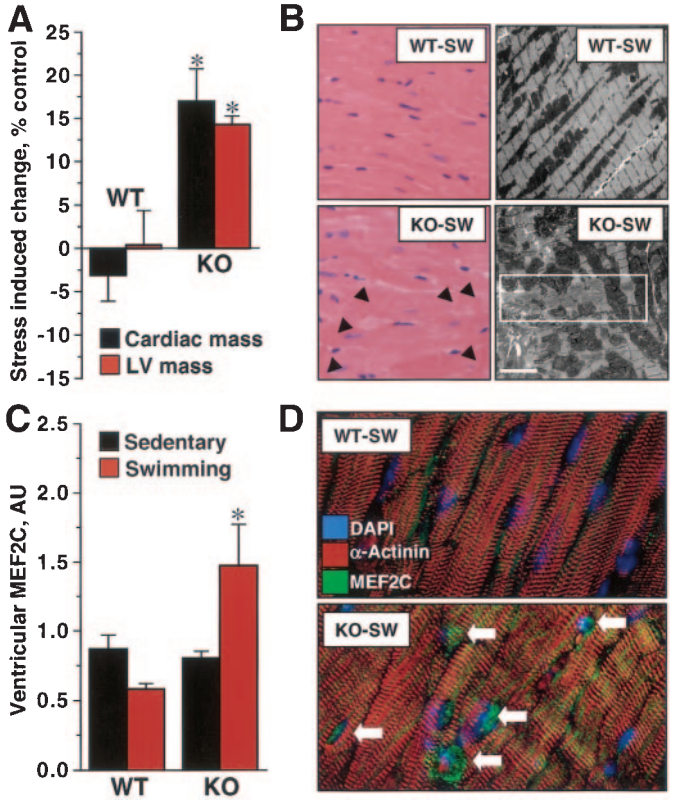

FIG. 5. Structural cardiac deficits in Kir6.2-KO with hypertrophy and focal contraction band necrosis following swimming. Unlike wild-type hearts (WT), Kir6.2-KO hearts (KO) displayed enlarged cardiac and left ventricular (LV) mass to swimming stress (*A*), as well as focal areas of contraction band necrosis seen on light (indicated by arrows in *B*) and electron (box in *B*) microscopy. SED, sedentary; SW, swimming. There was an increased expression of the transcription regulator of pathologic cardiac hypertrophy, MEF2C, in left ventricular tissue extracts (*C*), with evidence of nuclear localization of MEF2C on immunostaining confirmed by colocalization with the nuclear marker DAPI in α-actinin–stained tissue sections (*D*).

muscle under repetitive exercise stress (46). Indeed in heart muscle, in reaction to acute metabolic stresses such as hypoxia, ischemia, or adrenergic challenge, opening of myocardial K_{ATP} channels allows shortening of action potential duration, reducing energy demanding calcium influx and potentially toxic cytosolic calcium overload (29–31). Here, 1 month of a relatively modest swimming exercise protocol, insufficient to induce demonstrable physiologic cardiac hypertrophy in the wild-type mice, was tolerated extremely poorly by Kir6.2-KO mice. Underlying the observed survival disadvantage was a structurally damaged Kir6.2-KO heart with impaired contractile function. The contraction band necrosis present on left ventricular pathology was evidence for cytosolic calcium overload (40), a finding further supported by increased calcium-dependent hypertrophy in Kir6.2-KO hearts. There is growing evidence that K_{ATP} channels function to limit pathological structural remodeling in the heart under stress (47), with the recent identification in humans that defective channels induced by mutations in *ABCC9*, the gene encoding the cardiac sulfonylurea receptor subunit, confer susceptibility to heart failure (48). While the cause of death in the swum Kir6.2-KO mice was not established in this study, it is likely influenced by the observed cardiac deficits, which potentially formed a substrate for ventricular arrhythmia, to which calcium-loaded Kir6.2-KO stressed hearts are predisposed (31,49).

In conclusion, the findings in this study underscore the systemic advantage of Kir6.2-containing K_{ATP} channel activity in the response to the physiologic stress of a repetitive exercise protocol. Functional K_{ATP} channels are required not only for both the response to exercise and the attainment of the physiologic benefits of training, but also for the ability to adequately respond without acquiring muscular deficits.

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