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## Neuronal Nitric Oxide Synthase is Indispensable for the Cardiac Adaptive Effects of Exercise

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### Abstract

**Rationale**—Exercise results in beneficial adaptations of the heart that can be directly observed at the ventricular myocyte level. However, the molecular mechanism(s) responsible for these adaptations are not well understood. Interestingly, signaling via neuronal nitric oxide synthase (NOS1) within myocytes results in similar effects as exercise.

**Objective**—Thus, the objective was to define the role NOS1 plays in the exercise-induced beneficial contractile effects in myocytes.

**Methods and Results**—After an 8 week aerobic interval training program, exercise-trained (Ex) mice had higher  $VO_{2max}$  and cardiac hypertrophy compared to sedentary (Sed) mice. Ventricular myocytes from Ex mice had increased NOS1 expression and nitric oxide production compared to myocytes from Sed mice. Remarkably, acute NOS1 inhibition normalized the enhanced contraction (shortening and  $Ca^{2+}$  transients) in Ex myocytes to Sed levels. The NOS1 effect on contraction was mediated via greater  $Ca^{2+}$  cycling that resulted from increased phospholamban phosphorylation. Intriguingly, a similar aerobic interval training program on NOS1 knockout mice failed to produce any beneficial cardiac adaptations ( $VO_{2max}$ , hypertrophy, and contraction).

**Conclusions**—These data demonstrate that the beneficial cardiac adaptations observed after exercise training were mediated via enhanced NOS1 signaling. Therefore, it is likely that beneficial effects of exercise may be mimicked by the interventions that increase NOS1 signaling. This pathway may provide a potential novel therapeutic target in cardiac patients who are unable or unwilling to exercise.

### Keywords

myocyte;  $Ca^{2+}$ ; sarcoplasmic reticulum; high intense treadmill training; nitric oxide

## INTRODUCTION

Regular exercise has many beneficial effects [33], including direct adaptations of the heart that result in greater aerobic fitness ( $VO_{2max}$ ), physiological hypertrophy, enhanced contraction, and accelerated relaxation. These adaptations have clinical implications as exercise has been shown to decrease the risk for the development of cardiomyopathies [37].

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DISCLOSURES

None

In addition, exercise is used as a rehabilitation tool for cardiac patients to improve the quality of life and to decrease mortality [63]. These intrinsic exercise-induced cardiac adaptations can be observed at the myocyte level [25]. For example, ventricular myocytes from exercise-trained mice exhibit increased size, greater contraction due to enhanced sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  cycling, and faster contraction-relaxation rates. Previous studies have proposed that the contractile adaptations of exercise result from increases in SR  $\text{Ca}^{2+}$ -ATPase (SERCA) expression [23, 24]. However, it has been shown that the exercise-induced cardiac adaptations are still present in SERCA knockout mice [9], suggesting that mechanisms other than SERCA must also contribute to the beneficial cardiac adaptations of exercise.

In skeletal muscle, exercise training has been shown to increase nitric oxide (NO) produced via neuronal NO synthase (NOS1) [56]. Furthermore, exercise increased heart NOS1 expression in a nitric oxide-deficient hypertension model [19]. NOS1 is also constitutively expressed within ventricular myocytes, and its signaling leads to enhanced contraction and accelerated relaxation via increased SR  $\text{Ca}^{2+}$  cycling [1, 28, 58, 60]. As such, the contractile effects of NOS1 signaling are similar to the contractile adaptations of exercise. However, the contribution of NOS1 signaling to exercise-induced cardiac myocyte adaptations has not been investigated.

Therefore the purpose of the study was to assess whether NOS1 signaling contributes to the exercise-induced adaptations in the myocyte. We hypothesize that exercise training will 1) increase NOS1 expression levels and NO bioavailability, and 2) increase contraction and accelerate relaxation via enhanced SR  $\text{Ca}^{2+}$  cycling due to amplified NOS1 signaling in isolated ventricular myocytes.

## MATERIALS AND METHODS

To achieve exercise induced adaptations, C57Bl/6 (wildtype, WT), NOS1 knockout (KO) mice and canines underwent a treadmill training protocol (Table S1 and supplementary material) [18]. The effects of exercise were confirmed by  $\text{VO}_{2\text{max}}$  testing [61]. Post training, ventricular myocytes were isolated and  $\text{Ca}^{2+}$  transient and shortening were simultaneously measured as previously described [32, 58]. Inhibition of NOS1 was obtained by incubating isolated myocytes for 60 minutes with a specific NOS1 inhibitor, S-methyl-L-thiocitrulline (SMLT) [10]. Western blot analysis were performed with homogenized ventricular myocytes to measure NOS1 protein expression (Santa Cruz, Santa Cruz, CA) and PLB phosphorylation (Badrilla, Leeds, UK), as previously described [58]. NO bioavailability was measured via DAF-2 AM fluorescence. Data are presented as mean $\pm$ SEM. Differences between two groups were evaluated for statistical significance ( $P < 0.05$ ) by unpaired Student's *t* tests or by one way or two way ANOVA for multiple groups.

## RESULTS

### Exercise induced adaptations

Following an 8 week high intense, aerobic interval treadmill training (Table S1), exercise-trained (Ex) mice (C57Bl/6) had elevated  $\text{VO}_{2\text{max}}$  levels and physiological hypertrophy (Table 1) as compared to sedentary control (Sed) mice. Therefore, the training protocol had achieved a state of fitness and produced a “physiological” ventricular hypertrophy.

### Exercise increases myocyte NOS1 protein expression, NO bioavailability, and contraction/relaxation

Exercise training elicited a significant increase in NOS1 expression in isolated ventricular myocytes from Ex mice compared to Sed mice (Fig 1A). This was accompanied by an

increase in NO production in Ex myocytes (Fig 1B), that could be normalized by acute NOS1 inhibition (SMLT, 10  $\mu$ M). Our data suggest that in Sed myocytes that NOS1 accounts for ~35% of the cardiomyocyte NO production, while it increases to ~60% in Ex myocytes.

In agreement with previous studies [23, 24, 26], Ex myocytes also exhibited a greater contraction (observed as increased  $\text{Ca}^{2+}$  transient and shortening amplitudes) and a faster relaxation (measured as time to 50% relaxation,  $\text{RT}_{50}$ ) (faster  $\text{Ca}^{2+}$  transient and relengthening  $\text{RT}_{50}$ , Fig 1C-G). Remarkably, acute NOS1 inhibition (+SMLT) normalized Ex myocyte contraction ( $\text{Ca}^{2+}$  transients and cell shortening amplitude) and  $\text{Ca}^{2+}$  decline to Sed myocyte levels (Fig 1D-F). In addition, Ex myocytes had a greater functional response ( $\text{Ca}^{2+}$  transient and shortening amplitudes) to  $\beta$ -adrenergic receptor ( $\beta$ -AR) stimulation with isoproterenol (ISO) (Fig 1H), consistent with previous studies in animals and humans [34, 41, 53, 55]. As with basal contraction, acute NOS1 inhibition (+SMLT) abolished the increased functional response to low dose ISO in Ex myocytes. At higher doses of ISO with acute NOS1 inhibition (+SMLT), there was still a greater functional response in the Ex compared to Sed myocytes. We believe this is due to the increased expression of  $\text{G}\alpha_{15}$ . The force-frequency relationship (FFR), another important regulator of contraction [20, 42], was also enhanced via Ex and normalized with acute NOS1 inhibition (Figs S1). These data are consistent with the effects of Ex [26, 62] and NOS1 signaling [28, 58] on FFR.

### Effects of Ex and NOS1 signaling on SR $\text{Ca}^{2+}$ cycling

Since exercise enhances SR  $\text{Ca}^{2+}$  cycling [25], SR  $\text{Ca}^{2+}$  load and SR  $\text{Ca}^{2+}$  fractional release (twitch  $\text{Ca}^{2+}$  amplitude / SR  $\text{Ca}^{2+}$  load) were examined. Ex myocytes had both an increased SR  $\text{Ca}^{2+}$  load (similar to a previous study [54]), and an increased SR  $\text{Ca}^{2+}$  fractional release compared to Sed myocytes. These differences were normalized by acute NOS1 inhibition (Fig 2A and 2B). SR  $\text{Ca}^{2+}$  cycling was further examined with post-rest potentiation, which is the enhanced contraction following a rest period (Fig S2). Ex myocytes reached a greater  $\text{Ca}^{2+}$  transient and shortening amplitude compared to Sed on the first twitch following short periods of rest, which was also normalized by acute NOS1 inhibition (Fig S2). Thus, the Ex-induced increases in myocyte SR  $\text{Ca}^{2+}$  cycling resulted from enhanced NOS1 signaling.

Accelerated SR  $\text{Ca}^{2+}$  uptake occurs via increasing phosphorylation of phospholamban (PLB) [47, 48]. Previous studies have shown that Ex [36, 39] and NOS1 signaling increase PLB phosphorylation [58, 66]. In the present study, Ex myocytes (compared to Sed myocytes) had increased PLB serine16 phosphorylation, which is the PKA site, but not threonine17 phosphorylation, the CaMKII site (Sed:  $0.24 \pm 0.03$ , Ex:  $0.27 \pm 0.07$  A.U.). Once again, acute NOS1 inhibition normalized Ex serine16 phosphorylation to Sed levels (Fig 2C). There was no effect of NOS1 inhibition on threonine17 phosphorylation (Sed+SMLT:  $0.28 \pm 0.05$ , Ex+SMLT:  $0.28 \pm 0.05$  A.U.). Thus, these data suggest that the molecular mechanism responsible for the enhanced SR  $\text{Ca}^{2+}$  cycling induced by exercise is a NOS1 mediated increase in PLB phosphorylation.

The increase in serine16 phosphorylation with Ex suggest a change in PKA and/or phosphatase activity. Hence, we investigated the roles of PKA and phosphatase 1 and 2a on Ex and Sed myocyte contraction. Interestingly, contraction in Ex myocytes was normalized with either phosphatase inhibition (okadaic acid, OA) or PKA inhibition (PKI) (Fig 3). Thus, these data suggest that exercise shifts the kinase/phosphatase balance in myocytes.

### Effects of training NOS1 deficient mice

Since acute NOS1 inhibition reversed the adaptive contractile effects of exercise in WT mice, we examined the effects of Ex in a model deficient in NOS1. Thus, the cardiac effects of a similar exercise training protocol were evaluated in NOS1 knockout (KO) mice (Table S1). Notably, Ex-NOS1KO mice did not exhibit any of the cardiac adaptations that were observed in WT mice. That is, Ex-NOS1KO mice did not have increased  $VO_{2max}$  or hypertrophy compared to Sed-NOS1KO mice (Table 1). Furthermore, contraction, FFR, and post-rest potentiation were not increased in Ex-NOS1KO myocytes (Fig 4 and S3). Surprisingly, we observed  $Ca^{2+}$  mismanagement in the Ex-NOS1KO myocytes. That is, Ex-NOS1KO myocytes had significantly decreased  $Ca^{2+}$  transient amplitudes, SR  $Ca^{2+}$  loads, and slowed  $Ca^{2+}$  transient  $RT_{50}$  (Fig 4). Thus, exercise training did not produce beneficial cardiac adaptations in NOS1KO mice and actually caused deleterious effects on myocyte  $Ca^{2+}$  handling.

### Effects of Ex and NOS1 on canines

It is well documented that contractility is different between rodents and larger mammals (e.g., dog, humans). Therefore, we evaluated the contribution of the NOS1 signaling pathway to the cardiac adaptive response to exercise training in a canine model (see Supplementary material). Acute NOS1 inhibition (SMLT) had similar effects in isolated canine myocytes as were noted for murine myocytes (Fig 5). These data suggest a more universal role for exercise induced enhancement of NOS1 signaling in both large and small mammalian species.

### Enhanced role of NOS1 signaling with Ex

Since NOS1 inhibition normalized contractile function in Ex myocytes to Sed levels, the role of NOS1 with Ex was further investigated. NOS1 signals, in part, via peroxynitrite [31, 58]. FeTPPS, a peroxynitrite decomposition catalyst, had a similar effect on contraction as NOS1 inhibition in both Ex and Sed myocytes (Fig S4). However, the functional effects of FeTPPS were much greater in Ex vs Sed myocytes (Fig 6). This is consistent with the contractile effects of NOS1 inhibition (SMLT in murine and canine myocytes), PKA inhibition with PKI, and phosphatase inhibition with OA (smaller effect consistent with the kinase/phosphatase shift) (Fig 6). NOS1 inhibition also had a greater effect on its downstream target PLB. Specifically, NOS1 inhibition resulted in a greater decrease in serine16 PLB phosphorylation in Ex vs Sed myocytes ( $-0.40 \pm 0.05$  vs  $-0.18 \pm 0.03$  A.U.,  $P < 0.05$ ). Thus, these data suggest that NOS1 and its downstream signaling targets are the major mediators in the enhanced contraction and accelerated  $Ca^{2+}$  decline produced by exercise training.

## DISCUSSION

The molecular mechanism(s) by which the heart adapts to regular exercise is/are not completely known. This study demonstrates that NOS1 signaling plays an essential role in these adaptations via: 1) increased  $VO_{2max}$ , hypertrophy, and contraction in WT but not NOS1KO mice; 2) increased ventricular myocyte NOS1 expression and NO production; and 3) normalization and greater contractile effects of inhibiting NOS1 (and its downstream targets) in Ex myocytes.

### The role of NOS1 signaling in the exercise induced beneficial cardiac adaptations

The health benefits of exercise and the adverse consequences of inactivity are widely recognized. Regular exercise is associated with a lower risk for both cardiovascular and non-cardiovascular disease and increases the quality of life [33, 37, 63]. It may then be possible

to design therapies (either specific exercise protocols or pharmacological targets) that maximize the beneficial aspects of exercise. Thus, it is imperative that the molecular mechanisms responsible for the beneficial cardiac effects of exercise are identified.

Exercise training produces physiological hypertrophy, enhances  $VO_{2max}$  and myocyte contraction in both mice [26] and humans [63]. In the present study, in marked contrast to WT mice, aerobic interval training did not elicit these adaptations in NOS1KO mice (Table 1, Fig 1 & 4). While there are many contributing factors to  $VO_{2max}$ , it is mainly limited by maximal stroke volume [2]. Endogenous NO is able to modulate stroke volume [45]. Thus, the decreased myocyte contraction caused by NOS1 ablation may have contributed to the lack of improvement in trained NOS1KO. It is widely accepted that physiological hypertrophy is dependent upon the insulin-growth factor/PI3K/AKT pathway [5]. Our current data suggests that NOS1 is also essential for physiological hypertrophy. Interestingly, AKT has been shown to phosphorylate NOS1 in the brain [11], suggesting that NOS1 may be downstream of AKT. Future studies (e.g., IGF1/PI3K/AKT and calcineurin/NFAT pathways) are warranted to investigate the mechanism(s) for the lack of exercise induced hypertrophy in the NOS1KO mice. Overall, our observations strongly suggest that the NOS1 signaling pathway plays an essential role in cardiac adaptation to exercise.

Increased SR  $Ca^{2+}$  cycling and greater fractional shortening are also widely accepted as an adaptive response to exercise [25, 54] (Fig 1-3, 5). Previous studies focused on exercise-induced changes in excitation-contraction coupling protein profiles (i.e. SERCA2a) [24, 36, 39, 52]. The present study expands these findings, demonstrating that the NOS1 signaling pathway is also a key component in the exercise-induced contractile adaptations (Fig 1, 5 and 6). NOS1, which is localized to the SR, modulates SR  $Ca^{2+}$  cycling by targeting PLB (Fig 2). Studies have shown that increased PLB phosphorylation is responsible for the exercise induced increase in SR  $Ca^{2+}$  cycling [36, 39]. Our results extend this observation by demonstrating that enhanced NOS1 signaling is responsible for this effect. We believe that the molecular mechanism for the increased phosphorylation is a shift in the kinase/phosphatase balance (Fig 3) These data are consistent with previous reports that demonstrated increased PKA activity with Ex [54], while NOS1 signaling can activate PKA [31, 66] and inhibit phosphatase activity [66]. While Ex has been shown to increase NOS3 expression [14], NO produced via NOS3 does not contribute to the faster relengthening or the other observed contractile effects reported in the present study since NOS3 only modulates contractile function during  $\beta$ -AR stimulation and not basal function [38, 59]. Indeed, basal NO bioavailability was similar between Ex and Sed myocytes after NOS1 inhibition (Fig 1B). Taken together, these data suggest that exercise training increases contraction and accelerates  $Ca^{2+}$  decline at baseline due to augmented NOS1 and its downstream targets (Fig 6).

### Training NOS1 deficient mice results in $Ca^{2+}$ mishandling

Not only did exercise training fail to induce adaptive cardiac effects ( $VO_{2max}$  and hypertrophy) in the NOS1KO mice, but actually provoked detrimental actions on myocyte  $Ca^{2+}$  handling (Fig 4). As this is a global knockout, it is difficult to delineate the mechanism(s) responsible for this adverse cardiac response to exercise training. For example, the contribution of NO produced via NOS1 in the skeletal muscle cannot be overlooked, as demonstrated in other disease models [7]. In fact, mice deficient in NOS1 have increased skeletal muscle fatigue due to an immediate decrease in vasodilation after brief, mild exercise even though the strength of the muscle was sustained [30]. However, NOS1 signaling may not limit flow during sustained exercise [6]. As such, an increased vulnerability to fatigue could have reduced the exercise capacity (i.e., weekly training speeds) and thereby, potentially limiting the exercise-induced cardiac adaptations. However, this seems unlikely as training intensity was matched to similar levels in both the WT and

NOS1KO mice (i.e., mice were trained at ~90% of the speed of the peak oxygen consumption ( $VO_{2\text{peak}}$ ) (Fig S5). Since exercise induced cardiac adaptations are dependent upon training intensity [26], similar intensities between groups one would expect that the exercise training effects should yield similar cardiac adaptive responses in both WT and NOS1KO mice, regardless of any potential skeletal muscle fatigue. However, only the WT mice exhibited exercise-induced enhancement on  $VO_{2\text{max}}$ .

In a similar manner, NOS1 signaling also increases coronary blood flow [51]. Thus, NOS1KO mice may not have been able to increase coronary blood flow to match the increased myocardial oxygen consumption during exercise, resulting in ischemia and enhanced superoxide production [40]. Hence, implementing the 8 week training protocol in NOS1KO mice may have potentially exacerbated the already high cardiac superoxide levels [27, 29, 65] culminating in deleterious effects on SR  $Ca^{2+}$  cycling. While our data provide evidence that NOS1 is indispensable for the adaptive cardiac effect of exercise, other studies have shown that exercise results in other beneficial adaptations to the heart such as electrical remodeling (e.g.,  $K^+$  channels, L-type  $Ca^{2+}$  channel) and the mitochondria [22, 64]. While it's known that NO is able to modulate various ion channels and mitochondria function [3, 68], future studies are warranted to determine if electrical remodeling and mitochondrial adaptations with exercise still occur in NOS1KO mice.

### Beneficial effects of exercise

By understanding the natural/innate mechanisms by which a normal heart is remodeled into an athlete's heart, one might gain the knowledge necessary to design therapies to heal the diseased heart. Alternates to exercise must be identified to treat those cardiac patients that are mentally unwilling or physically unable to exercise. This might be achieved by pharmacological approaches to target the beneficial signaling pathways that are activated by exercise training in order to mimic "exercise in a pill" [12, 46]. One could term this new-age approach: Exercise-Like Induced Medicine (ELIM). Our results showing that NOS1 is indispensable for the beneficial cardiac effects of exercise and earlier studies demonstrating a cardioprotective role of NO [13, 16, 17, 44, 50] would suggest that manipulation of NOS1 protein expression or activation of components of the NOS1 signaling pathway might represent one such pharmacological approach. In this regards, it is interesting that studies have shown that NOS1 knockout mice do worse (adverse remodeling, contractility, mortality) after myocardial infarction [8, 49] while NOS1 overexpression is protective after ischemia/reperfusion, transverse aortic constriction, and heart failure [4, 35, 43]. It should be noted that the Burkard et al (2010) study [4] showed that NOS1 overexpression resulted in decreased contraction (opposite of our current results). We believe that this is due to the overexpressed NOS1 being localized to the caveolae (instead of the SR). Studies have shown although NO is a diffusible gas, its signaling is highly compartmentalized [67]. Thus, since the overexpressed NOS1 is at the caveolae, its negative effect on contraction is consistent with that of NOS3, which is also localized to caveolae and cardioprotective [21, 57, 59]. These results further suggest that NOS1 overexpression may mimic the beneficial effects of exercise and could potentially be a novel therapeutic (ELIM) treatment.

In conclusion, as far as we are aware, the present study is the first to show that NOS1 signaling contributes to the adaptive cardiac effects of exercise. Specifically, exercise increases ventricular myocyte NOS1 expression and NO bioavailability, which is essential for aerobic fitness, hypertrophy and enhanced contraction/relaxation.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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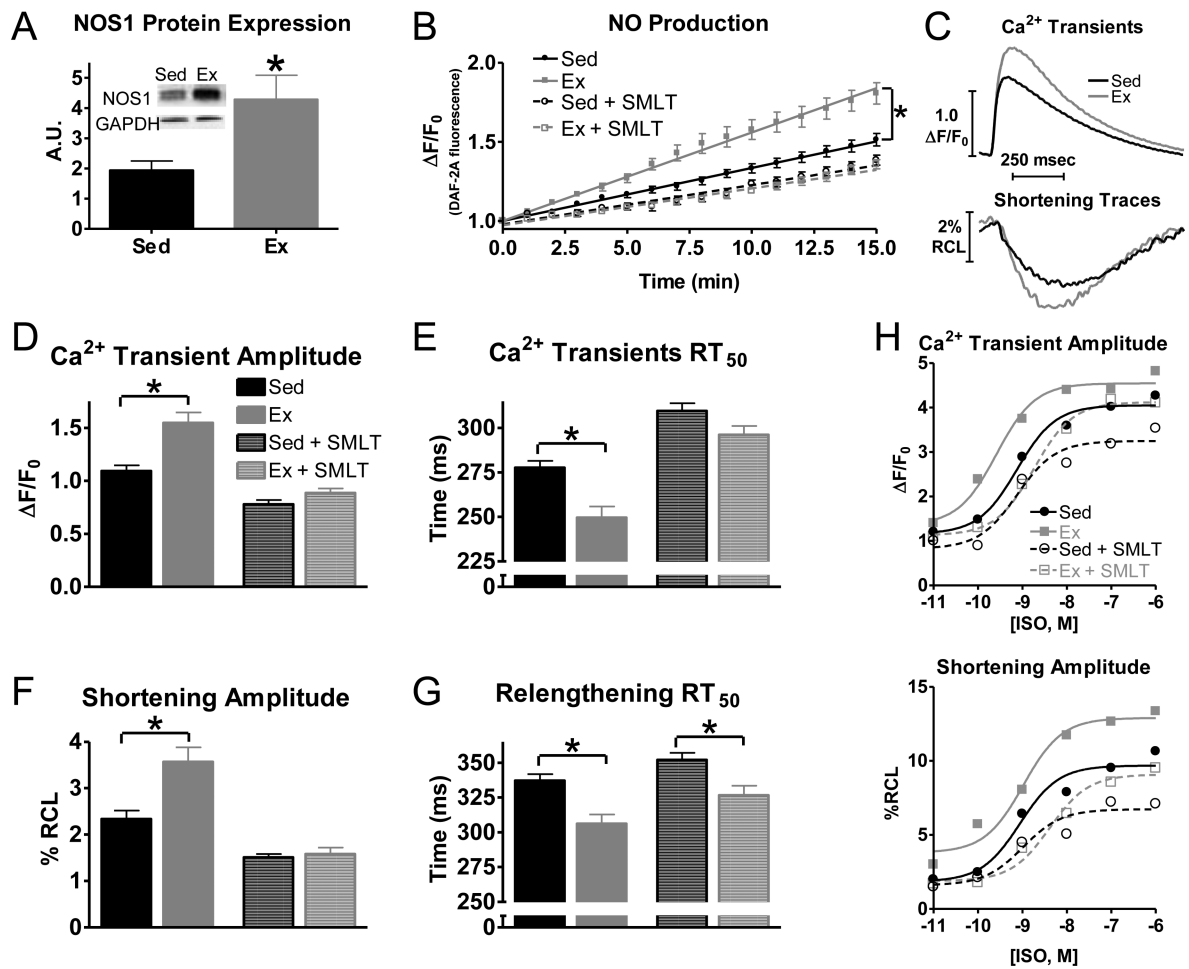
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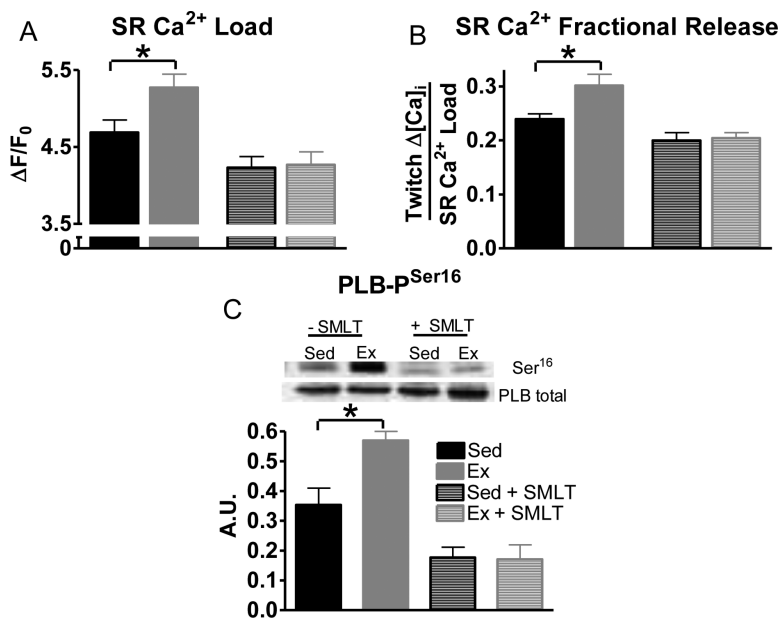
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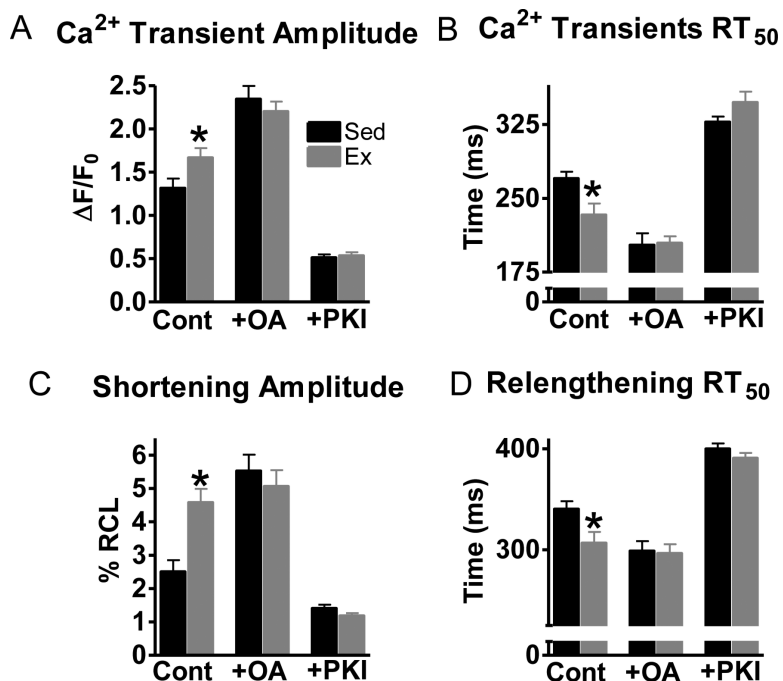
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**Fig 1.**

Exercise increases myocyte NOS1 expression, nitric oxide (NO) production and enhances myocyte contraction, which is normalized by acute NOS1 inhibition. A) Summary data of NOS1 protein expression (A.U.–arbitrary units), n=12 mice/group. B) Summary data of NO production over a 15 minute time period ( $\pm$ acute NOS1 inhibition, SMLT, dashed), n=19-26 cells/3 hearts. C) Representative traces of Ca<sup>2+</sup> transients and shortening. Summary data of Ca<sup>2+</sup> transient amplitudes (D), Ca<sup>2+</sup> decline to 50% of its peak (RT<sub>50</sub>)(E), shortening amplitudes (F), and relengthening RT<sub>50</sub> (G) in exercise (Ex-grey) and sedentary (Sed-black)myocytes ( $\pm$ acute NOS1 inhibition, SMLT, striped), n=39-42 cells/7-10 hearts, (H) Summary data of Ca<sup>2+</sup> transient (top) and shortening (bottom) amplitudes to various concentrations of ISO, n=12-39 myocytes/4 hearts, \**P* < 0.05 vs corresponding Sed.

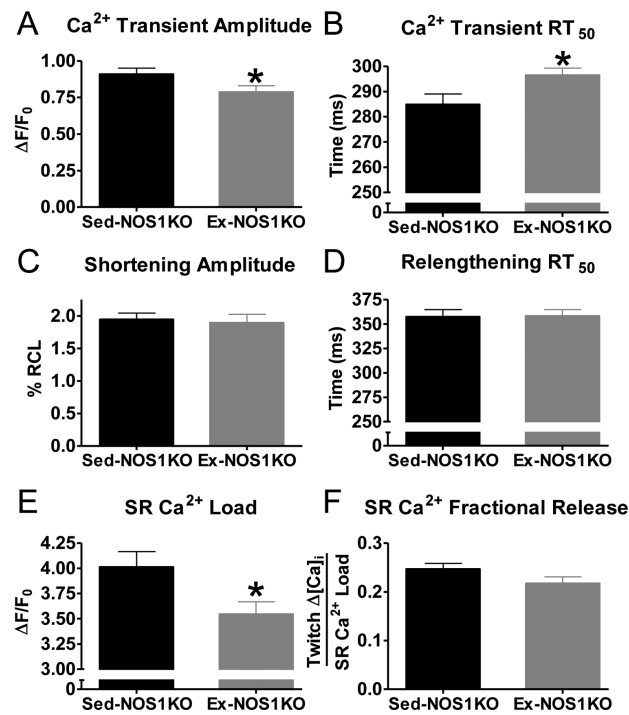


**Fig 2.** Exercise enhances SR Ca<sup>2+</sup> cycling and is normalized by acute NOS1 inhibition. Summary data of SR Ca<sup>2+</sup> load (A) and SR Ca<sup>2+</sup> fractional release (B), n=26-35 cells/11-12 hearts. C) Summary data of PLB Serine16 phosphorylation (n=6 hearts) in exercise (Ex-grey) and sedentary (Sed-black) myocytes (±acute NOS1 inhibition, SMLT, striped), \*P< 0.05 vs corresponding Sed.



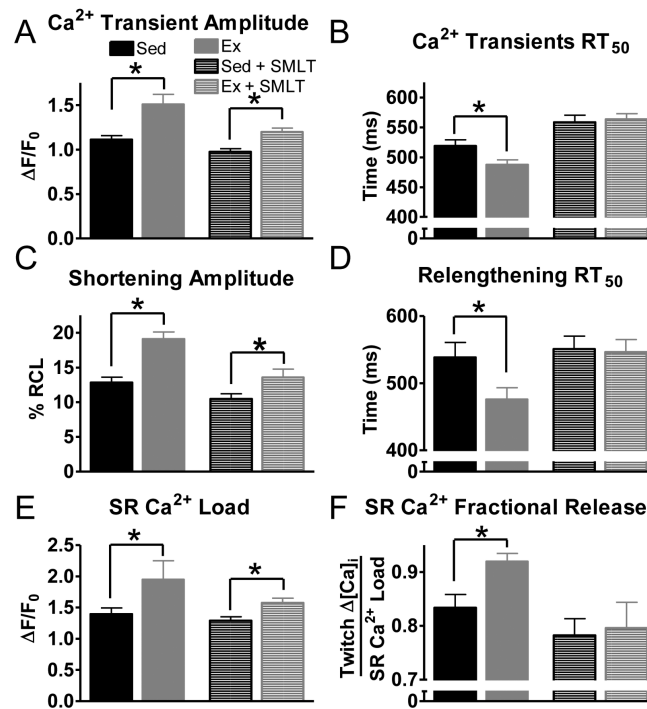
**Fig 3.** Protein phosphatase 1 and 2a inhibition (okadaic acid, OA) and PKA inhibition (PKI) abrogated the contractile difference between Sed and Ex myocytes. Summary data of Ca<sup>2+</sup> transient amplitudes (A), Ca<sup>2+</sup> transient RT<sub>50</sub> (B), shortening amplitudes and (C), relengthening RT<sub>50</sub> (D) in exercise (Ex-grey) and sedentary (Sed-black) myocytes. n=10-22 cells/4 hearts, \*P< 0.05 vs corresponding Sed.

## NOS1KO



**Fig 4.** Negative effects of exercising on Ca<sup>2+</sup> handling in NOS1 deficient myocytes. Summary data of Ca<sup>2+</sup> transient amplitudes (A), Ca<sup>2+</sup> transient RT<sub>50</sub> (B), shortening amplitudes (C), relengthening RT<sub>50</sub> (D), SR Ca<sup>2+</sup> load (E), and SR Ca<sup>2+</sup> fractional release (F) in exercise (Ex-NOS1KO-grey) and sedentary (Sed-NOS1KO-black), n=22-42 cells/5-6 hearts, \*P < 0.05 vs corresponding Sed.

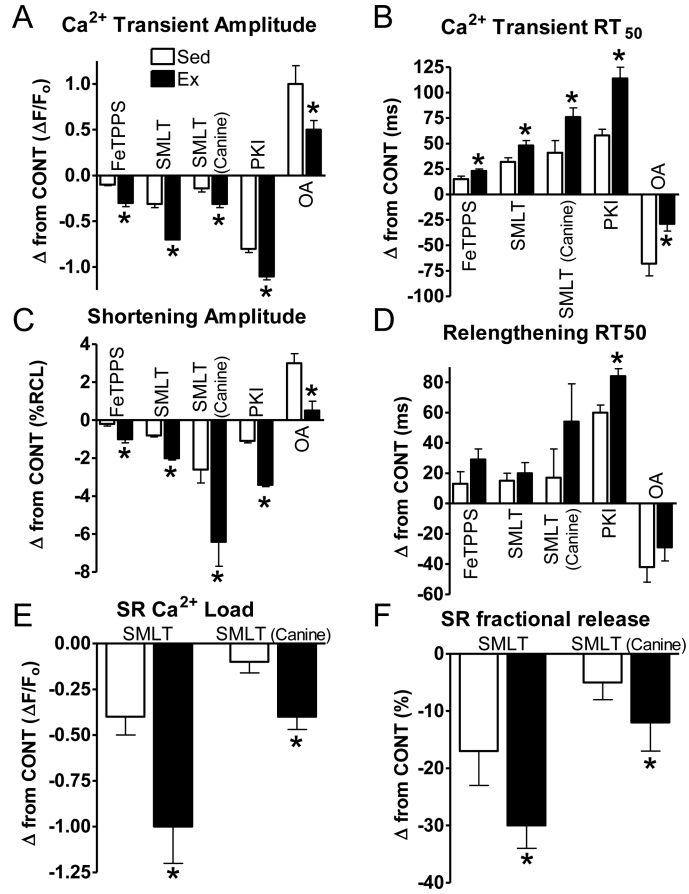
## Canine



**Fig 5.**

Exercise in a trained canine model elicited similar results with NOS1 inhibition on myocyte contraction. Summary data of Ca<sup>2+</sup> transient amplitudes (A), Ca<sup>2+</sup> transient RT<sub>50</sub> (B), shortening amplitudes (C), relengthening RT<sub>50</sub> (D), SR Ca<sup>2+</sup> load (E), and SR Ca<sup>2+</sup> fractional release (F) in exercise (Ex-grey) and sedentary (Sed- black), ( $\pm$ acute NOS1 inhibition, SMLT, striped), n = 10-33 cells/3-4 hearts, \* $P < 0.05$  vs corresponding Sed.





**Fig 6.** NOS1 and its downstream signaling targets have a greater effect with exercise. Summary data shown as change from control of Ca<sup>2+</sup> transient amplitudes (A), Ca<sup>2+</sup> decline to 50% of its peak (RT<sub>50</sub>) (B), shortening amplitudes (C), and relengthening RT<sub>50</sub> (D), SR Ca<sup>2+</sup> load (E), and SR Ca<sup>2+</sup> fractional release (F) in exercise (Ex-grey) and sedentary (Sed-black) myocytes, \*P < 0.05 vs corresponding Sed.

**Table 1**

Summary data of maximal oxygen consumption ( $VO_{2max}$ ) (n=4-6 mice), heart weight to body weight ratio and heart weight to tibia length (n=12 mice) in exercise (Ex) and sedentary (Sed) WT and NOS1KO mice.

	Sed	Ex	Sed-NOS1KO	Ex-NOS1KO
Heart weight (mg)	121 ± 2	129 ± 3 *	112 ± 3	111 ± 4
Body weight (g)	22.2 ± 0.4	20.4 ± 0.2 *	21.8 ± 0.5	20.2 ± 0.5 *
Heart to body ratio (mg/g)	5.5 ± 0.1	6.3 ± 0.1 *	5.2 ± 0.1	5.5 ± 0.2
Tibia length (mm)	22.0 ± 0.1	21.9 ± 0.1	21.0 ± 0.2	21.2 ± 0.4
Heart to tibia ratio (mg/mm)	5.8 ± 0.1	6.2 ± 0.2 *	5.4 ± 0.1	5.2 ± 0.2
$VO_{2max}$ (mL/kg <sup>-0.75</sup> /min)	52.7 ± 2.9	62.0 ± 1.4 *	50.4 ± 2.4	51.1 ± 1.6

\*  $P < 0.05$  vs corresponding control.