Prévention par l'exercice des effets délétères d'une exposition au CO sur l'homéostasie calcique cardiomyocytaire : implication dans la sensibilité à l'ischémie-reperfusion

Exercise prevents impaired Ca^{2+} handling in heart of CO exposed rat: implication for sensitivity to ischemia-reperfusion

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III. Etude n°3

1. Résumé article 3

1.1. Contexte scientifique :

La plus grande sensibilité du myocarde à l'IR est expliquée par le remodelage phénotypique délétère des cardiomyocytes de rats exposés pendant 4 semaines au CO (Etude n°1). Ce remodelage est notamment caractérisé par une moindre capacité de défense enzymatique antioxydante et par une surcharge calcique plasmatique cardiomyocytaire. La pratique régulière d'exercice à intensité modérée est une stratégie de prévention largement reconnue aujourd'hui comme permettant notamment d'améliorer la protection du muscle cardiaque contre la survenue d'événements pro-oxydants aigus (French et al., 2006 ; French et al., 2008 ; Kavazis et al., 2008 ; Powers et al., 2008). Par ailleurs, l'exercice est reconnu comme permettant d'améliorer le pronostic vital cardiaque dans le cadre du développement de cardiomyopathies, notamment via une normalisation de l'homéostasie calcique cardiomyocytaire (French et al., 2008 ; Kemi et Wisloff, 2010). Le développement d'un phénotype cellulaire de type pathologique semblant secondaire à l'augmentation du stress oxydant dans notre modèle. L'objectif de cette étude n°3 était de répondre aux interrogations suivantes:

Quels sont les effets protecteurs d'un entraînement en endurance sur le remodelage phénotypique cardiomyocytaire associé à une période d'exposition prolongée au CO ? Cette stratégie préventive influence–t'elle la sensibilité du myocarde, de rats exposés au CO, au syndrome d'IR ?

1.2. Méthodologie :

Pour répondre à ces questions, une troisième population de rats entraînée pendant 4 semaines avant d'être exposée au CO (rats CO-Ex) a été ajoutée aux deux groupes de rats sédentaires (rats Ctrl et rats CO). Suite aux différents protocoles d'exposition et/ou d'entraînement, une évaluation du statut enzymatique antioxydant et des mouvements calciques intracellulaires au cours du couplage excitation-contraction a été réalisée. Enfin, afin d'évaluer l'implication des différentes modifications phénotypiques sur la sensibilité du cœur à l'IR, un protocole d'IR régionale sur cœur isolé perfusé était conduit.

1.3. Résultats majeurs :

Le résultat majeur de notre étude est la prévention, par l'entraînement régulier à intensité modérée conduit préalablement à l'exposition au CO, du développement d'un phénotype cellulaire cardiaque pathologique. En effet, les conséquences délétères d'une exposition au CO sur l'activité enzymatique antioxydante sont prévenues par l'exercice en endurance. De plus, un tel entraînement permet également d'inhiber les effets délétères du CO sur l'expression de SERCA-2a et ainsi de préserver l'homéostasie calcique cellulaire chez les rats exposés au CO. Enfin, ces effets bénéfiques de l'exercice, rapportés à l'étage cellulaire,

ont permis de normaliser la sensibilité du myocarde au syndrome d'IR chez la population de rats CO.

L'entraînement à intensité modérée permet de prévenir le développement d'un phénotype cardiomyocytaire pathologique chez la population de rats exposés au CO, et ainsi de normaliser la sensibilité myocardique au syndrome d'IR.

2. Article n°3 :

Exercise prevents impaired Ca^{2+} handling in heart of CO exposed rat: implication for sensitivity to ischemia-reperfusion

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Abstract

Sustained urban carbon monoxide (CO) exposure exacerbates heart vulnerability to ischemia reperfusion via deleterious effects on the antioxidant status and Ca^{2+} homeostasis of cardiomyocytes. The aim of this work was to evaluate whether moderate exercise training prevents these effects. Wistar rats were randomly assigned to control group and to CO groups, living during 4 weeks in simulated urban CO pollution (30-100 ppm, 12 hours/day) with (CO-Ex) or without exercise (CO-Sed). Exercise procedure began 4 weeks before CO exposure and was maintained twice a week in standard filtered air during CO exposure. On a first set of rats, myocardial ischemia (30 min) and reperfusion (120 min) were performed on isolated perfused rat hearts. On another set of rats, myocardial antioxidant status and Ca^{2+} handling were evaluated following environmental exposure. Exercise training prevented CO-induced myocardial phenotypical changes. Indeed, exercise induced myocardial antioxidant status recovery in CO exposed rats, which is accompanied by normalization of SERCA-2a expression and then of Ca^{2+} handling. Importantly, in CO exposed rats, the normalization of cardiomyocytes phenotype with moderate exercise was associated with restored sensitivity of the myocardium to IR. Indeed, CO-Ex rats present a lower infarct size and a significant decrease of reperfusion arrhythmias when compared to their sedentary counterparts. To conclude, moderate exercise, by preventing CO-induced Ca^{2+} handling and myocardial antioxidant status alterations, reduces heart vulnerability to IR.

Keywords: Myocardial infarction, Environmental pollution, Endurance training, antioxidant status

1. Introduction

The severity of myocardial infarction results from a complex interplay between genetic, pathological and environmental factors (20, 24). Among environmental factors, numerous epidemiological studies have demonstrated that carbon monoxide (CO) pollution correlated with hospital admissions for cardiovascular diseases (3, 15), as well as cardiovascular mortality (25). We recently reported that sustained low level CO exposure, similar to that found in an urban environment, induced a pathological remodelling of the myocardium (1) rendering the heart more vulnerable to ischemia-reperfusion (IR) (16). This remodelling involves a marked alteration of enzymatic antioxidant status associated with marked changes in Ca^{2+} handling (1), promoting cardiomyocyte death and severe ventricular arrhythmias (16).

Today, amongst numerous cardioprotective strategies used to prevent deleterious myocardial remodelling associated with several pathological states, and/or to reduce the vulnerability of the heart to acute ischemic events, regular endurance exercise training is reported as one of the most practicable and sustainable methods (2, 7, 11, 22, 23). Although mechanisms responsible for exercise-induced cardioprotection remain unclear, many studies suggest that increased enzymatic antioxidant status plays an important role (7, 10, 32). In addition, exercise training is also reported to normalize Ca^{2+} homeostasis in the pathological myocardium (26, 29). Therefore, we have hypothesized that regular exercise training started prior to sustained CO exposure could prevent pathological cardiac remodelling and/or modulate heart vulnerability to IR.

The aim of this study was to evaluate potential cardioprotective effects of regular bouts of endurance training in an experimental rat model exposed to

simulated sustained urban CO pollution. Especially, we focused on the effects of exercise training on Ca^{2+} handling, myocardial enzymatic antioxidant status alterations and consequences on heart vulnerability to IR. The major results showed that regular bouts of endurance training prevented the pathological cardiac remodelling and the higher vulnerability to IR of the hearts of rats exposed to chronic CO.

2. Methods

All investigations complied with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publications No. 85-23, revised 1996) and with the approval of the French Ministry of Agriculture. All experiments have been approved by the local research ethics committee (Comité Régional d'Ethique).

2.1. Animals

Adult male Wistar rats (n=57; 384±8 g; Charles Rivers Laboratories) were randomly assigned to three experimental groups: 1) sedentary control group (Ctrl-Sed rats), 2) sedentary CO exposed group (CO-Sed rats), 3) trained CO exposed group (CO-Ex rats). All experimental groups were maintained on a 12:12 hour light-dark cycle and provided rat chow and water *ad libitum*.

2.2. Exercise training protocol

Exercise training protocol was performed on a motor driven treadmill for 4 weeks, 5 days/week at a relative work rate corresponding to 50 % of maximal aerobic velocity (20 m/min; 40 min/day). During the 4 following weeks, corresponding to CO exposure period, to preserve exercise training benefits, exercise training was maintained in CO-Ex rats on a frequency of 2 days/week.

2.3. Carbon monoxide exposure

CO groups were exposed to simulated CO urban pollution for 4 weeks, 12 hours/day during the night phase. CO rats were housed in an airtight exposure container and exposure was performed as follows: i) During CO exposure, a CO concentration of 30 ppm was maintained in the airtight container and monitored with an aspirative CO analyzer (CHEMGARD Infrared Gas Monitor NEMA 4 Version, MSA), this initial concentration was completed with five 1 hour peaks at 100 ppm CO; ii) During ambient air exposure, animals were placed in the laboratory animal-house at a CO concentration of 0 ppm. Throughout this CO exposure period, Ctrl-Sed rats were confined in the laboratory animal-house and were manipulated daily. At the end of the 4 weeks of CO exposure, the rats were housed for 24 hours in standard filtered air before euthanasia in order to avoid acute effects of CO on the myocardium.

2.4. Ca^{2+} handling in cardiomyocytes

In the first set of rats (n=4/group), evaluation of exercise training and CO exposure on excitation-contraction was performed on single ventricular cardiomyocytes isolated by enzymatic digestion (19). Unloaded cell shortening and Ca^{2+} concentration (Indo-1 dye) were measured using field stimulation (0.5 Hz, 22°C, 1.8 mM external Ca^{2+}). Sarcomere length (SL) and fluorescence (405 and 480 nm) were simultaneously recorded (IonOptix system, Hilton, USA) (1).

2.5. Regional myocardial ischemia-reperfusion protocol on isolated perfused heart

In a second set of rats (n=10/group), a regional myocardial IR protocol on isolated-perfused heart was performed (16). The coronary occlusion-induced

myocardial regional ischemia was maintained for 30 min. Subsequently, the heart was allowed to reperfuse during 120 min. Lactate dehydrogenase (LDH) release, incidence of ventricular fibrillation and infarct size were evaluated.

2.6. Biochemical assays

2.6.1. Heart antioxidant enzyme activity

In order to assess the effects of exercise training and/or CO exposure on the antioxidant capacity, heart enzymatic antioxidant status was measured in the third set of rats as previously described (n=5/group), (16). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymatic activities were evaluated.

2.6.2. Lactate dehydrogenase activity in coronary effluents

LDH activity, used as an index of cell membrane damage, was measured in coronary effluents at 5 min of reperfusion. LDH activity was measured spectrophotometrically using a LDH kit (LDH-P, BIOLABO SA, France).

2.6.3. Western blot analysis

Proteins were separated using 4-20 % SDS-PAGE and blotted onto a nitrocellulose membrane (Protran, Schleichen and Schuele, Dassel, Germany). Membranes were incubated overnight at 4°C with the SERCA-2a antibody (A010-20, Badrilla, UK), and levels were expressed relative to glyceraldehydes 3-phosphate dehydrogenase (GAPDH) content on the same membrane. Immunodetection was

carried out using the ECL Plus system (Amersham Pharmacia, Little Chalfont Buckinghamshire, England).

2.7. Statistics

Data were analyzed using either one-way factorial or repeated measures ANOVA. When significant interactions were found, a Student-Newman-Keuls test was applied. Binomially distributed variables (such as incidence of ventricular fibrillations) were analyzed using a non parametric Yates' chi square test (Statview; Adept scientific, Letchworth, UK). A level of $p<0.05$ was considered statistically significant. Data are expressed as group means or group mean fractions of baseline ± S.E.

3. Results

3.1. Contraction and Ca^{2+} handling in single cells

Chronic exposure to CO pollution decreased SL shortening (Fig. 1 AB), increased diastolic cytosolic Ca^{2+} (Fig. 1 CD), and decreased the amplitude of the Ca^{2+} transient (Fig. 1 CE). In addition, the decay kinetics (tau) of the Ca^{2+} transient were impaired (Fig. 1 F), which was explained by a decrease in SERCA-2a expression in CO rats (Fig. 1 G). Exercise training prevented both SERCA-2a reduction (Fig. 1 G) and Ca^{2+} handling alterations (Fig. 1 DEF). Exercise training therefore preserved normal SL shortening, which was similar to that of rats living in standard filtered air (Fig. 1 AB).

3.2. Myocardial enzymatic antioxidant status.

After 4 weeks of sustained CO exposure, myocardial enzymatic antioxidant status was depressed. The SOD and GPx activities were reduced in CO-Sed rats compared to Ctrl-Sed rats (Fig. 2 AB). Exercise training prevented these deleterious effects as SOD and GPx activities did not differ between CO-Ex rats and Ctrl-Sed rats. In contrast, no effect from CO exposure or exercise training was reported on CAT activity (Fig. 2 C).

3.3. Infarct size and myocardial cells death.

Cardiac cells death induced by IR was aggravated by prolonged CO exposure (Fig. 3 AB). Indeed, infarct size was higher in CO rats than in controls. In addition, LDH release, measured in coronary effluents at the time of post-ischemic myocardial reperfusion and used as an index of cell membrane damage, was higher in CO exposed rats than in their counterparts. The promoting effect of CO exposure on myocardial necrosis was fully prevented by regular exercise as no difference in infarct size was observed between CO-Ex and Ctrl-Sed rats (Fig. 3 AB). Consistently, the same result was obtained regarding the effects of exercise on LDH release during post-ischemic reperfusion (Fig. 3 C).

3.4. Myocardial reperfusion arrhythmias.

Although no difference was observed in the incidence of VF (33 %, Ctrl-Sed *vs.* 50 % CO-Sed), chronic CO exposure was responsible for a marked increase in the severity of post-ischemic reperfusion VF. Indeed, sustained VF (Fig. 3 D) occurred in 25 % of CO-Sed rats, whereas this phenomenon was not observed in Ctrl-Sed rats (Fig. 3 E). Interestingly the pronounced deleterious effect of CO exposure on the severity of reperfusion arrhythmias was fully prevented by exercise training since no VF was reported in CO-Ex rats (Fig. 3 E).

4. Discussion

The major results of this study are that endurance training prevents the deleterious effects of sustained CO exposure on myocardial antioxidant status, cellular Ca^{2+} handling and myocardial vulnerability to IR injury.

4.1. CO exposure and cardioprotective effects of exercise training

In line with previous reports, including ours, we confirmed here deleterious effects of simulated urban CO exposure on enzymatic antioxidant status and cardiomyocyte Ca^{2+} handling (1, 4). Low and sustained levels of CO exposure promoted pathological cardiac remodelling with impaired Ca^{2+} handling due to increased diastolic Ca^{2+}, decreased Ca^{2+} transient and Ca^{2+} reuptake in the sarcoplasmic reticulum (SR) due to reduced SERCA-2a expression (1, 4). These changes are related to CO-induced oxidative stress (16, 34), being associated with altered redox (1) and enzymatic antioxidant statuses (1, 16).

One main result of the present study was that exercise training conducted prior to CO exposure, successfully prevented the deleterious effects of CO exposure on myocardial enzymatic antioxidant activities (SOD and GPx). Even if the underlying mechanisms are not fully understood yet, exercise training is well-recognized today as one of the most efficient cardioprotective strategies, notably through the enhancement of myocardial antioxidant status (2, 7, 12-14). Consistently, exercise preserved antioxidant status and, thereby, prevented cytosolic Ca^{2+} overload and depressed Ca^{2+} transient in CO-trained rats, in line with various reports of beneficial effects of exercise training on Ca^{2+} handling in pathological population (19, 26, 29).

Maintenance of normal SERCA-2a expression mainly explained this benefit on Ca^{2+} homeostasis. Although direct effects of exercise training on SERCA-2a expression could not be ruled out (19, 30), our results are consistent with indirect effects mediated by the normalization of enzymatic antioxidant status on this redox sensitive protein (8, 18, 28, 31).

4.2. Endurance training and sensitivity to IR consecutive to CO exposure

We recently reported that sustained CO-exposure increased the vulnerability to IR (16). We showed here that occurrence of sustained ventricular fibrillations and cellular death were enhanced after IR in CO-Sed rats. Our results are in line with the well-reported determinant role of oxidative stress and Ca^{2+} overload in the severity of post-ischemic reperfusion arrhythmias, cardiac dysfunction, and irreversible cardiomyocyte damages (5, 6, 20, 33). A major result of our study was that endurance training, by preventing CO-induced cellular alterations, fully prevented the worsened sensitivity of CO rats to IR. This is in accordance with numerous studies supporting the successful cardioprotective effect of exercise training against IR injuries (2, 7, 11, 22, 23). This beneficial effect has been largely proposed to reflect improved myocardial enzymatic antioxidant status (7, 9, 22, 32). In our work, normalization of enzymatic antioxidant activities certainly contributed to decrease the sensitivity of CO-rat hearts to IR. In addition, various studies also reported the preservation of Ca^{2+} homeostasis as being a key factor for improvement in ischemic heart disease tolerance (27). In particular, the functional level of SERCA-2a is one of the factors that determines intracellular Ca^{2+} overload following IR injuries (21, 27). Taken together, our results show that indirect effects (mediated by antioxidant

effects) or/and direct beneficial effects of exercise on SERCA-2a expression play a major role to normalize heart vulnerability to IR in rats exposed to sustained CO.

Taken together, our results demonstrate that regular exercise training mainly prevents the toxicity of prolonged exposure to environmental CO due to urban pollution. These results point out the essential role of CO-induced cellular Ca^{2+} handling and antioxidant status alterations in the higher vulnerability of CO rats' myocardium to IR and the prevention of these alterations by exercise training. Endurance training, recognized as an efficient antioxidant strategy, seems to be a relevant cardioprotective approach capable of preventing higher cardiac vulnerability to ischemic stress. Given that exposure to air pollutants is an important health issue, responsible for 800,000 premature deaths worldwide each year and with increases in the risk of mortality from cardiovascular disease by 76% (17), such a workable preventive strategy is very attractive and deserves further interests.

5. Grants

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6. Author Disclosure Statement.

No competing financial interests exist

7. Bibliography

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Legends to figures

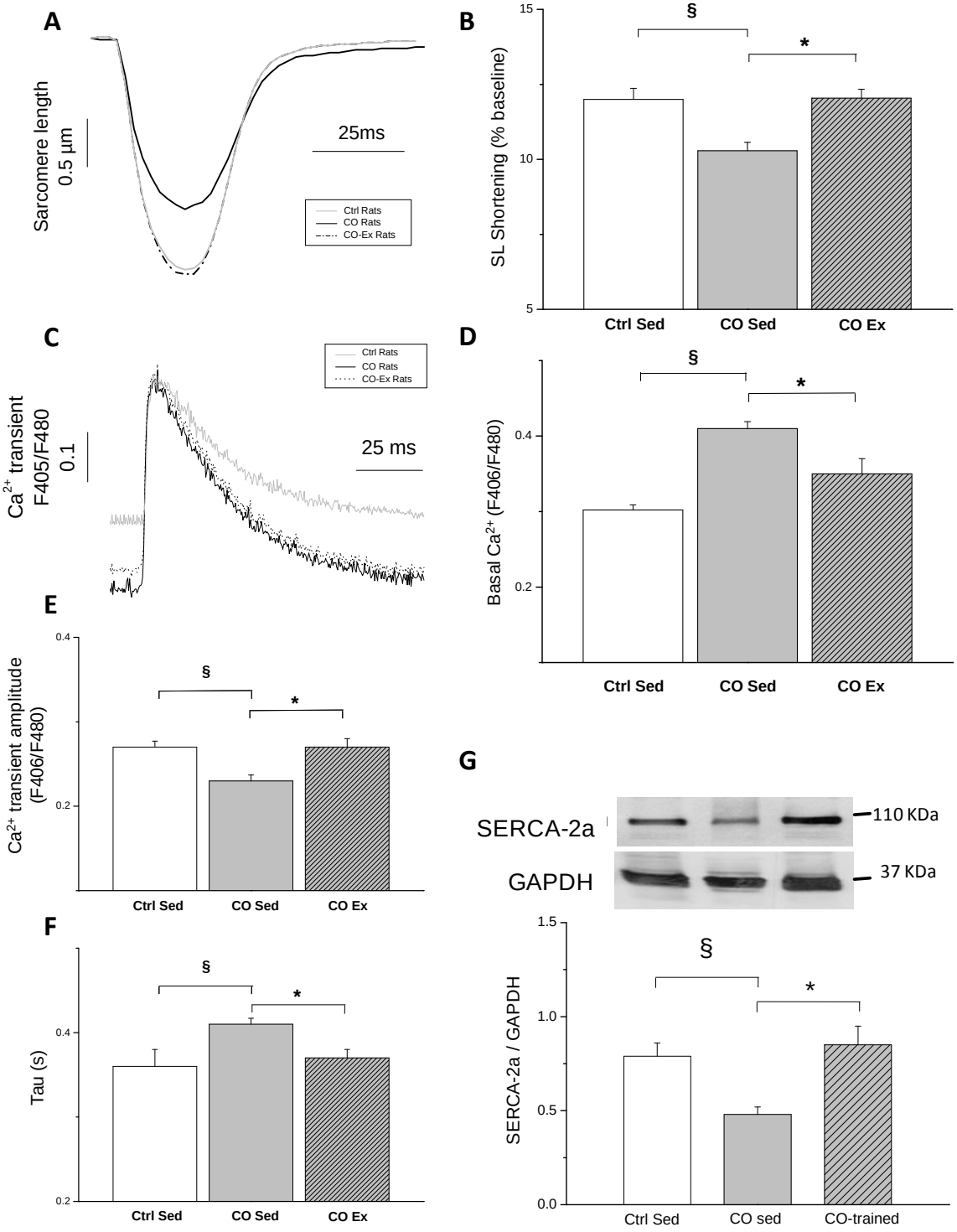
Fig. 1. Effects of exercise training and CO exposure on sarcomere length (SL) shortening and Ca^{2+} handling in cardiomyocytes. A, Representative contraction of intact cardiomyocytes measured by sarcomere shortening at 0.5 Hz. B, Amplitude of sarcomere length shortening. Data are presented as percentage of baseline. C, Representative Ca^{2+} transient during cardiomyocyte excitation-contraction. D, Diastolic intracellular Ca^{2+}. E, Ca^{2+} transient amplitude. F, Ca^{2+} reuptake kinetics (Tau). G, Cardiac expression of SERCA-2a. The GAPDH blot (37KDa) was used as a loading control to normalize SERCA-2a protein expression. Data are presented as mean ± S.E. (n=5 per group, one way ANOVA; §, $p<0.05$ Ctrl-Sed *vs*. CO-Sed, *, $p<0.05$ CO-Sed *vs*. CO-Ex).

Fig. 2. Effects of exercise training and CO exposure on myocardial antioxidant enzyme activities. A, Superoxide dismutase (SOD) activity; B, Glutathione peroxidase (GPx) activity; C, Catalase (CAT) activity. Activities are expressed in U/mg of protein. Data are presented as mean ± S.E. (n=5 per group, one way ANOVA; §, $p<0.05$ Ctrl-Sed *vs*. CO-Sed, *, $p<0.05$ CO-Sed *vs*. CO-Ex).

Fig. 3. Effects of exercise training and CO exposure on IR-induced cellular death and post-ischemic ventricular fibrillations. A, Representative sections of area at risk and infarct size of rat hearts stained respectively with Evans Blue and triphenyltetrazolium chloride (TTC) after 30 min regional ischemia and 120 min reperfusion from isolated

heart experiments in each experimental group. B, Infarct sizes expressed as percentage of area at risk. Data are presented as mean ± S.E. (Ctrl-Sed n=7, CO-Sed n=6, CO-Ex- n=6, one way ANOVA; §, $p<0.05$ Ctrl-Sed vs CO-Sed, *, $p<0.05$ CO-Sed vs CO-Ex). C, LDH activity observed in coronary effluents at 5 min of reperfusion and used as a marker of cell death. Data are presented as mean ± S.E.(Ctrl-Sed n=9, CO-Sed n=7, CO-Ex n=10, one way ANOVA; §, $p<0.05$ Ctrl-Sed *vs*. CO-Sed, *, $p<0.05$ CO-Sed *vs*. CO-Ex). D, Representative plot of ventricular fibrillation (VF). E, Incidence of ventricular fibrillations occurring during the 5 first minutes of reperfusion. Data are presented as percentage of rats per experimental group. (Ctrl-Sed n=6, CO-Sed n=8, CO-Ex n=9, non parametric Yates' chi square test; §, $p<0.05$ Ctrl-Sed *vs.* CO-Sed, *, $p<0.05$ CO-Sed *vs*. CO-Ex).

Fig. 1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*
Ctrl Sed
CO Sed
CO Ex
E
Ca2+ transient amplitude
(F406/F480)
0.4
0.2
§
*
Ctrl Sed
CO Sed
CO Ex
F
Tau (s)
0.4
0.2
§
*
Ctrl Sed
CO Sed
CO Ex
G
SERCA-2a
GAPDH
110 KDa
37 KDa
SERCA-2a / GAPDH
1.5
1.0
0.5
0.0
§
*
Ctrl Sed
CO sed
CO-trained

Fig. 2

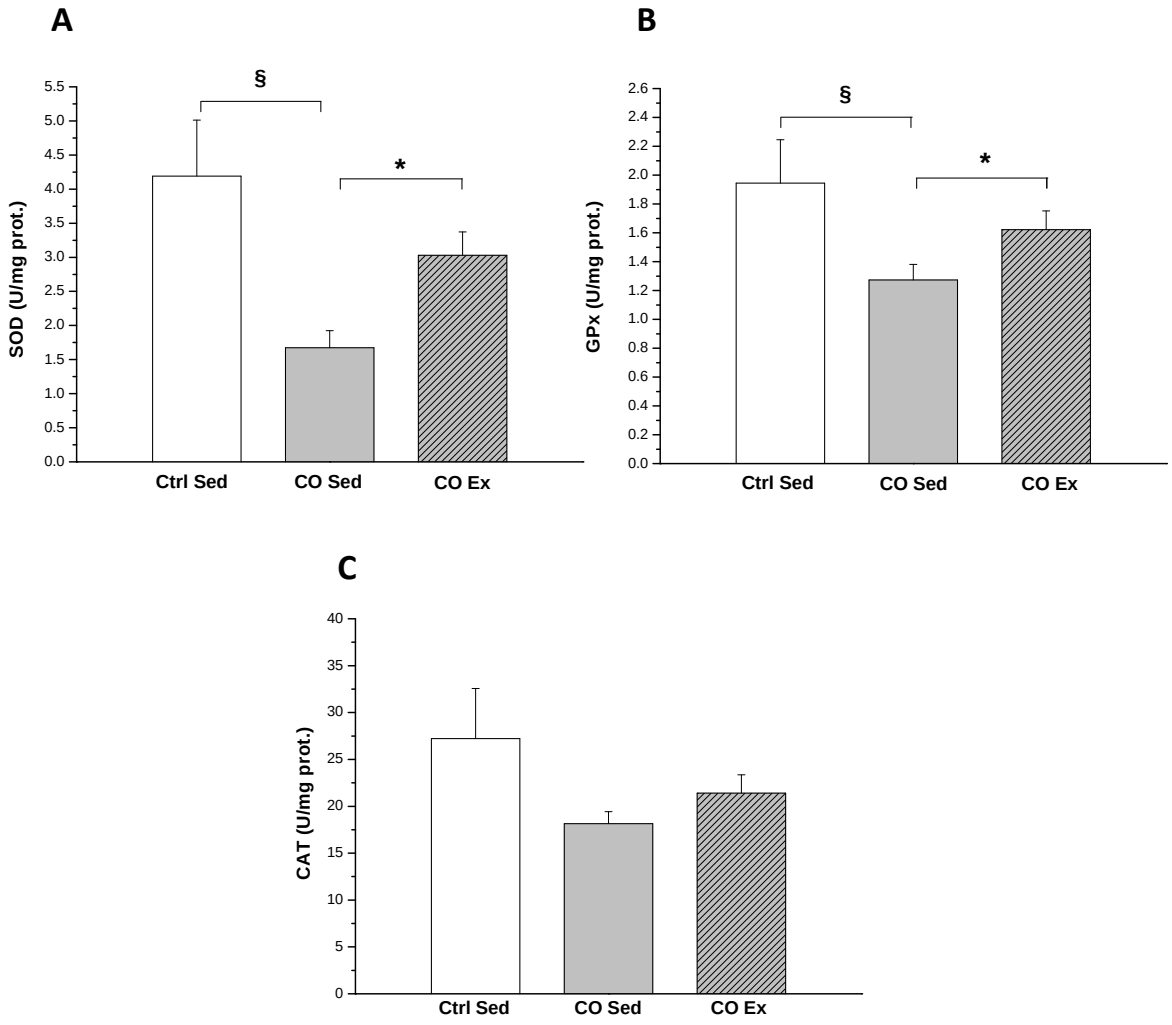
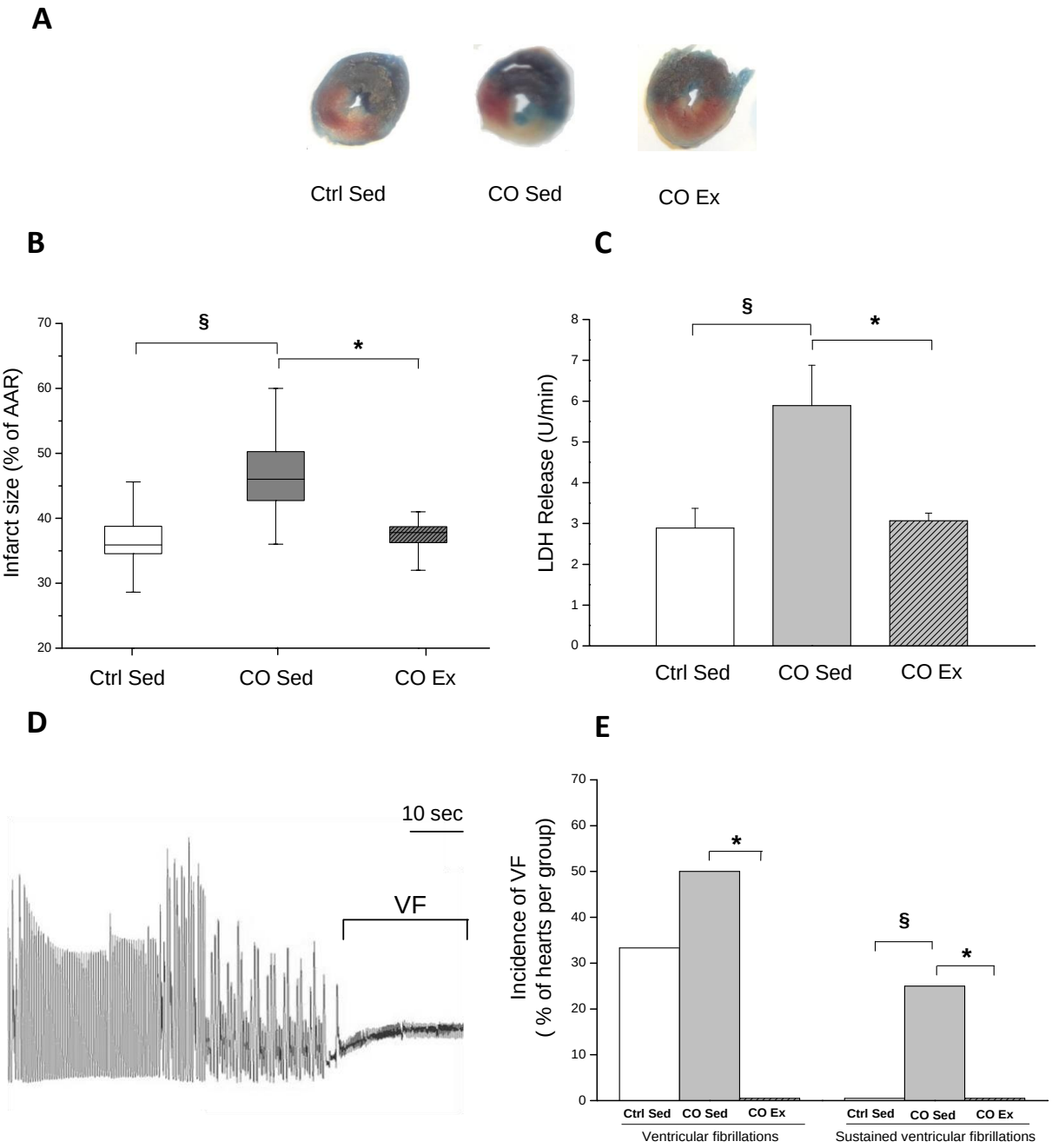

A
§
*
SOD (U/mg prot.)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Ctrl Sed
CO Sed
CO Ex
B
§
*
GPx (U/mg prot.)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ctrl Sed
CO Sed
CO Ex
C
CAT (U/mg prot.)
40
35
30
25
20
15
10
5
0
Ctrl Sed
CO Sed
CO Ex

Fig. 3


A
Ctrl Sed
CO Sed
CO Ex
B
Infarct size (% of AAR)
§
*
Ctrl Sed
CO Sed
CO Ex
C
LDH Release (U/min)
§
*
Ctrl Sed
CO Sed
CO Ex
D
10 sec
VF
E
Incidence of VF
(% of hearts per group)
*
§
*
Ctrl Sed
CO Sed
CO Ex
Ventricular fibrillations
Ctrl Sed
CO Sed
CO Ex
Sustained ventricular fibrillations