

Moderate exercise prevents impaired Ca²⁺ handling in heart of CO-exposed rat: implication for sensitivity to ischemia-reperfusion

C. Farah, G. Meyer, L. André, J. Boissiere, S. Gayrard, Olivier Cazorla, Sylvain Richard, F. Boucher, S. Tanguy, P. Obert, et al.

► **To cite this version:**

C. Farah, G. Meyer, L. André, J. Boissiere, S. Gayrard, et al.. Moderate exercise prevents impaired Ca²⁺ handling in heart of CO-exposed rat: implication for sensitivity to ischemia-reperfusion. *AJP - Heart and Circulatory Physiology*, American Physiological Society, 2010, 299 (6), pp.H2076 - H2081. 10.1152/ajpheart.00835.2010 . hal-01824341

HAL Id: hal-01824341

<https://hal.umontpellier.fr/hal-01824341>

Submitted on 13 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Moderate exercise prevents impaired Ca²⁺ handling in heart of CO-exposed rat: implication for sensitivity to ischemia-reperfusion

C. Farah,^{1*} G. Meyer,^{1*} L. André,² J. Boissière,¹ S. Gayrard,¹ O. Cazorla,² S. Richard,² F. Boucher,³ S. Tanguy,¹ P. Obert,¹ and C. Reboul¹

¹Research Laboratory EA 4278, Physiology and Physiopathology of Cardiovascular Adaptations to Exercise, Faculty of Sciences, Avignon University, Avignon; ²Research Laboratory, Institut National de la Santé et de la Recherche Médicale U637, Cardiovascular Physiopathology, Montpellier1 University, Faculty of Medicine, Montpellier; ³Research Laboratory, Centre National de la Recherche Scientifique UMR5525 Physiologie Respiratoire Expérimentale Théorique et Appliquée-TIMC, Grenoble University Joseph Fourier, Grenoble, France

Farah C, Meyer G, André L, Boissière J, Gayrard S, Cazorla O, Richard S, Boucher F, Tanguy S, Obert P, Reboul C. Moderate exercise prevents impaired Ca²⁺ handling in heart of CO-exposed rat: implication for sensitivity to ischemia-reperfusion.

—Sustained urban carbon monoxide (CO) exposure exacerbates heart vulnerability to ischemia-reperfusion via deleterious effects on the antioxidant status and Ca²⁺ homeostasis of cardiomyocytes. The aim of this work was to evaluate whether moderate exercise training prevents these effects. Wistar rats were randomly assigned to a control group and to CO groups, living during 4 wk in simulated urban CO pollution (30–100 parts/million, 12 h/day) with (CO-Ex) or sedentary without exercise (CO-Sed). The exercise procedure began 4 wk before CO exposure and was maintained twice a week in standard filtered air during CO exposure. On one 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 Ca²⁺ handling were evaluated following environmental exposure. As a result, exercise training prevented CO-induced myocardial phenotypical changes. Indeed, exercise induced myocardial antioxidant status recovery in CO-exposed rats, which is accompanied by a normalization of sarco(endo)plasmic reticulum Ca²⁺-ATPase 2a expression and then of Ca²⁺ handling. Importantly, in CO-exposed rats, the normalization of cardiomyocyte phenotype with moderate exercise was associated with a restored sensitivity of the myocardium to ischemia-reperfusion. Indeed, CO-Ex rats presented a lower infarct size and a significant decrease of reperfusion arrhythmias compared with their sedentary counterparts. To conclude, moderate exercise, by preventing CO-induced Ca²⁺ handling and myocardial antioxidant status alterations, reduces heart vulnerability to ischemia-reperfusion.

carbon monoxide; myocardial infarction; environmental pollution; endurance training; antioxidant status

THE SEVERITY OF myocardial infarction results from a complex interplay between genetic, pathological, and environmental factors (22, 27). Among the environmental factors, numerous epidemiological studies have demonstrated that carbon monoxide (CO) pollution correlated with hospital admissions for cardiovascular diseases (3, 17), as well as cardiovascular mortality (28). We recently reported that a sustained low-level CO exposure, similar to that found in an urban environment,

induced a pathological remodeling of the myocardium (1), rendering the heart more vulnerable to ischemia-reperfusion (I/R) (18). This remodeling involves a marked alteration of enzymatic antioxidant status associated with marked changes in Ca²⁺ handling (1), promoting cardiomyocyte death and severe ventricular arrhythmias (18).

Today, among numerous cardioprotective strategies used to prevent deleterious myocardial remodeling 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, 25, 26). Although the mechanisms responsible for exercise-induced cardioprotection remain unclear, many studies suggest that increased enzymatic antioxidant status plays an important role (7, 10, 35). In addition, exercise training is also reported to normalize Ca²⁺ homeostasis in the pathological myocardium (29, 32). Therefore, we have hypothesized that regular exercise training started before sustained CO exposure could prevent pathological cardiac remodeling and/or modulate heart vulnerability to I/R.

The aim of this study was to evaluate the 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 Ca²⁺ handling, myocardial enzymatic antioxidant status alterations, and the consequences on heart vulnerability to I/R. The major results showed that regular bouts of endurance training prevented the pathological cardiac remodeling and the higher vulnerability to I/R of the hearts of rats exposed to chronic CO.

METHODS

All investigations complied with the *Guide for the Care and Use of Laboratory Animals*, published by the 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'Éthique).

Animals

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

* C. Farah and G. Meyer contributed equally to this work.

Address for reprint requests and other correspondence: C. Reboul, Research Laboratory, EA 4278, Physiology and Physiopathology of Cardiovascular Adaptations to Exercise, Faculty of Sciences, Avignon Univ., F-84000 Avignon, France (e-mail: cyril.reboul@univ-avignon.fr).

Exercise Training Protocol

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

CO Exposure

The CO groups were exposed to simulated CO urban pollution for 4 wk, 12 h/day, during the night phase. CO rats were housed in an airtight exposure container, and exposure was performed as follows: 1) during CO exposure, a CO concentration of 30 parts/million (ppm) was maintained in the airtight container and monitored with an aspirative CO analyzer (CHEMGARD Infrared Gas Monitor NEMA 4 Version, MSA), and this initial concentration was completed with five 1-h peaks at 100 ppm CO; and 2) during ambient air exposure,

the 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 subjected to the same restraint as the CO-Sed rats. At the end of the 4 wk of CO exposure, the rats were housed for 24 h in standard filtered air before euthanasia to avoid any acute effects of CO on the myocardium.

Ca²⁺ Handling in Cardiomyocytes

In the first set of rats ($n = 4$ rats/group; 10 myocytes/rat), an evaluation of exercise training and CO exposure on excitation-contraction was performed on single ventricular cardiomyocytes isolated by enzymatic digestion (21). Unloaded cell shortening and Ca²⁺ concentration (Indo-1 dye) were measured using field stimulation (0.5 Hz, 22°C, 1.8 mM external Ca²⁺). Sarcomere length (SL) and fluorescence (405 and 480 nm) were simultaneously recorded (Ion-Optix system, Hilton) (1).

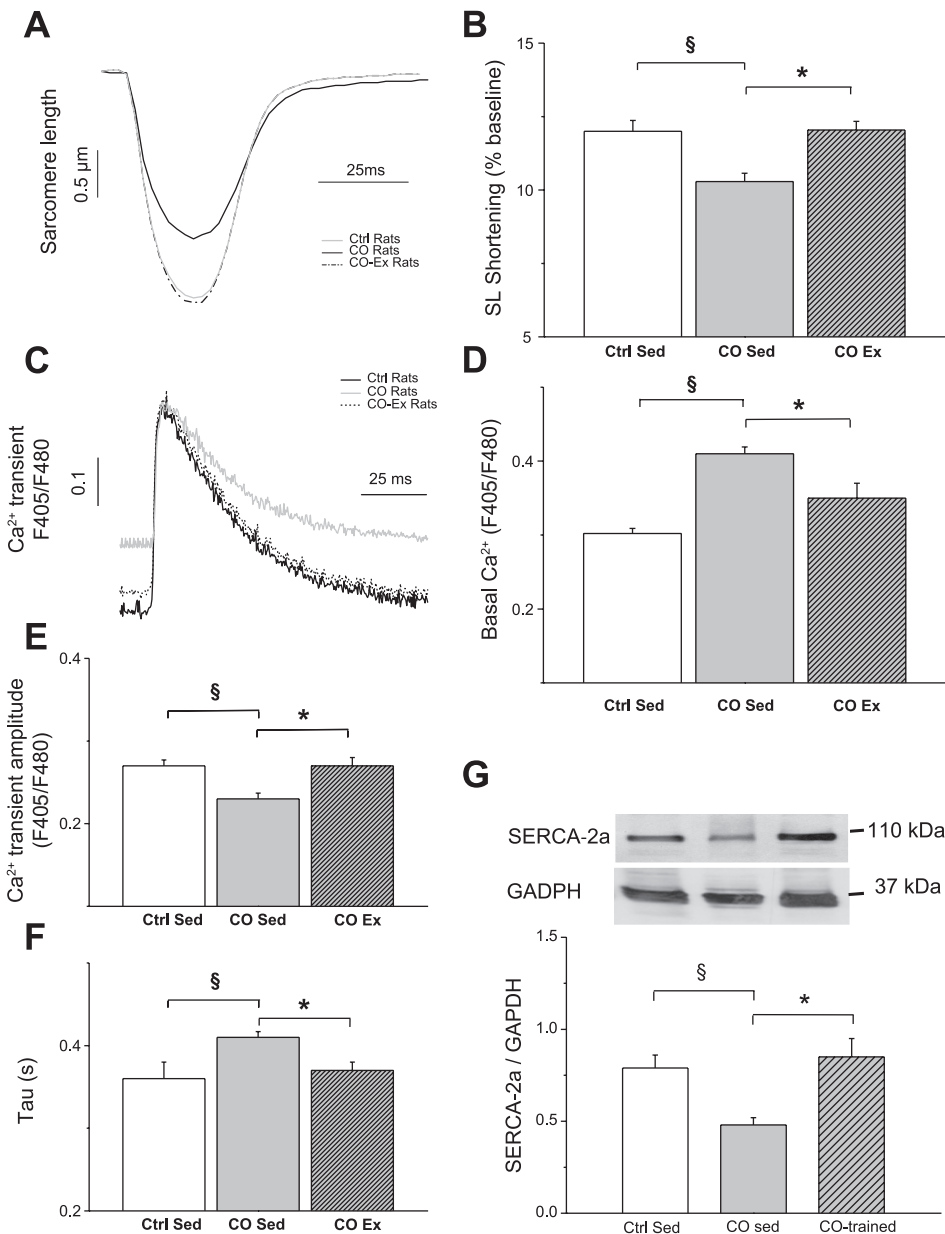


Fig. 1. Effects of exercise training and carbon monoxide (CO) exposure on sarcomere length (SL) shortening and Ca²⁺ handling in cardiomyocytes. *A*: representative contraction of intact cardiomyocytes measured by sarcomere shortening at 0.5 Hz. *B*: amplitude of SL shortening. Data are presented as percentages of baseline. *C*: representative Ca²⁺ transient during cardiomyocyte excitation-contraction. F₄₀₅/F₄₈₀, ratio of fluorescence at 405 to 480 nm. *D*: diastolic intracellular Ca²⁺. *E*: Ca²⁺ transient amplitude. *F*: Ca²⁺ reuptake kinetics (τ). *G*: cardiac expression of sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA2a). The GAPDH blot (37 kDa) was used as a loading control to normalize SERCA2a protein expression. Data are presented as means \pm SE ($n = 5$ per group, one-way ANOVA). § $P < 0.05$, control (Ctrl)-sedentary (Sed) vs. CO-Sed; * $P < 0.05$, CO-Sed vs. CO-exercise trained (Ex).

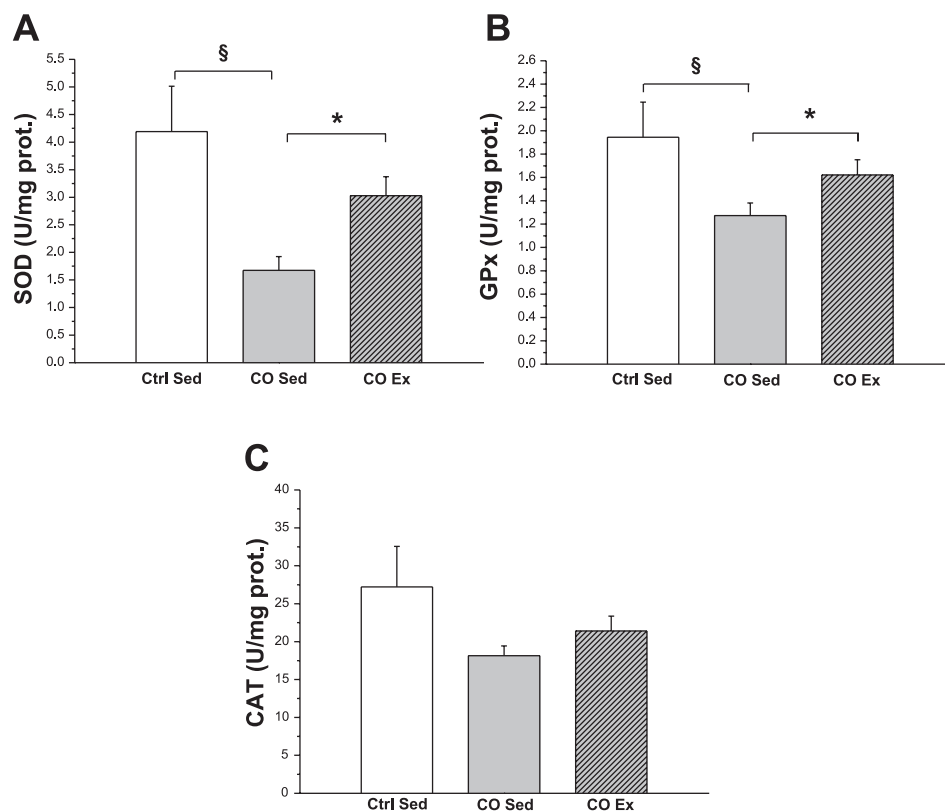


Fig. 2. Effects of exercise training and CO exposure on myocardial antioxidant enzyme activities. *A*: superoxide dismutase (SOD) activity. *B*: glutathione peroxidase (GPx) activity. *C*: catalase (Cat) activity. Activities are expressed in U/mg of protein (Prot). Data are presented as means \pm SE ($n = 5$ per group, one-way ANOVA). § $P < 0.05$, Ctrl-Sed vs. CO-Sed; * $P < 0.05$, CO-Sed vs. CO-Ex.

Regional Myocardial I/R Protocol on Isolated Perfused Heart

In a second set of rats ($n = 10$ /group), a regional myocardial I/R protocol on an isolated-perfused heart was performed (18). This model was chosen to abrogate the potential effects of CO exposure on hormonal, circulating, and nervous parameters. 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 and incidence of ventricular fibrillation (VF) was evaluated during the reperfusion period. At the end of the perfusion, the hearts were divided into five slices perpendicular to the apex-base axis and triphenyltetrazolium chloride staining (0.5 mg/ml for 20 min at 37°C) was used to assess myocardial tissue viability and to determine myocardial infarct size. The tissue slices were photographed, and the risk and infarcted areas were then determined using a computer-based system (ImageJ, NIH).

Biochemical Assays

Heart antioxidant enzyme activity. To assess the effects of exercise training and/or CO exposure on the antioxidant capacity, the heart enzymatic antioxidant status was measured in the third set of rats as previously described ($n = 5$ /group) (18). Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) enzymatic activities were evaluated.

LDH 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 an LDH kit (LDH-P, BIOLABO SA, France).

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 sarco(endo)plasmic reticulum Ca^{2+} -ATPase 2a (SERCA2a) antibody (A010-20, Badrilla, UK), and the levels were expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) content on the same membrane. Immunodetection was carried out using the ECL Plus system (Amersham Pharmacia, Little Chalfont Buckinghamshire, UK).

drogenase (GAPDH) content on the same membrane. Immunodetection was carried out using the ECL Plus system (Amersham Pharmacia, Little Chalfont Buckinghamshire, UK).

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 VFs) were analyzed using a nonparametric Yates' χ^2 -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 \pm SE.

RESULTS

Contraction and Ca^{2+} Handling in Single Cells

Chronic exposure to CO pollution decreased SL shortening (Fig. 1, *A* and *B*), increased diastolic cytosolic Ca^{2+} (Fig. 1, *C* and *D*), and decreased the amplitude of the Ca^{2+} transient (Fig. 1, *C* and *E*). In addition, the decay kinetics (τ) of the Ca^{2+} transient were impaired (Fig. 1*F*), which was explained by a decrease in SERCA2a expression in CO rats (Fig. 1*G*). Exercise training prevented both SERCA2a reduction (Fig. 1*G*) and Ca^{2+} -handling alterations (Fig. 1, *D–F*). Exercise training therefore preserved normal SL shortening, which was similar to that of rats living in standard filtered air (Fig. 1, *A* and *B*).

Myocardial Enzymatic Antioxidant Status

After 4 wk of sustained CO exposure, the myocardial enzymatic antioxidant status was depressed. The SOD and GPx activities were reduced in CO-Sed rats compared with Ctrl-Sed

rats (Fig. 2, A and B). Exercise training prevented these deleterious effects since 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 catalase activity (Fig. 2C).

Infarct Size and Myocardial Cells Death

Cardiac cell death induced by I/R was aggravated by prolonged CO exposure (Fig. 3, A and B). Indeed, infarct size was higher in CO rats than in controls. In addition, LDH release, measured in coronary effluents at the time of postischemic 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 since no difference in infarct size was observed between CO-Ex and Ctrl-Sed rats (Fig. 3, A and B). Consistently, the same result was obtained regarding the effects of exercise on LDH release during postischemic reperfusion (Fig. 3C).

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 postisch-

emic reperfusion VF. Indeed, sustained VF (Fig. 3D) occurred in 25% of CO-Sed rats, whereas this phenomenon was not observed in Ctrl-Sed rats (Fig. 3E). 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. 3E).

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 I/R injury.

CO Exposure and Cardioprotective Effects of Exercise Training

In line with previous reports, including ours, we confirmed here the 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 remodeling 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 SERCA2a expression (1, 4). These changes are related to CO-induced oxidative stress (18, 37)

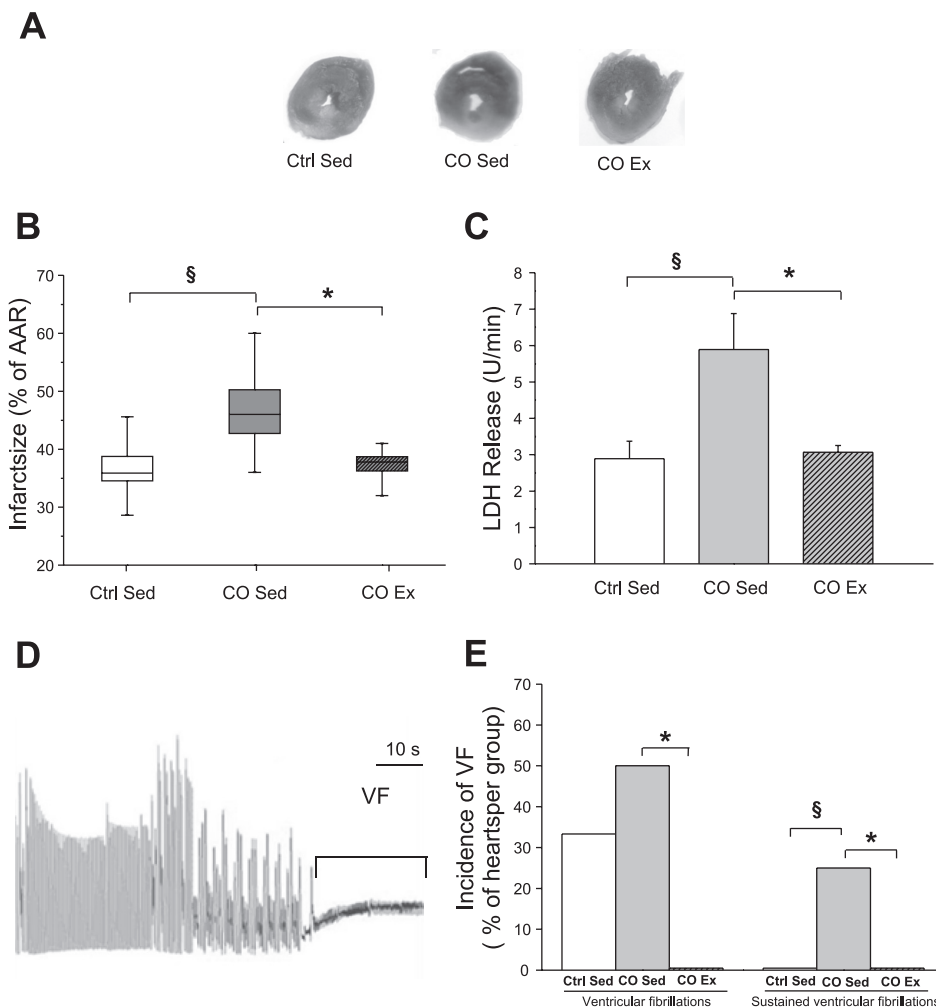


Fig. 3. Effects of exercise training and CO exposure on ischemia-reperfusion-induced cellular death and postischemic ventricular fibrillations (VFs). *A*: representative sections of area at risk (AAR) and infarct size of rat hearts stained, respectively, with Evans blue and triphenyltetrazolium chloride after 30 min regional ischemia and 120 min reperfusion from isolated heart experiments in each experimental group. *B*: infarct sizes expressed as percentages of AAR. Data are presented as means \pm SE (Ctrl-Sed, $n = 7$; CO-Sed, $n = 6$; and CO-Ex, $n = 6$; one-way ANOVA). *C*: LDH activity observed in coronary effluents at 5 min of reperfusion and used as a marker of cell death. Data are presented as means \pm SE (Ctrl-Sed, $n = 9$; CO-Sed, $n = 7$; and CO-Ex, $n = 10$; one-way ANOVA). *D*: representative plot of VF. *E*: incidence of VFs occurring during the first 5 min of reperfusion. Data are presented as percentages of rats per experimental group (Ctrl-Sed, $n = 6$; CO-Sed, $n = 8$; and CO-Ex, $n = 9$; nonparametric Yates' χ^2 -test). § $P < 0.05$, Ctrl-Sed vs. CO-Sed; * $P < 0.05$, CO-Sed vs. CO-Ex.

being associated with altered redox (1) and enzymatic antioxidant statuses (1, 18).

One main result of the present study was that moderate exercise training conducted before 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, 15, 16). 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 the beneficial effects of exercise training on Ca^{2+} handling in a pathological population (21, 29, 32). The maintenance of normal SERCA2a expression mainly explained this benefit on Ca^{2+} homeostasis. Although the direct effects of exercise training on SERCA2a expression could not be ruled out (21, 33), our results are consistent with the indirect effects mediated by the normalization of enzymatic antioxidant status on this redox-sensitive protein (8, 20, 31, 34).

Endurance Training and Sensitivity to I/R Consecutive to CO Exposure

We recently reported that a sustained CO exposure increased the vulnerability to I/R (18). Although lower infarct sizes in CO rats were observed in this study compared with the previous one (18), the present work confirms that CO exposure is associated with an increased severity of myocardial I/R injuries, characterized by an exacerbated occurrence of sustained VFs and cardiomyocyte death. These results are in line with the well-reported determinant role of oxidative stress and Ca^{2+} overload in the severity of postischemic reperfusion arrhythmias, cardiac dysfunction, and irreversible cardiomyocyte damages (5, 6, 22, 36). 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 I/R. This is in accordance with numerous studies supporting the successful cardioprotective effect of exercise training against I/R injuries (2, 7, 11, 25, 26). This beneficial effect has been largely proposed to reflect the improved myocardial enzymatic antioxidant status (7, 9, 25, 35). In our work, the normalization of enzymatic antioxidant activities certainly contributed to decrease the sensitivity of CO rat hearts to I/R. In addition, various studies also reported the preservation of Ca^{2+} homeostasis as being a key factor for improvement in ischemic heart disease tolerance (30). In particular, the functional level of SERCA2a is one of the factors that determines intracellular Ca^{2+} overload following I/R injuries (23, 30). Taken together, our results show that the indirect effects (mediated by antioxidant effects) and/or the direct beneficial effects of exercise on SERCA2a expression play a major role to normalize heart vulnerability to I/R in rats exposed to sustained CO.

Finally, it has to be noted that in this work, no specific effect of low-intensity exercise training was reported in Ctrl-Ex rats compared with Ctrl rats (data not shown). This result is not surprising since it is well known today that high-intensity exercise training is more effective to induce adaptations in healthy rat hearts (13, 14). Therefore, one of the main findings

of the present work is that an exercise training protocol, specifically designed to follow the recommendation of the World Health Organization [30 min, 5 days/wk, at low intensity (33a)] was shown to be efficient in preventing the development of a myocardial vulnerable phenotype in CO-exposed rats.

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 rat myocardium to I/R 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% (19), such a workable preventive strategy is very attractive and deserves further interest.

GRANTS

This work was supported by a French National Research Agency grant (COMYOCARD).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. Andre L, Boissiere J, Reboul C, Perrier R, Zalvidea S, Meyer G, Thireau J, Tanguy S, Bideaux P, Hayot M, Boucher F, Obert P, Cazorla O, Richard S. Carbon monoxide pollution promotes cardiac remodeling and ventricular arrhythmia in healthy rats. *Am J Respir Crit Care Med* 181: 587–595, 2010.
2. Ascensao A, Ferreira R, Magalhaes J. Exercise-induced cardioprotection—biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. *Int J Cardiol* 117: 16–30, 2007.
3. Burnett RT, Cakmak S, Brook JR, Krewski D. The role of particulate size and chemistry in the association between summertime ambient air pollution and hospitalization for cardiorespiratory diseases. *Environ Health Perspect* 105: 614–620, 1997.
4. Bye A, Sorhaug S, Ceci M, Hoydal MA, Stolen T, Heinrich G, Tjonna AE, Najjar SM, Nilsen OG, Catalucci D, Grimaldi S, Contu R, Steinshamn S, Condorelli G, Smith GL, Ellingsen O, Waldum H, Wisloff U. Carbon monoxide levels experienced by heavy smokers impair aerobic capacity and cardiac contractility and induce pathological hypertrophy. *Inhal Toxicol* 20: 635–646, 2008.
5. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* 18: 655–673, 2000.
6. Dumitrescu C, Biondi R, Xia Y, Cardounel AJ, Druhan LJ, Ambrosio G, Zweier JL. Myocardial ischemia results in tetrahydrobiopterin (BH₄) oxidation with impaired endothelial function ameliorated by BH₄. *Proc Natl Acad Sci USA* 104: 15081–15086, 2007.
7. French JP, Hamilton KL, Quindry JC, Lee Y, Upchurch PA, Powers SK. Exercise-induced protection against myocardial apoptosis and necrosis: MnSOD, calcium-handling proteins, and calpain. *FASEB J* 22: 2862–2871, 2008.
8. Grover AK, Samson SE, Robinson S, Kwan CY. Effects of peroxynitrite on sarcoplasmic reticulum Ca^{2+} pump in pig coronary artery smooth muscle. *Am J Physiol Cell Physiol* 284: C294–C301, 2003.
9. Hamilton KL, Quindry JC, French JP, Staib J, Hughes J, Mehta JL, Powers SK. MnSOD antisense treatment and exercise-induced protection against arrhythmias. *Free Radic Biol Med* 37: 1360–1368, 2004.
10. Hamilton KL, Staib JL, Phillips T, Hess A, Lennon SL, Powers SK. Exercise, antioxidants, and HSP72: protection against myocardial ischemia/reperfusion. *Free Radic Biol Med* 34: 800–809, 2003.

11. **Kavazis AN.** Exercise preconditioning of the myocardium. *Sports Med* 39: 923–935, 2009.
12. **Kavazis AN, McClung JM, Hood DA, Powers SK.** Exercise induces a cardiac mitochondrial phenotype that resists apoptotic stimuli. *Am J Physiol Heart Circ Physiol* 294: H928–H935, 2008.
13. **Kemi OJ, Ellingsen O, Smith GL, Wisloff U.** Exercise-induced changes in calcium handling in left ventricular cardiomyocytes. *Front Biosci* 13: 356–368, 2008.
14. **Kemi OJ, Wisloff U.** Mechanisms of exercise-induced improvements in the contractile apparatus of the mammalian myocardium. *Acta Physiol (Oxf)* 199: 425–439, 2010.
15. **Lennon SL, Quindry JC, French JP, Kim S, Mehta JL, Powers SK.** Exercise and myocardial tolerance to ischaemia-reperfusion. *Acta Physiol Scand* 182: 161–169, 2004.
16. **Lew H, Quintanilha A.** Effects of endurance training and exercise on tissue antioxidative capacity and acetaminophen detoxification. *Eur J Drug Metab Pharmacokinet* 16: 59–68, 1991.
17. **Mann JK, Tager IB, Lurmann F, Segal M, Quesenberry CP Jr, Lugg MM, Shan J, Van Den Eeden SK.** Air pollution and hospital admissions for ischemic heart disease in persons with congestive heart failure or arrhythmia. *Environ Health Perspect* 110: 1247–1252, 2002.
18. **Meyer G, Andre L, Tanguy S, Boissiere J, Farah C, Lopez-Lauri F, Gayrard S, Richard S, Boucher F, Cazorla O, Obert P, Reboul C.** Simulated urban carbon monoxide air pollution exacerbates rat heart ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 298: H1445–H1453, 2010.
19. **Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD.** Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med* 356: 447–458, 2007.
20. **Morris TE, Sulakhe PV.** Sarcoplasmic reticulum Ca^{2+} -pump dysfunction in rat cardiomyocytes briefly exposed to hydroxyl radicals. *Free Radic Biol Med* 22: 37–47, 1997.
21. **Mou YA, Reboul C, Andre L, Lacampagne A, Cazorla O.** Late exercise training improves non-uniformity of transmural myocardial function in rats with ischaemic heart failure. *Cardiovasc Res* 81: 555–564, 2009.
22. **Murphy E, Steenbergen C.** Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev* 88: 581–609, 2008.
23. **Niwano K, Arai M, Koitabashi N, Watanabe A, Ikeda Y, Miyoshi H, Kurabayashi M.** Lentiviral vector-mediated SERCA2 gene transfer protects against heart failure and left ventricular remodeling after myocardial infarction in rats. *Mol Ther* 16: 1026–1032, 2008.
25. **Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, Shanely RA, Jessup J.** Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. *Am J Physiol Regul Integr Comp Physiol* 275: R1468–R1477, 1998.
26. **Powers SK, Quindry JC, Kavazis AN.** Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury. *Free Radic Biol Med* 44: 193–201, 2008.
27. **Ruiz-Meana M, Garcia-Dorado D.** Translational cardiovascular medicine (II). Pathophysiology of ischemia-reperfusion injury: new therapeutic options for acute myocardial infarction. *Rev Esp Cardiol* 62: 199–209, 2009.
28. **Samoli E, Touloumi G, Schwartz J, Anderson HR, Schindler C, Forsberg B, Vigotti MA, Vonk J, Kosnik M, Skorkovsky J, Katsouyanni K.** Short-term effects of carbon monoxide on mortality: an analysis within the APHEA project. *Environ Health Perspect* 115: 1578–1583, 2007.
29. **Stolen TO, Hoydal MA, Kemi OJ, Catalucci D, Ceci M, Aasum E, Larsen T, Rolim N, Condorelli G, Smith GL, Wisloff U.** Interval training normalizes cardiomyocyte function, diastolic Ca^{2+} control, and SR Ca^{2+} release synchronicity in a mouse model of diabetic cardiomyopathy. *Circ Res* 105: 527–536, 2009.
30. **Talukder MA, Zweier JL, Periasamy M.** Targeting calcium transport in ischaemic heart disease. *Cardiovasc Res* 84: 345–352, 2009.
31. **Viner RI, Krainev AG, Williams TD, Schoneich C, Bigelow DJ.** Identification of oxidation-sensitive peptides within the cytoplasmic domain of the sarcoplasmic reticulum Ca^{2+} -ATPase. *Biochemistry* 36: 7706–7716, 1997.
32. **Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O.** Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+} sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res* 54: 162–174, 2002.
33. **Wisloff U, Loennechen JP, Falck G, Beisvag V, Currie S, Smith G, Ellingsen O.** Increased contractility and calcium sensitivity in cardiac myocytes isolated from endurance trained rats. *Cardiovasc Res* 50: 495–508, 2001.
- 33a. **World Health Organization/Fédération Internationale de Médecine du Sport Committee on Physical Activity for Health.** Exercise for health. *Bull World Health Organ* 73: 135–136, 1995.
34. **Xu KY, Zweier JL, Becker LC.** Hydroxyl radical inhibits sarcoplasmic reticulum Ca^{2+} -ATPase function by direct attack on the ATP binding site. *Circ Res* 80: 76–81, 1997.
35. **Yamashita N, Hoshida S, Otsu K, Asahi M, Kuzuya T, Hori M.** Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. *J Exp Med* 189: 1699–1706, 1999.
36. **Zhao X, Chen YR, He G, Zhang A, Druhan LJ, Strauch AR, Zweier JL.** Endothelial nitric oxide synthase (NOS3) knockout decreases NOS2 induction, limiting hyperoxygenation and conferring protection in the postischemic heart. *Am J Physiol Heart Circ Physiol* 292: H1541–H1550, 2007.
37. **Zuckerbraun BS, Chin BY, Bilban M, d'Avila JC, Rao J, Billiar TR, Otterbein LE.** Carbon monoxide signals via inhibition of cytochrome c oxidase and generation of mitochondrial reactive oxygen species. *FASEB J* 21: 1099–1106, 2007.