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RESEARCH PAPER

Effects of hyperoxia and cardiovascular risk factors on myocardial ischaemia–reperfusion injury: a randomized, sham-controlled parallel study

Angela Acheampong[1] | Christian Mélot[1] | Mariam Benjelloun[1] | Maxime Cheval[1] | Florence Reye[2] | Cédric Delporte[2] | Pierre van Antwerpen[2] | Thierry Franck[3] | Kathleen Mc Entee[2] | Philippe van de Borne[1]

[1] Hospital Erasme, Anderlecht, Belgium

[2] Faculty of Pharmacy, Therapeutic Chemistry and Analytical Platform, Université Libre de Bruxelles, Bruxelles, Belgium

[3] Centre de l'oxygène: Recherche et développement (C.O.R.D.), University of Liège, Liege, Belgium

Correspondence
Angela Acheampong, Route de Lennik 808, Anderlecht 1070, Belgium.
Email: acheampong_angela@hotmail.com

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Abstract

Recent studies on O_2 supplementation in acute coronary syndrome patients are equivocal. We tested the hypothesis that oxidative stress is increased in rodents with cardiovascular risk factors and enhances ischaemia–reperfusion injury in the presence of hyperoxia. A total of 43 Wistar rats (WR), 30 spontaneously hypertensive rats (SHR) and 33 obese Zucker rats (ZR) were randomized in a sham procedure (one-third) or underwent a left anterior descending ligation of the coronary artery for 60 min (two-thirds). This was followed by 3 h of reperfusion while animals were randomized either in a hyperoxic (HR) or a normoxic reperfusion (NR) group. Myocardial infarction size and oxidative stress biomarkers (myeloperoxidase (MPO), malondialdehyde and total free thiols) were assessed in blood samples. Baseline troponin T was higher in SHR and ZR than in WR (both $P < 0.001$). Baseline total MPO was elevated in ZR in comparison to SHR and WR (both $P < 0.001$). SHR had lower thiol concentration compared to WR and ZR ($P < 0.000001$). HR was associated with a lower troponin T rise in SHR and ZR than in NR (both $P < 0.001$), while the reverse occurred in WR ($P < 0.001$). In SHR, HR limited total MPO increase as compared to NR ($P = 0.0056$) and the opposite effect was observed with total MPO in WR ($P = 0.013$). NR was associated with a drastic reduction of total thiols as compared to HR both in SHR and in ZR (both $P < 0.001$). Despite a heightened baseline oxidative stress level, HR limited myocardial necrosis and anti/pro-oxidant imbalance in SHR and ZR whereas this effect was exacerbated in healthy WR.

KEYWORDS
acute coronary ischaemia–reperfusion, antioxidant and prooxidant markers, hyperoxia, normoxia

1 | INTRODUCTION

Cardiovascular diseases are the leading cause of death globally (Roth et al., 2015), especially in the elderly and foremost in patients with cardiovascular risk factors. Indeed, individual risk factors account for about 70% of cardiovascular disease cases. The main contributors are hypertension and dyslipidaemia (Yusuf et al., 2019). Ischaemic heart diseases are associated with high morbidity and mortality as one-quarter of acute ischaemic heart diseases are fatal (Grey et al., 2017). Ischaemic heart disease mortality rates (Iqbal & Fox, 2010) have been

falling in recent years, and elderly people above 65 years account for more than 80% of these patients (Rosamond et al., 2008).

ST-elevation myocardial infarction (STEMI) as a result of an acute blood flow decrease in the coronary artery leads to localized necrosis of myocardial tissue. The treatment of choice of a STEMI is to reperfuse the myocardium as soon as possible (James & Spertus, 2013). Although reperfusion is essential for viable myocardium salvage, the process of reperfusion can also induce myocardial injury and death of cardiomyocytes that were still viable at the end of ischaemia. Ischaemia–reperfusion injury (IRI) is a paradoxical tissue response following the restoration of blood flow and tissue oxygenation in an ischaemic organ. Reperfusion injury is one of the main factors that contributes to the morbidity and mortality in a variety of clinical entities (Hausenloy & Yellon, 2013) and can contribute up to 50% of the final myocardial infarct size (Yellon & Hausenloy, 2007).

The predominant role of oxidative stress in the pathophysiology of reperfusion injury is now well established (Granger & Kvietys, 2015). The abrupt re-entry of oxygen by reperfusion may lead to a burst of oxidative reactions through production of reactive oxygen species (ROS) from cardiomyocytes and endothelial cells, which in turn amplify the local inflammatory response and can lead to a vicious feedback loop of ROS production. Therefore, there are reasons to believe that in the presence of acute myocardial necrosis, excessive oxygen supplementation might exacerbate ROS generation, and thereby enhance coronary vasoconstriction, endothelial vascular dysfunction and myocardial infarct size.

Studies on the effects of hyperoxia in patients with myocardial infarction resulted in equivocal results. The randomized AVOID study (Stub et al., 2012) showed that excessive hyperoxia during STEMI-reperfusion was associated with larger myocardial infarction size. Conversely, in the DETOX-2-AMI study (Hofmann et al., 2017), hyperoxia did not alter prognosis in patients with acute coronary syndrome. Meanwhile the more recent NZOTACTS study (Stewart, 2019) even suggested some favourable effects of hyperoxia in STEMI. The observations of the AVOID study contrasted with previous experimental studies in rats (Mariero et al., 2012), rabbits (Shnier et al., 1991) and dogs (Kelly et al., 1995) where hyperoxia during early myocardial infarction reperfusion failed to increase the infarct size. It should be emphasized, however, that studies in young and healthy animals may not reflect the pathophysiology in humans. While Yellon (Yellon & Hausenloy, 2007) emphasized more than 10 years ago that IRI should be investigated in older animals with cardiovascular risk factors, to the best of our knowledge, hyperoxic myocardial reperfusion has not been assessed in such circumstances. This is somewhat surprizing as ageing (Liu et al., 2012), hypertension (Harrison & Gongora, 2009), diabetes (Wang et al., 2018) and dyslipidaemia (Bhalodia et al., 2010) are associated with increased reperfusion injury. Moreover, in humans myocardial infarction prognosis is also strongly and inversely correlated with the age of the patient (Mozaffarian et al., 2015). Although the most recent recommendations indicate that oxygen should not be administered in acute coronary syndrome patients with a S_{pO_2} above 90% (Ibanez et al., 2018; O'Gara et al., 2013), the effects of oxygen in the setting of myocardial infarction remain uncertain.

New Findings

- **What is the central question of this study?**
  The beneficial effects of supplemental oxygen in patients with acute myocardial infarction are still uncertain: what are the effects of ischaemia–reperfusion injury during hyperoxia and normoxia in mature rats with and without cardiovascular risk factors?
- **What is the main finding and its importance?**
  Despite elevated baseline oxidative stress in rodents with cardiovascular risk factors, hyperoxic reperfusion limited myocardial necrosis and anti/pro-oxidant imbalance in spontaneously hypertensive and Zucker rats. In contrast, this effect was exacerbated in healthy Wistar rats. These results suggest that oxygen supplementation may not be harmful in patients with acute myocardial injury.

Evaluating myocardial IRI in mature animals with cardiovascular risk factors would represent an important step towards the implementation of experimental conditions that better resemble the clinical conditions of STEMI patients. To this end, we designed a large randomized, sham-controlled, parallel study to test the hypothesis that (1) oxidative stress is increased in rodents with cardiovascular risk factor as compared to their control counterparts, and (2) that an elevated baseline oxidative stress level enhances the toxicity of IRI in the presence of hyperoxia. Healthy Wistar rats (WR), spontaneously hypertensive rats (SHR) and obese Zucker rats (ZR), older than those that had been investigated previously (Harrison & Gongora, 2009; Liu et al., 2012), underwent an experimental STEMI followed by normoxic (NR) or hyperoxic reperfusion (HR). The balance between ROS production and antioxidant activity was assessed by means of biomarker changes in blood samples and myocardial infarction size was evaluated.

2 | METHODS

2.1 | Ethics approval

The experimental study was approved by the institutional ethics committee for animal well-being (CEBEA ULB – Agreement LA 1230334 Code 661N) and was in accordance with the Act of 14 August 1986 on the protection and welfare of animals and the Royal Decree on

the protection of experimental animals of 29 May 2013 (Belgium). This work complies with the animal ethics checklist of *Experimental Physiology*.

2.2 | Housing and husbandry

Three male adult rat models were utilized: WR, obese ZR and SHR. WR and SHR were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and ZR from Charles Rivers Laboratories (Saint Germain Nuelles, France). All animals were reared and transported under conditions specified in the EU Council Regulation No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations. They had free access to standard rodent food except for ZR, which were fed with a high-fat diet from weaning (Rodent Diet with 45 kcal% fat from Research Diets, Inc., New Brunswick, USA). WR were used as controls in our study. Obese ZR were used to assess the effect of obesity, hypertriglyceridaemia and a high level of insulin on the heart, which are independent risk factors for coronary artery diseases (Bugger & Abel, 2009). SHR were used as a model of systemic hypertension, atherosclerosis and hypertensive cardiomyopathy (Doggrell & Brown, 1998).

2.3 | Sample size analysis

We estimated the number of rats required for our study according to a previous publication where significant differences in the size of myocardial infarcts were observed when hyperoxia was administered before induction of myocardial ischaemia in groups of 11 animals ($P \leq 0.05$) (Pourkhalili et al., 2012). We expected an animal loss of 5% (Samsamshariat et al., 2005), so the number of animals needed per experimental arm was 12.

2.4 | Animal preparation and anaesthesia

Mature male WR, SHR and ZR were anaesthetized with isoflurane in an induction chamber. The animals were intubated with a 16-G (human intravenous) catheter that was connected to an invasive mechanical ventilation system (UNO Micro Ventilator-03 machine, Zevenaar, The Netherlands) with 2% isoflurane in ambient air. Respiratory parameters (respiratory rate, tidal volume and positive end expiratory pressure) were adjusted according to animal weight and capnometer values to maintain end-tidal P_{CO_2} between 30 and 35 mmHg (CapnoTrue system from Carfill Quality (Oud-Turnhout, Belgium) and pulse oximeter). Animals were placed on a rat rigid thermostatic pad. Rectal temperature was maintained between 37°C and 37.5°C. Rats received a subcutaneous injection of buprenorphine 0.01 mg/kg above the right anterior thigh muscle. Subcutaneous needles were inserted in the four limbs to obtain instantaneous ECG (DI, DII and DIII) (Rodent Surgical Monitor from Uno (Zevenaar, The Netherlands)). The left femoral artery was cannulated for the measurement of arterial pressure and blood sampling. Vital parameters were recorded and analysed with a data acquisition system (Powerlab 8/35 and LabChart Pro from ADInstruments, Oxford, United Kingdom).

2.5 | Surgery and ischaemia–reperfusion

Pectoral muscles were incised between the fourth and fifth left ribs to gain access to the thoracic cavity. The pericardium was dissected and opened, and a 5/0 silk suture was placed into the myocardium under the left anterior descending (LAD) coronary artery approximately 2 mm distal to its eminence behind the pulmonary trunk. The animals were allowed 10 min of stabilization and were assigned to either the sham group or the ischaemic group (Figure 1). The unligated thread was kept in the myocardium for 4 h in the sham group. The LAD coronary artery was tied in a reversible way in the ischaemic group and ischaemia was maintained for 60 min. Ischaemia was confirmed by an elevation of ST segment on the ECG (DI) and/or the fading of the myocardial anterior wall. After ischaemia, the suture was loosened for a 3 h reperfusion period while animals were randomized either in the HR group (100% O_2) or the room air (normoxic) reperfusion group. Reperfusion was confirmed by the recolouring of the anterior wall and/or the reduction of ST elevation (on DI). Animals were monitored during the whole procedure. Animals were killed at the end of procedure by exsanguination.

2.6 | Exclusion criteria

Animals that developed ventricular tachycardia and rats whose S_{pO_2} decreased below 90% during the ischaemic period were excluded from the study. Animals in the hyperoxic group with S_{pO_2} below 99% were excluded from the study. Animals that did not reach the end of the reperfusion period were excluded for infarct size and oxidative stress analyses. Mortality rate and the moment and cause of death were recorded.

2.7 | Blood sample

Blood samples (2 ml) were taken from the right femoral artery before surgery and at the end of the experiment. The blood samples were centrifuged immediately following collection (14 min at 3000 *g*) and plasma was stored at −80°C until analysis.

2.8 | Measurements

2.8.1 | Biological assessment of infarct size

Cardiac troponin T (cTnT) was determined by the ELISA troponin T assay from Roche Diagnostics (Basel, Switzerland) as a surrogate for myocardial infarction size.

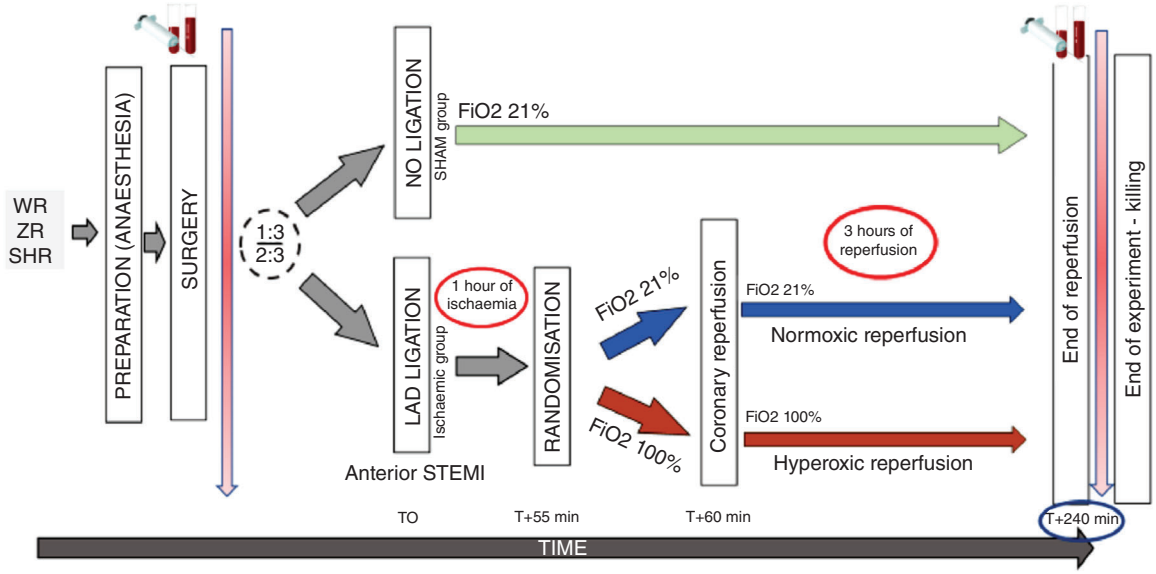

FIGURE 1 Study design. In each arm (WR, ZR and SHR), a third of the animals were allocated in the sham group (surgery but no LAD coronary artery ligation; pale green arrow) and two-thirds in the ischaemic group that underwent a LAD coronary artery ligation for 60 min. The animals were randomized to either the isocapnic normoxic arm (blue arrow) or the isocapnic hyperoxic reperfusion arm (brown arrow). LAD, left anterior descending; SHR, spontaneously hypertensive rat; WR, Wistar rats; ZR, Zucker rats

2.8.2 | Oxidative stress biomarkers

Given the evanescent nature of ROS, we measured stable by-products resulting from oxidative stress that have entered the circulation. Malondialdehyde (MDA) and myeloperoxidase (MPO) activity were measured as surrogates for oxidative stress level.

Lipid peroxidation with unsaturated lipids generates a variety of oxidation products. Among these, MDA is the most mutagenic product of lipid peroxidation and has been extensively studied as a biomarker of oxidative stress for its simple reaction with thiobarbituric acid (TBA) (Esterbauer & Cheeseman, 1990). The TBA-reacting substances test (TBARS) is centred on the reactivity of TBA toward MDA to generate a strong coloured chromogen fluorescent red complex that is measured by fluorometry (Conti et al., 1991).

MPO is an enzyme expressed in polymorphonuclear neutrophils and macrophages and is released into extracellular fluid during inflammation. MPO catalyses the conversion of chloride and hydrogen peroxide to hypochlorite (Loria et al., 2008) and serves as a marker of oxidative stress. As shown in the CAPTURE trial, MPO serum levels in patients with acute coronary syndrome powerfully predict an increased risk for cardiovascular events and prognosis (Baldus et al., 2003). MPO acting as a master enzyme in generation of ROS was measured by ELISA (HycultBiotech Rat MPO HK105, Uden, The Netherlands).

Gluthathione peroxidase (GPx) is an antioxidant enzyme that reduces hydrogen peroxide to water and oxygen. It also reduces lipid hydroxyperoxide to alcohols and oxygen and oxidizes glutathione to glutathione disulphide. Yoshida et al. (1997) showed that *GPx1* gene knockout mice were more susceptible to myocardial IRI compared to a non-transgenic control group. Free total thiols were measured as a surrogate for GPx by spectrophotometry at 412 nm according to Riddles et al. (1979) using Ellman's reagent method.

2.9 | Statistical analysis

The statistical analysis was performed using Statistix 9 (Analytical Software, Tallahassee, FL, USA). The normality of the variable distribution was checked using the Shapiro–Wilk test. The variables were presented as means ± standard deviation (SD), if Gaussian, or median (interquartile range), if non-Gaussian. A chi-square test was performed to test relationships between categorical variables. Thereafter, data were analysed using a three-factor analysis of variance (phenotype, group and time) with repeated measures on time and interaction phenotype × group. When $F < 0.05$, pairwise comparisons for repeated measures on time (before and after) were made using either a modified paired Student's *t*-test or Wilcoxon's signed-rank test. A Bonferroni correction for multiple comparisons was applied (nine comparisons). To study changes in biological markers over time, we performed linear regression analyses (time; independent and biological variables; dependent variable) and realized pairwise comparison of variable–time coefficient of regression (slopes) using Student's *t*-test (Altman et al., 2000). A *P*-value <0.05 was considered significant.

3 | RESULTS

3.1 | Baseline characteristics of the three strains

WR were older than SHR and ZR, while ZR were older than SHR. ZR and WR were heavier than SHR and ZR had hypertriglyceridaemia.

TABLE 1 Baseline characteristics of the three strains of rats used in this study

	WR[1] ($n = 41$)	SHR[2] ($n = 29$)	ZR[3] ($n = 33$)
Age (months)	9.0 (7.0–10.0)	6.0 (6.0–8.3)[1 vs. 2]	7.0 (6.0–8.0)[1 vs. 3, 2 vs. 3]
Weight (mg)	590 (553–646)	436 (419–485)[1 vs. 2]	670 (622–716)[2 vs. 3]
Systolic arterial pressure (mmHg)	146 (120–174)	165 (120–197)	188 (169–215)[1 vs. 3]
Heart rate (beat/min)	284 (268–310)[1 vs. 2]	244 (216–270)	254 (230–288)[1 vs. 3]
Troponin T (ng/l)	13 (9–20)	42 (33–60)[1 vs. 2]	33 (23–53)[2 vs. 3]
MDA (nmol)	0.10 (0.04)	0.09 (0.06)	0.14 (0.10)[2 vs. 3]
Total MPO (ng/ml)	2889.7 (932.1)	2684.0 (681.7)	4479.7 (728.0)[1 vs. 3, 2 vs. 3]
Free total thiols (μM)	222.6 (213.9–240.5)	40.2 (5.3–60.2)[1 vs. 2]	82.3 (17.5–235.7)[2 vs. 3]

Both paired *t*-test and Wilcoxon signed rank test were used. Variables are shown as mean (standard deviation) or median (interquartile range). Pairwise tests for WR versus SHR (1 vs. 2), WR versus ZR (1 vs. 3) and SHR versus ZR (2 vs. 3) were $P < 0.05$. WR were older than SHR and ZR. ZR were older than SHR. The ZR were more obese than SHR and more hypertensive than WR. Baseline troponin T was larger in SHR than ZR and WR. ZR had higher level of triglycerides than SHR (1062 (683–2459) vs. 87 (64–110) mg/dl, respectively, with $P < 0.05$). Triglycerides were not measured in WR. Total MPO was more elevated in ZR than SHR and WR. Oxidative stress (malondialdehyde and myeloperoxidase) was higher in ZR than SHR and total thiols were lowest in SHR. SHR, spontaneously hypertensive rats, WR, Wistar rats, ZR, Zucker rats.

TABLE 2 Baseline measurements within strains

	SHAM[A]		NR[B]		HR[C]	
	n	Mean (SD)/median (IQR)	*n*	Mean (SD)/median (IQR)	*n*	Mean (SD)/median (IQR)
WR						
Troponin T (ng/l)	11	7 (6–19)	17	13 (10–18)	14	19 (12–34)
MDA (nmol)	7	0.08 (0.02)	8	0.10 (0.05)	6	0.12 (0.04)
Thiols (μM)	12	212.2 (2.0–223.5)	10	237.7 (222.6–258.3)[AB]	7	238.3 (219.2–240.5)
Total MPO (ng/ml)	10	3546.5 (1173.5)	8	2520.9 (345.5)[AB]	7	2373.1 (243.0)[AC]
SHR						
Troponin T (ng/l)	7	40 (23–76)	11	42 (31–59)	10	48 (36–65)
Triglycerides (mg/dl)	8	78.5 (44.5–98.5)	9	80 (64–100)	9	99 (88–177)
MDA (nmol)	9	0.09 (0.04)	11	0.07 (0.06)	10	0.10 (0.08)
Thiols (μM)	9	5.3 (4.7–32.2)	11	51.7 (5.0–93.6)	10	48.8 (37.6–94.2)
Total MPO (ng/ml)	9	2360.7 (493.2)	11	2720.0 (598.6)	10	2935.3 (840.6)
ZR						
Troponin T (ng/l)	8	46 (29.5–61.5)	13	32 (24–42)	10	27.5 (23–68)
Triglycerides (mg/dl)	7	1468 (399–4338)	13	844 (683–1323)	11	1256 (717–2643)
MDA (nmol)	9	0.20 (0.04)	12	0.11 (0.11)	10	0.13 (0.09)
Thiols (μM)	9	17.5 (11.7–233.3)	12	183.4 (44.5–237.9)	10	79.6 (41.7–171.8)
Total MPO (ng/ml)	9	4870.0 (411.8)	12	4359.9 (744.1)	11	4291.0 (839.4)

Both a paired *t*-test and Wilcoxon signed rank test were performed. Variables are shown as mean (standard deviation) or median (interquartile range). Pairwise tests were $P < 0.05$ for sham versus NR (A vs. B), sham versus HR (A vs. C) and NR versus HR (B vs. C). Baseline troponin T, MDA, total MPO and thiols were not different between sham, normoxic reperfusion (NR) and hyperoxic reperfusion (HR) animals within the same strain, except for total MPO that was higher in sham-WR than in NR-WR and HR-WR and higher thiols in NR-WR than in sham-WR. MDA, malondialdehyde; MPO, myeloperoxidase; SHR, spontaneously hypertensive rats; WR, Wistar rats; ZR, Zucker rats.

Basal heart rate was higher in WR than in the two other strains and ZR were hypertensive compared to WR (Table 1). In SHR and ZR, the two strains with cardiovascular risk factors, baseline cTnT was increased compared to WR and they were in a pro-oxidant state (Table 1). Indeed, oxidative stress was increased in ZR as demonstrated by elevated MDA and total MPO. On the other hand, SHR had a lower total thiols concentration compared to WR and ZR (Table 1). Within a same strain, baseline cTnT, MDA, total MPO and thiols were not different between sham, NR and HR animals except for total MPO, which was higher in sham-WR than in NR-WR and HR-WR, and higher thiols were observed for NR-WR than in sham-WR (Table 2).

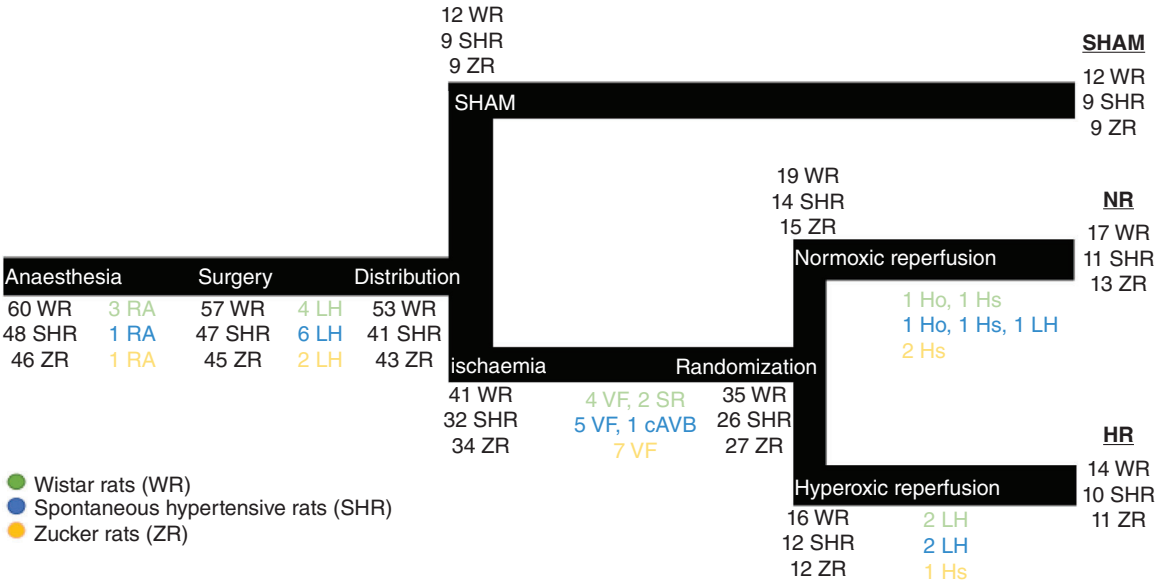

FIGURE 2 Study design and mortality. Chi-squared test of independence was used to assess the relationship between phenotype (WR, SHR and ZR) with mortality and occurrence of lethal ventricular fibrillation. No difference was observed in mortality between WR, SHR and ZR ($P = 0.521$). VF occurrence was similar in the three strains ($P = 0.470$). cAVB, complete A-V block; Ho, unexplained hypoxaemia; HR, hyperoxic reperfusion; Hs, haemodynamic shock; LH, lethal haemorrhage; NR, normoxic reperfusion; RA, respiratory arrest; SR, septal rupture; VF, ventricular fibrillation

3.2 | Clinical data and mortality during ischaemia–reperfusion

No difference between strains was observed for ischaemia-induced global mortality (28.3%, 37.5% and 28.3% in WR, SHR and ZR, respectively; $P = 0.521$) and mortality from ventricular fibrillation (9.8%, 15.6% and 20.6% in WR, SHR and ZR, respectively; $P = 0.470$) (Figure 2). Mortality rate during the reperfusion period was similar whatever the mode of reperfusion (100% O_2 vs. room air) (14.6 % and 12.5% in NR and HR, respectively; $P = 0.78$). A total of 43 WR, 30 SHR and 33 ZR underwent the entire experimental protocol (Figure 2). Systolic arterial pressure dropped after ligation of the LAD artery ($P < 0.05$ in all groups, except in HR-WR) but stabilized at 1 h after reperfusion, with no effect of the group ($P = 0.2$) or group–time interaction ($P = 0.6$) (Figure 3). There was no difference in heart rate between end of ischaemia and the first hour of reperfusion (Figure 4).

3.3 | Effects of ischaemia–reperfusion on cTnT

Time, strain, reperfusion mode and the interaction strain–reperfusion mode affected cTnT concentration ($P < 0.001$, $P < 0.001$, $P = 0.011$ and $P < 0.001$, respectively). Regression analysis of cTnT increases over time showed that cTnT increased in sham-operated rats and there were no differences between strains. Whatever the strain, ischaemia–reperfusion produced a larger rise in cTnT than the sham surgery (Tables 3 and 4). Whatever the mode of reperfusion, the procedure induced a more severe cTnT elevation in SHR than in ZR. Troponin rise did not differ between WR-NR and ZR-NR as well as between WR-HR and SHR-HR (Tables 3 and 4). In WR, HR induced a larger rise in cTnT than by NR (Tables 3 and 4 and Figure 5), while a reverse response was observed in SHR and ZR. In these latter two strains with cardiovascular risk factors, HR reduced the cTnT elevation compared to their respective NR arm (Tables 3 and 4 and Figure 5).

3.4 | Effects of ischaemia–reperfusion on oxidative stress

Time, strain, reperfusion mode and strain–reperfusion mode interaction influenced the total MPO measure (all at $P < 0.001$). When we compare WR and SHR, the two strains with similarly low total MPO at baseline, regression analysis of total MPO changes over time showed that ischaemia–reperfusion produced a larger total MPO rise in SHR than in WR, as well as a larger total MPO rise after NR compared to HR, whatever the strain. On the other hand, ischaemia–reperfusion induced a smaller increase of total MPO in ZR, the strain with high basal MPO, with no difference between the two modes of reperfusion (Tables 3 and 4). MDA was influenced by the strain ($P < 0.002$) and with interactions between the strain and the group ($P = 0.003$). Regression analysis of MDA changes over time showed that ischaemia–reperfusion induced an increase in MDA in SHR with a larger rise observed in the NR group compared to the sham and HR groups (Tables 3 and 4). Total thiols were affected by the strains and the groups (both $P < 0.001$). Regression analysis of changes in total thiols over time showed that NR was associated with a decrease in total thiols in the three strains. The decrease in total thiols

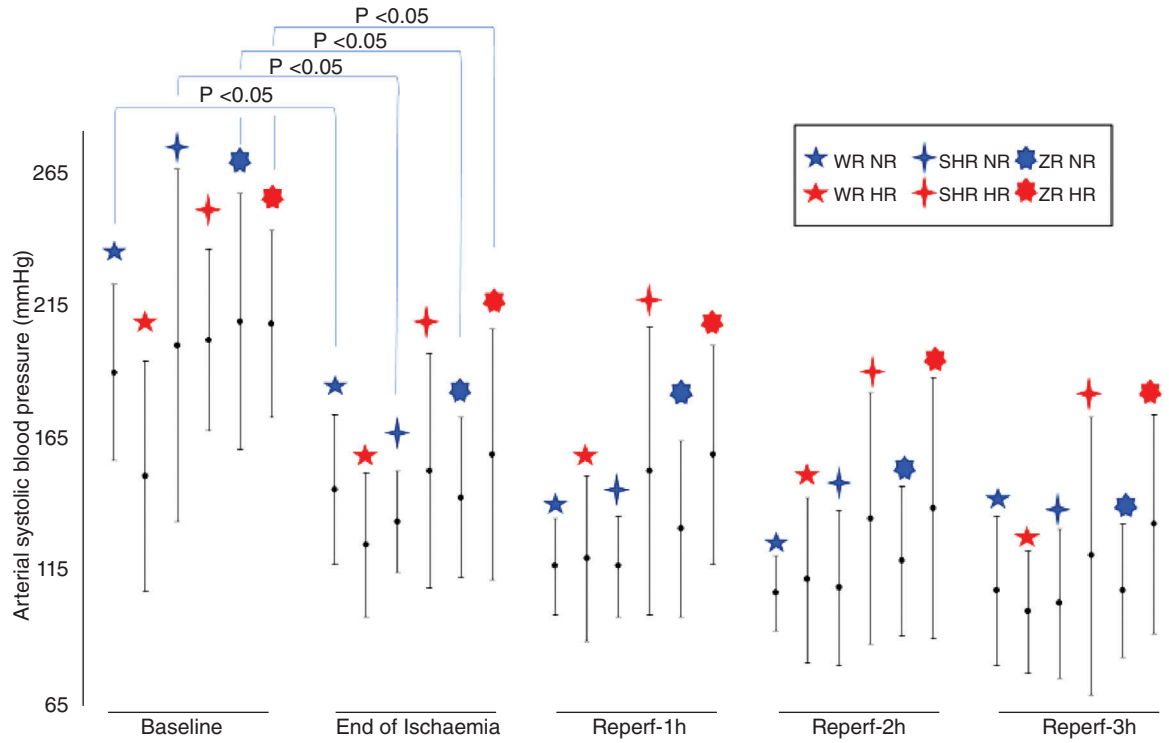

FIGURE 3 Arterial systolic blood pressure in ischaemic animals over time. Distributions of arterial blood pressure in WR, SHR and ZR at baseline were not normal. We presented mean ± standard deviation to facilitate graphical presentation. Non-Gaussian distribution of baseline values were taken into consideration for statistical analysis. A pairwise comparison using a Wilcoxon test is shown. Two-factor ANOVA (group and time) with repeated measures on time and interaction group × time. Variable tested was systolic blood pressure. There was an effect of time on systolic blood pressure ($P = 1.83 \times 10^{-11}$). The systolic arterial pressure dropped after ligation of the LAD ($P < 0.05$ in all groups, except in WR-HR) but stabilized after 1 h reperfusion onset with no effect of the group ($P = 0.2$) or group × time interaction ($P = 0.6$). Baseline systolic blood pressure in WR did not differ between NR and HR ($P = 0.32$). HR, hypertensive rats; LAD, left anterior descending; NR, normoxic reperfusion; SHR, spontaneously hypertensive rats; WR, Wistar rats; ZR, Zucker rats

observed in ZR-HR animals was smaller than the response observed in ZR-NR animals (Tables 3 and 4).

4 | DISCUSSION

This study assessed the effects of cardiovascular risk factors on baseline oxidative stress in a large study involving three different rat strains, and the subsequent effects of normoxia and hyperoxia on myocardial IRI. The main finding of this study was that, contrary to our initial hypothesis, HR limited myocardial necrosis and anti/pro-oxidant imbalance in SHR and ZR in comparison to NR despite elevated baseline levels of oxidative stress relative to healthy WR.

Reducing myocardial reperfusion injury is an essential component of managing acute myocardial ischaemia. Many therapies have been studied and identified as cardioprotective to reduce myocardial IRI (Davidson et al., 2019). Myocardial IRI is associated with numerous clinical complications such as arrhythmias, myocardial stunning, microvascular obstruction and LV systolic dysfunction (Hausenloy & Yellon, 2013). It is therefore essential to treat and prevent reperfusion injury as intensively as ischaemia. Cardiovascular risk factors such as hypertension, obesity and diabetes are known to cause an excessive production of oxidative stress factors. This is the first study to assess the effect of hyperoxia on myocardial reperfusion injury in animals with cardiovascular risk factors.

4.1 | Myocardial infarction in experimental models with cardiovascular risk factors

Past experimental studies on myocardial reperfusion injury were mainly performed on young and healthy animals. However, ageing is associated with increased susceptibility to myocardial ischaemia, increased rates of mitochondrial free radical production and elevated mitochondrial DNA mutations (Hosseini et al., 2020; Lucas & Szweda, 1998). Hypertension is associated with decreased antioxidant capacity due to decreased superoxide dismutase and GPx activities in newly diagnosed patients (Pedro-Botet et al., 2000). Obesity is associated with increased oxidative stress (Marseglia et al., 2014). Hence, if mature rat strains with cardiovascular risk factors also demonstrate a heightened anti/pro-oxidant imbalance, then these animals are also more relevant for studies on myocardial reperfusion injury.

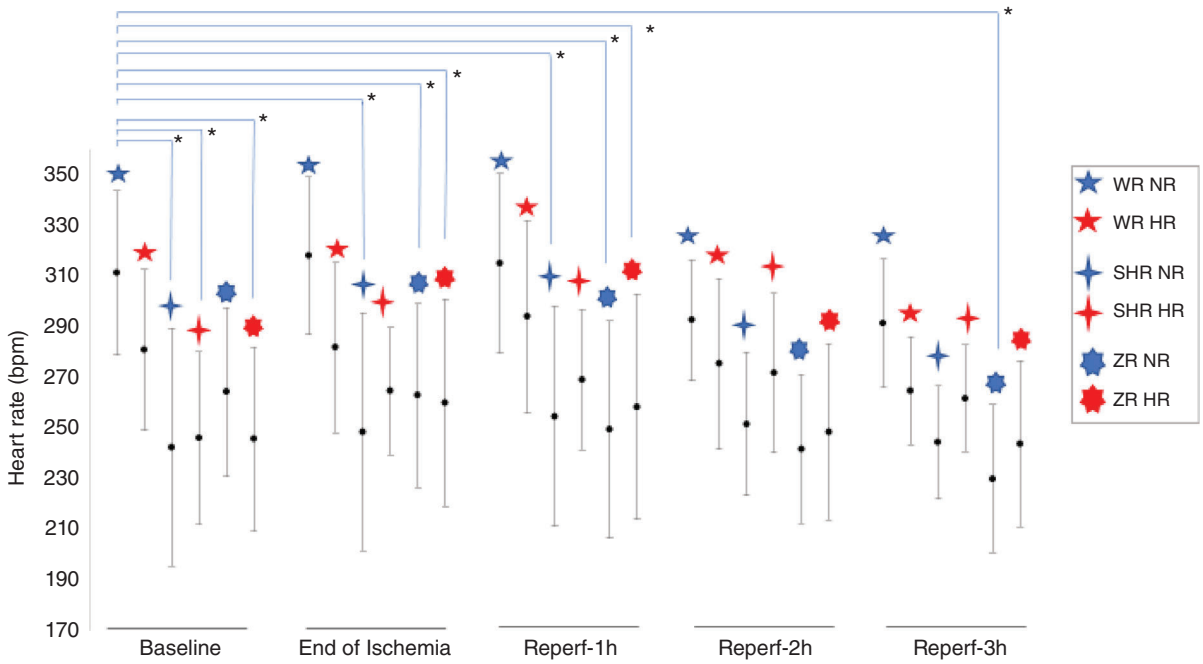

FIGURE 4 Heart rate in ischaemic animals over time. Two-way ANOVA (group, time and interaction group–time) with repeated measurement on time revealed that there was an effect of group, time and interaction group × time on heart rate ($P = 1.74 \times 10^{-5}$, $P = 1.70 \times 10^{-4}$ and $P = 1.67 \times 10^{-2}$, respectively). Pairwise comparisons were performed using a *t*-test. There was no difference in heart rate between baseline and the end of ischaemia in all groups. There was no difference in heart rate between end of ischaemia and the first hour of reperfusion. Heart rate increased in SHR HR between baseline and the first hour of reperfusion ($P = 0.017$). In WR NR and ZR NR, heart rate decreased between baseline and the second hour of reperfusion ($P = 0.008$) and third hour of reperfusion ($P < 0.001$); it increased in SHR HR from baseline to the second hour of reperfusion ($P = 0.009$). There was a decrease of HR in WR NR and WR HR between the first and the third hour of reperfusion ($P = 0.009$ and $P = 0.002$, respectively). bpm, beats per minute; reperf, reperfusion; SHR HR, spontaneously hypertensive rats-hyperoxic reperfusion; SHR NR, spontaneously hypertensive rats–normoxic reperfusion; WR HR, Wistar rats–hyperoxic reperfusion; WR NR, Wistar rats–normoxic reperfusion; ZR HR, Zucker rats–hyperoxic reperfusion; ZR NR, Zucker rats–normoxic reperfusion. *$P < 0.05$. Variables are shown as means ± standard deviation

In this study, WR were the oldest and ZR were older than SHR. ZR and SHR had higher baseline oxidative stress levels relative to mature WR. Thus, in the rat strains investigated here, cardiovascular risk factors appeared more deleterious than ageing *per se* on oxidative stress. Baseline thiols were lower in SHR than in WR, which is in line with the study of Pedro-Botet et al. (2000) where newly diagnosed hypertensive patients had a lower level of GPx. ZR are a pertinent model for metabolic syndrome (Marseglia et al., 2014). In our study, ZR had highly elevated triglyceride levels (10- to 15-fold higher than either SHR or WR). We did not assess triglycerides in WR since this has already been previously reported. Normal values reported by the animal supplier are ±120 mg/dl (Burgeiro et al., 2017; Eleftheriades et al., 2014; Karami et al., 2014). It has been shown that obese patients present higher MPO (Borato et al., 2016), increased MDA (Sankhla et al., 2012) and decreased glutathione (Goyal et al., 2011) as compared to non-obese patients. This is analogous to our results where ZR had higher baseline total MPO and MDA compared to SHR and WR.

Dobrian et al. (2001) showed that a high-fat diet (32 kcal% fat) induced obesity and mild hypertension in 50% of obesity-prone versus obesity-resistant Sprague–Dawley rats. The obesity-prone group had higher body weight, mild hypertension and higher level of MDA as compared with obese-resistant rats. Moreover, Friedman (Friedman et al., 2003) and his team compared oxidative stress levels in WR, SHR, non-insulin-dependent Cohen diabetic rats and Cohen–Rosenthal diabetic hypertensive rats. These authors assessed lipid peroxides by spectrophotometry and lipid oxidability. Lipid peroxide formation and lipid oxidability were more marked in SHR than WR.

Our present study is in agreement with previous evidence of higher baseline oxidative stress levels in animals with cardiovascular risk factors. We further observed that myocardial infarction resulted in a greater MPO increase in SHR than ZR. Therefore, our data suggest that hypertension-related atherosclerosis enhanced further myocardial damage after STEMI more than obesity, hyperlipaemia and diabetes in ZR. Importantly, troponin I and T are markers of myocardial damage and can be chronically elevated in patients with cardiovascular risk factors and without pre-existing cardiac disease. Indeed, obesity is independently associated with elevated troponin in human studies (Ndumele et al., 2014). Likewise in the study of Aeschbacher et al. (2015), troponin I was independently associated with systolic blood pressure and left ventricular hypertrophy in a young and healthy population. In the two strains with cardiovascular risk factors we investigated, we observed that baseline cTnT was increased relative to WR.

TABLE 3 Regression lines for biological variables of strains

		Regression line	Slope SE
Troponin			
WR	SHAM	$y = 1036.60x - 1076.28$	73.30
	NR	$y = 2171.29x - 2255.99$	153.53
	HR	$y = 2979.28x - 3101.41$	156.80
SHR	SHAM	$y = 1254.18x - 1145.77$	80.62
	NR	$y = 4106.93x - 3839.05$	290.40
	HR	$y = 2956.44x - 3041.47$	209.05
ZR	SHAM	$y = 1268.32x - 1279.39$	89.68
	NR	$y = 2256.10x - 2324.65$	173.37
	HR	$y = 1660.40x - 1707.11$	117.41
Total MPO			
WR	SHAM	$y = 498.02x + 3025.57$	35.21
	NR	$y = 496.04x + 2002.64$	35.08
	HR	$y = 314.85x + 2043.98$	22.26
SHR	SHAM	$y = 946.53x + 1371.87$	66.93
	NR	$y = 1113.86x + 1609.02$	78.76
	HR	$y = 599.01x + 2309.04$	42.36
ZR	SHAM	$y = 409.90x + 4441.65$	28.98
	NR	$y = 134.65x + 4219.29$	9.52
	HR	$y = 166.34x + 4117.19$	11.76
MDA			
WR	SHAM	$y = 5.94 \times 10^{-3}x + 7.58 \times 10^{-2}$	4.20×10^{-4}
	NR	$y = -6.93 \times 10^{-3}x + 0.10$	4.93×10^{-4}
	HR	$y = -1.19 \times 10^{-2}x + 0.13$	8.40×10^{-4}
SHR	SHAM	$y = 5.94 \times 10^{-3}x + 8.18 \times 10^{-2}$	4.20×10^{-4}
	NR	$y = 4.16 \times 10^{-2}x + 2.35 \times 10^{-2}$	2.94×10^{-3}
	HR	$y = 9.90 \times 10^{-4}x + 0.11$	7.00×10^{-5}
ZR	SHAM	$y = -2.48 \times 10^{-2}x + 0.23$	1.75×10^{-3}
	NR	$y = -2.57 \times 10^{-2}x + 0.14$	1.82×10^{-3}
	HR	$y = -3.17 \times 10^{-2}x + 0.16$	2.24×10^{-3}
Total thiol			
WR	SHAM	$y = -1.49x + 213.75$	0.11
	NR	$y = -2.87x + 240.70$	0.20
	HR	$y = 6.44x + 231.57$	0.46
SHR	SHAM	$y = 1.39x + 3.85$	0.10
	NR	$y = -27.72x + 80.67$	1.96
	HR	$y = 2.48x + 46.21$	0.18
ZR	SHAM	$y = -.30x + 17.81$	2.10
	NR	$y = -90.79x + 278.28$	6.42
	HR	$y = -6.34x + 86.22$	0.45

Regression lines of troponin T, total MPO, MDA and total thiol in all groups. HR, hyperoxic reperfusion; NR, normoxic reperfusion; SE, standard error; HR, spontaneously hypertensive rats; WR, Wistar rats; *x*, time (independent variable); *y*, biological variable (dependent parameter); ZR, Zucker rats.

Compared to younger and healthy animals without cardiovascular risk factors assessed previously, our data indicate that rat strains with cardiovascular risk factors better resemble the anti/pro-oxidant imbalance of patients at risk for myocardial infarction. This is in agreement with previous work (Dobrian et al., 2001; Friedman et al., 2003).

4.2 | Oxygen therapy and myocardial infarction

Oxygen therapy in patients critically ill with STEMI (Lellouche, 2018) is a continuing concern as studies have been contradictory to date. Although the AVOID study (Stub et al., 2012) concluded that hyperoxia was associated with more extensive myocardial infarction (assessed in grams), this was opposed by the observation that myocardial infarct size normalized to left ventricular mass did not differ between normoxic or hyperoxic conditions (Bulluck & Hausenloy, 2016).

Our study showed the unanticipated finding that HR limited myocardial necrosis and anti/pro-oxidant imbalance in rats with cardiovascular risk factors versus NR, as compared to healthy WR. Whether ischaemic preconditioning played a role in our findings is unclear. It is widely known that pre-conditioning (short episodes of ischaemia–reperfusion before sustained ischaemia) and post-conditioning (short ischaemia–reperfusion cycles at reperfusion onset) reduce myocardial infarct size by lessening IRI (Xia et al., 2016). It has been predicted that 0.2–2% of oxygen consumed in the cell is emitted as ROS (Balaban et al., 2005) and ROS plays an essential role in IRI. However, ROS generated by the mitochondrial respiratory chain can also trigger conditions resembling preconditioning and postconditioning by several pharmacological mechanisms (Kalogeris et al., 2014; Penna et al., 2009). This dual-nature role of ROS, and especially its ability to induce cell survival pathways that increase the resistance of tissues and organs to the detrimental effects of IRI, has been reported for cardiomyocytes *in vitro*, isolated organs and healthy animals. However, preconditioning and postconditioning in animals with comorbidities has not be proven to be either beneficial or efficient (Ferdinandy et al., 2007). Indeed, when the left main coronary artery was occluded for 35 min followed by 120 min of reperfusion in excised hearts of SHR and WR in a Langendorff perfusion system, preconditioning was absent in SHR through independent contributions of both hypertension and ageing (Ebrahim et al., 2007). In a clinical study, preconditioning elicited by prodromal angina was lessened in patients with hypertension in cases where left ventricular hypertrophy inhibited ischaemic preconditioning effects upon acute myocardial infarction (Takeuchi et al., 2011). Likewise, diabetic patients were unresponsive to both preconditioning (Ishihara et al., 2001; Kristiansen et al., 2004) and postconditioning (Przyklenk et al., 2011).

While these observations reinforce our belief that studies in older animals with cardiovascular risk factors are more relevant to understand human cardiac ischaemic physiopathology, our study remains nevertheless in overall agreement with previous findings in healthy animals. For example, in rabbits, HR after a 45 min occlusion of the anterolateral marginal coronary artery did not increase myocardial

TABLE 4 Pairwise comparison of variable-time coefficient of regression (slopes) between strains

		WR			SHR			ZR	
Troponin		**SHAM**	**NR**	**HR**	**SHAM**	**NR**	**HR**	**SHAM**	**NR**
WR	NR	0.004							
	HR	0.001	<0.001						
SHR	SHAM	0.118	0.007	0.001					
	NR	0.001	0.005	<0.001	0.002				
	HR	0.002	0.004	0.932	0.003	<0.001			
ZR	SHAM	0.115	0.008	0.002	0.908	0.002	0.003		
	NR	0.004	0.729	<0.001	0.007	0.007	<0.001	0.008	
	HR	<0.001	<0.001	0.004	0.045	0.026	0.007	<0.001	<0.001
MDA		**WR**			**SHR**			**ZR**	
		SHAM	**NR**	**HR**	**SHAM**	**NR**	**HR**	**SHAM**	**NR**
WR	NR	<0.001							
	HR	<0.001	0.008						
SHR	SHAM	1.000	<0.001	<0.001					
	NR	0.001	<0.001	<0.001	0.001				
	HR	0.001	<0.001	<0.001	0.001	<0.001			
ZR	SHAM	<0.001	0.002	0.004	<0.001	<0.001	<0.001		
	NR	<0.001	0.001	0.004	<0.001	<0.001	<0.001	0.713	
	HR	<0.001	0.001	0.002	<0.001	<0.001	<0.001	0.071	0.108
Total MPO		**WR**			**SHR**			**ZR**	
		SHAM	**NR**	**HR**	**SHAM**	**NR**	**HR**	**SHAM**	**NR**
WR	NR	0.969							
	HR	0.013	0.013						
SHR	SHAM	0.005	0.005	0.002					
	NR	0.003	0.003	0.002	0.180				
	HR	0.140	0.134	0.005	0.013	0.006			
ZR	SHAM	0.125	0.131	0.060	0.003	0.002	0.022		
	NR	0.001	0.001	0.003	0.001	<0.001	0.001	0.002	
	HR	0.002	0.002	0.005	0.001	0.001	0.002	0.002	0.104
Free total thiols		**WR**			**SHR**			**ZR**	
		SHAM	**NR**	**HR**	**SHAM**	**NR**	**HR**	**SHAM**	**NR**
WR	NR	0.005							
	HR	<0.001	<0.001						
SHR	SHAM	<0.001	<0.001	0.001					
	NR	<0.001	<0.001	<0.001	<0.001				
	HR	<0.001	<0.001	0.002	0.007	<0.001			
ZR	SHAM	0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
	NR	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	
	HR	0.001	0.003	<0.001	<0.001	0.001	<0.001	<0.001	<0.001

Variables shown are *P*-values. Assessment of biological marker changes over time performed through linear regression (time, independent variable; and biological variable, dependent variable). Pairwise comparison of variable-time coefficient of regression (slopes) were performed using Student's *t*-test. HR, hyperoxic reperfusion; NR, normoxic reperfusion; SHR, spontaneously hypertensive rats; WR, Wistar rats; ZR, Zucker rats.

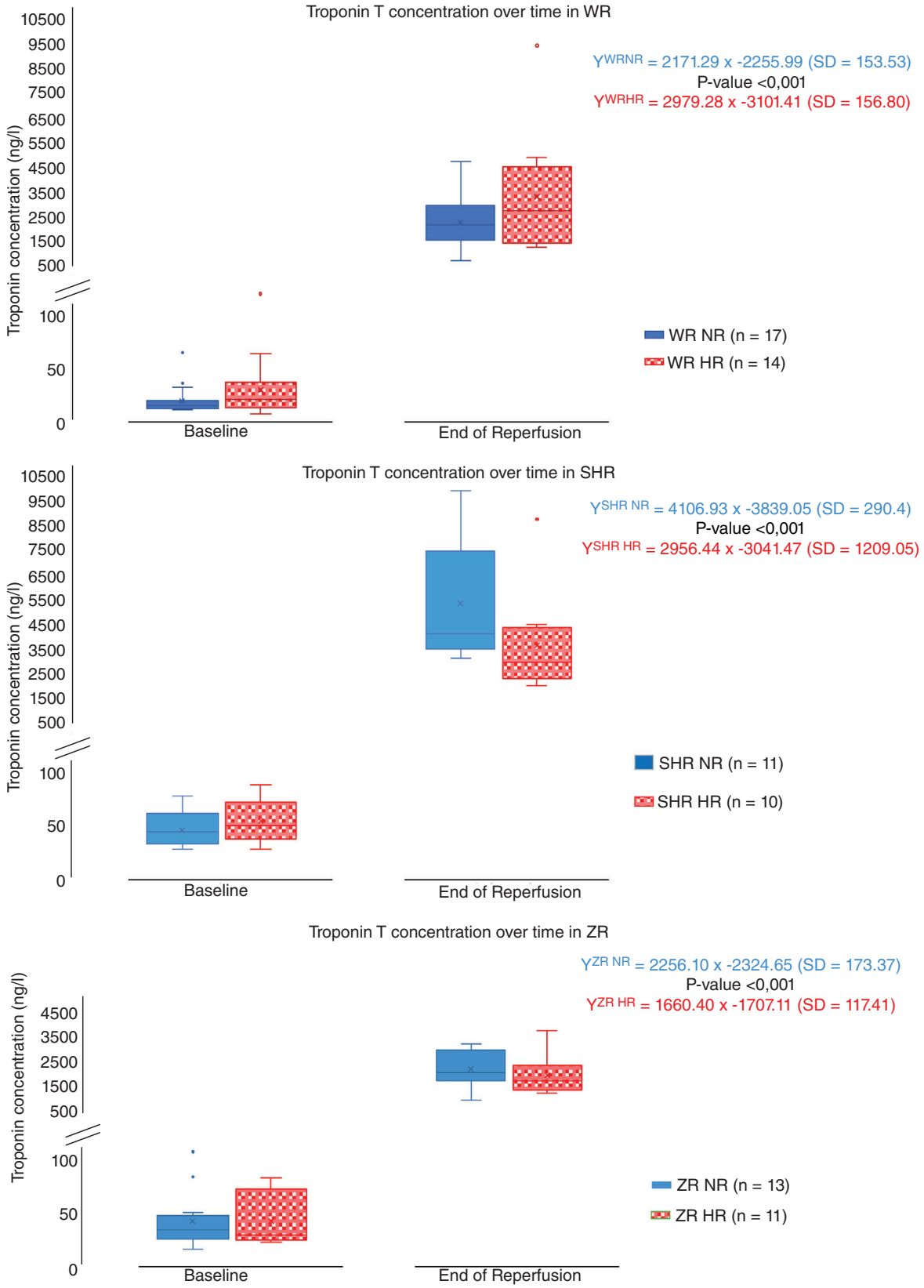

FIGURE 5 Troponin T concentration evolution slopes. Linear regression (time as the independent variable and troponin T as the dependent variable) and pairwise comparisons of troponin T-time coefficient of regression (slopes) were performed using Student's *t*-test. Myocardial infarct size was assessed by the rise in troponin T, which was greater with NR than HR in the animals with cardiovascular risk factors in contrast to Wistar rats. HR, hyperoxic reperfusion; NR, normoxic reperfusion; SHR, spontaneously hypertensive rats; WR, Wistar rats; ZR, Zucker rats

infarct size compared with NR (Shnier et al., 1991). In male mongrel dogs that underwent 90 min myocardial ischaemia by experimental occlusion of the left descending coronary artery followed by HR (100% F_{iO_2}) for 3 h, HR was associated with a smaller infarct size compared to ambient air reperfusion after 72 h (Kelly et al., 1995). Finally, in WR (Mariero et al., 2012), normobaric HR after 40 min myocardial ischaemia did not increase IRI.

In clinical studies, two reports showed no differences in patients with acute coronary syndrome who received hyperoxia or normoxia (Hofmann et al., 2017; Stewart, 2019). In a sub-group of STEMI patients, hyperoxia was associated with lower 30-day mortality compared to normoxia (8.8 vs. 10.6%, $P = 0.016$), suggesting that hyperoxia might even be beneficial (Stewart, 2019). These data, taken together with the results of this study, indicate that the possible hazards associated with hyperoxia in patients with myocardial infarction should be reconsidered (Ibanez et al., 2018; O'Gara et al., 2013).

5 | LIMITATIONS

Our study comports several limitations. The procedure used to induce STEMI in this study differs markedly from the spontaneous plaque rupture and subsequent coronary occlusion in patients. Also, the animals were not medicated similarly to STEMI patients, and all animals were fully anaesthetized. The experimental conditions differed completely from patients who experience a myocardial infarction. The mortality rate due to ventricular fibrillation in the ischaemic group was high (10–20%) and might be biased from the assessment of the final troponin concentration. A different rat strain was used to assess the effects of obesity but a high fat diet-induced obese WR may have been a better model for this study. The age of the animals within the rat strains differed. Despite WR being older, however, SHR and ZR had the highest levels of oxidative stress. Nevertheless, Abete et al. (1999) studied the tolerance of hearts from ZR aged 3, 6 and 12 months to oxidative stress through the infusion of ROS (hydrogen peroxide). They showed that there was no difference in haemodynamics, the occurrence of arrhythmia and oxidative stress level in hearts of rats between 6 and 12 months of age. Therefore, the age difference between WR (9 months) and SHR/ZR (6/7 months, respectively) is unlikely to affect our results. Another limitation may be that the follow-up after myocardial infarction was limited to 3 h. Finally, further studies are needed to confirm our observations.

6 | CONCLUSON

Contrary to our initial hypothesis, despite an elevated baseline oxidative stress level in rodents with cardiovascular risk factors, HR limited myocardial necrosis and anti/pro-oxidant imbalance in SHR and ZR, and the reverse was observed with healthy WR. These results may prove relevant for accumulating evidence that indicates the ineffectiveness of oxygen supplementation on clinical outcome in patients with a myocardial infarction.

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AUTHOR CONTRIBUTIONS

A.A. conducted the experiments and performed data analysis. M.B. and M.C. aided with the in vivo experiments. F.R., C.D., P.A. and T.F. contributed to the oxidative stress assessment. C.M. performed the statistical analysis and interpretation of the data. A.A., K.M. and P.B. designed the research, interpreted data and prepared the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Angela Acheampong https://orcid.org/0000-0003-1628-9446
Christian Mélot https://orcid.org/0000-0002-6172-0330
Mariam Benjelloun https://orcid.org/0000-0002-9514-8926

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