Dose-dependent effects of perfluorocarbon-based blood substitute on cardiac function in myocardial ischemia–reperfusion injury

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- A separate organizational unit, which serves as an innovation center on the one hand for the transfer of knowledge and technologies, and on the other as a research center.

Ischemia/Reperfusion injury

- Coronary heart disease is the leading cause of death and disability worldwide;
- The effects of coronary heart disease are usually attributable to the detrimental effects of acute myocardial ischemia-reperfusion injury;
- Ischemia-reperfusion injury typically arises in patients presenting with an acute ST-segment elevation myocardial infarction (STEMI);
- The most effective therapeutic intervention in those patients for reducing acute myocardial ischemic injury and limiting the size of myocardial infarction is timely and effective myocardial reperfusion using either thrombolytic therapy or primary percutaneous coronary intervention (PPCI).

Ischemia/Reperfusion injury

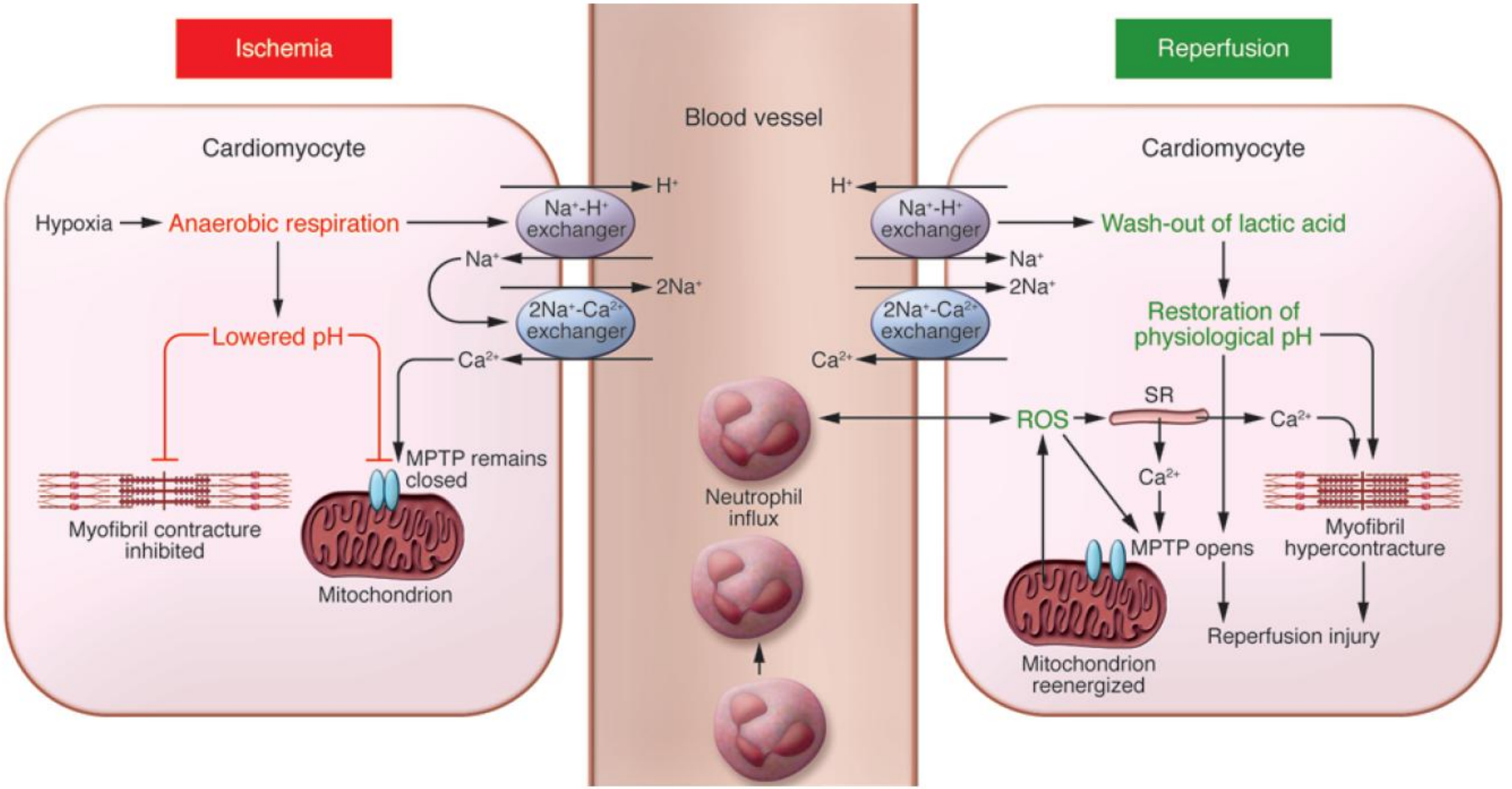
- The process of myocardial reperfusion can itself induce further cardiomyocyte death, a phenomenon known as **myocardial reperfusion injury**;

- Acute occlusion of the coronary artery in the STEMI patient subjects the myocardium supplied by that vessel to acute myocardial ischemia;

- If the period of acute myocardial ischemia is prolonged a “wave front” of cardiomyocyte death begins in the subendocardium and extends transmurally over time toward the epicardium;

- The deprivation of oxygen and nutrient supply results in a series of abrupt biochemical and metabolic changes within the myocardium.

Ischemia/Reperfusion injury



Ischemia/Reperfusion injury

- After the onset of acute myocardial ischemia in patients with STEMI, timely myocardial reperfusion is essential to salvage viable myocardium;
- On the other hand, the reperfusion of acutely ischemic myocardium can independently induce cardiomyocyte death;
- The four forms of myocardial reperfusion injury are recognized, the first two reversible and the second two irreversible: 1) *Reperfusion-induced arrhythmias,* 2) *Myocardial stunning,* 3) *Microvascular obstruction,* and 4) *Lethal myocardial reperfusion injury.*

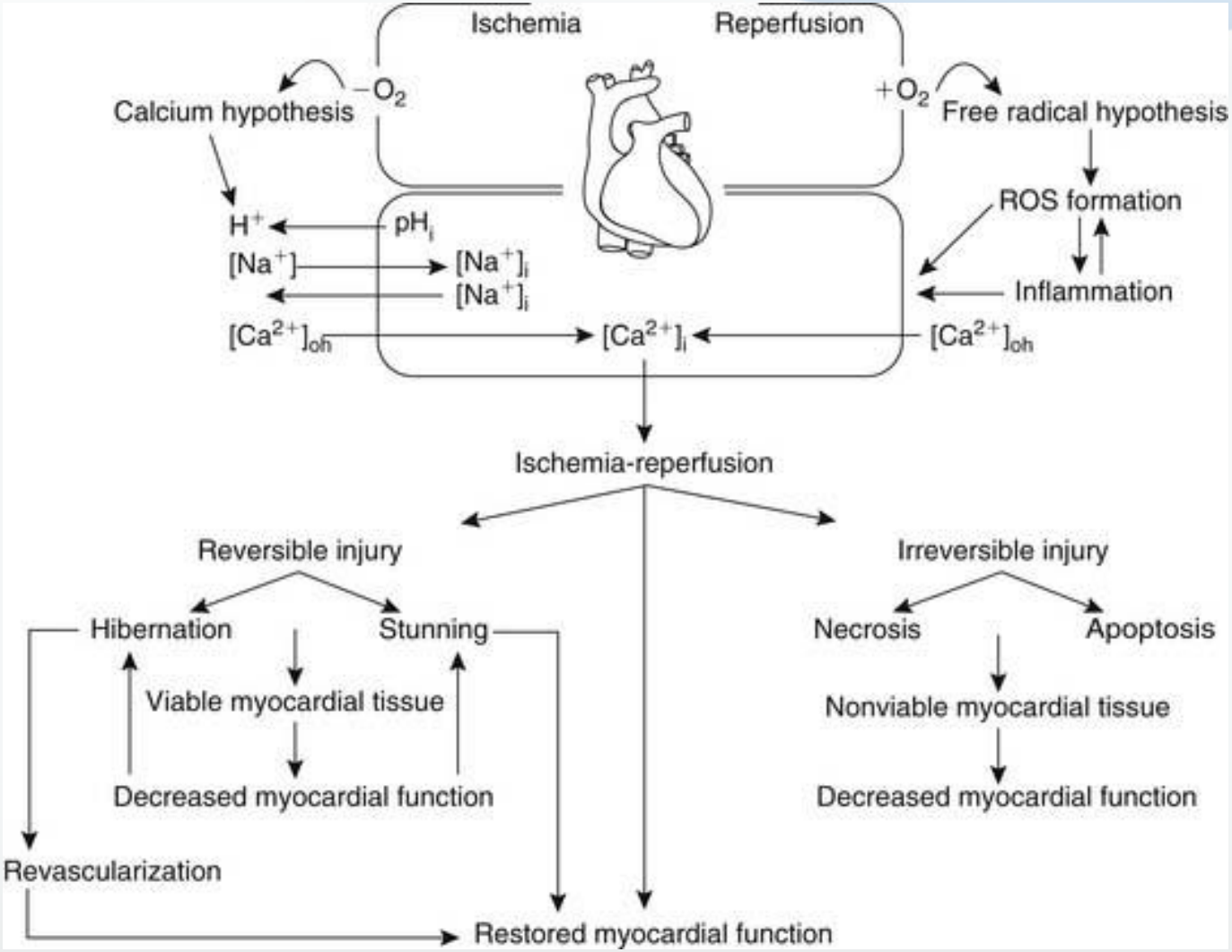
Ischemia/Reperfusion injury

- Experimental studies have identified several critical factors that act in concert to mediate the detrimental effects of myocardial reperfusion injury;

- In the first few minutes of myocardial reperfusion, a burst of **oxidative stress** is produced by a variety of sources, which mediates myocardial injury and cardiomyocyte death through a number of different mechanisms;

- **Intracellular and mitochondrial Ca^{2+} overload** begins during acute myocardial ischemia and is exacerbated at the time of myocardial reperfusion due to disruption of the plasma membrane, oxidative stress-induced damage to the sarcoplasmic reticulum, and mitochondrial re-energization.

Ischemia/Reperfusion injury



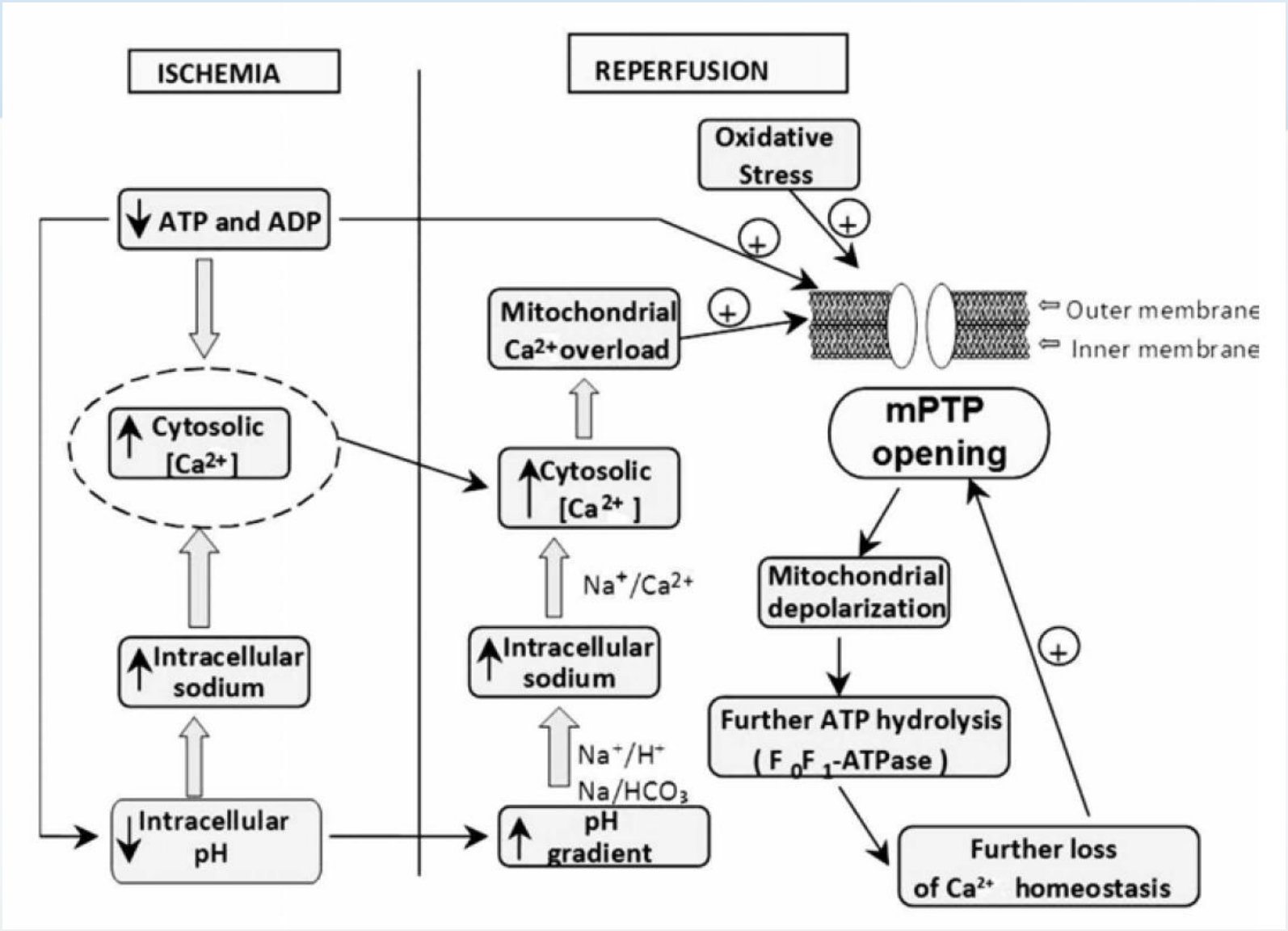
Ischemia/Reperfusion injury

- During acute myocardial ischemia the intracellular **pH decreases** to less than 7.0, whereas at reperfusion, physiological **pH is rapidly restored** by the washout of lactate and the activation of the Na^+-H^+ exchanger as well as the Na^+-HCO^- symporter;

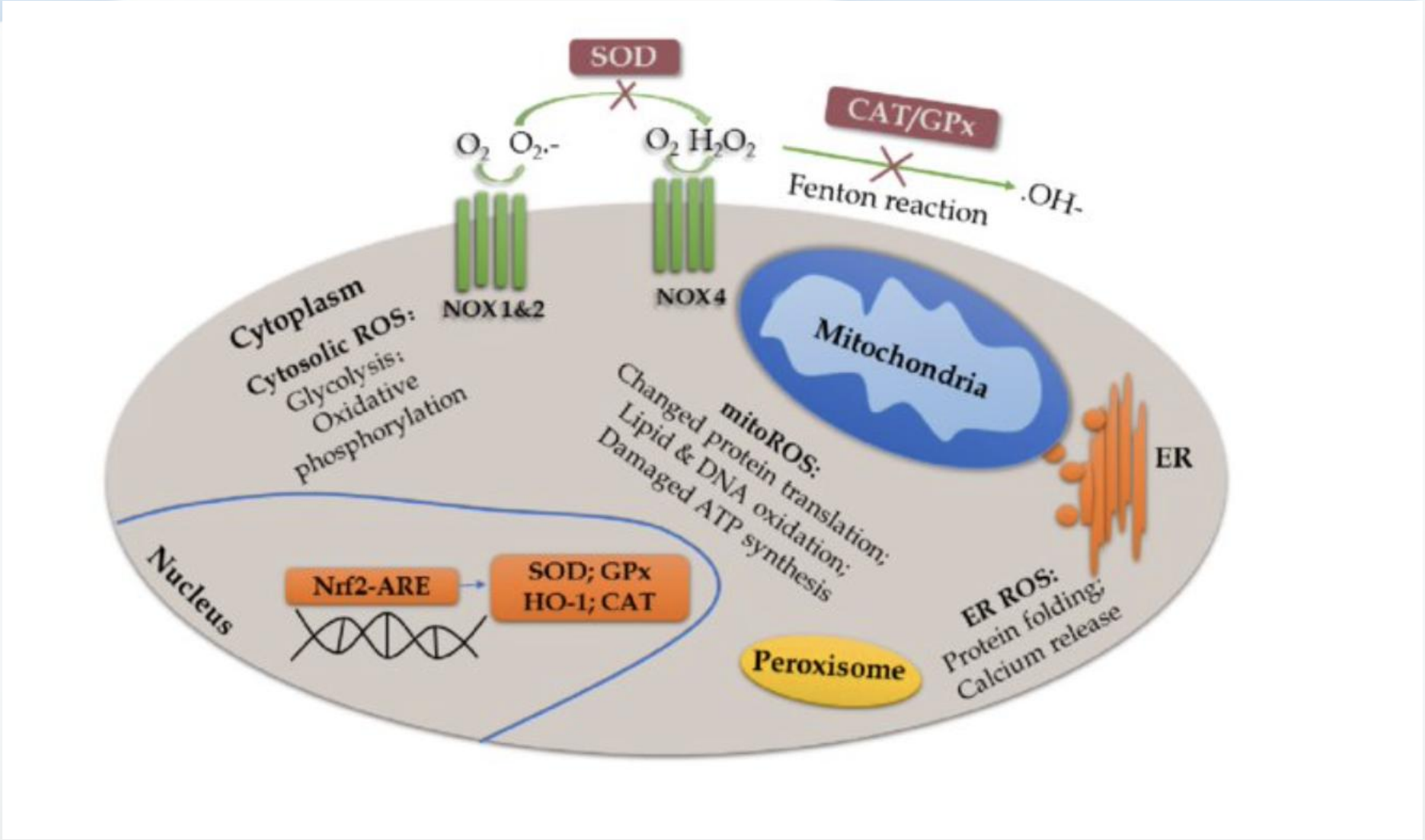
- This **pH shift** contributes to the cardiomyocyte death of lethal myocardial reperfusion injury by permitting MPTP opening and cardiomyocyte rigor hypercontracture in the first few minutes of reperfusion.

- The MPTP is a nonselective channel of the inner mitochondrial membrane, the opening of which results in **mitochondrial membrane depolarization and uncoupling of oxidative phosphorylation**, leading to ATP depletion and cell death

Ischemia/Reperfusion injury



Oxidative imbalance in ischemic conditions



AIMS OF THE STUDY

- The main goal of this study was to investigate the cardio-protective properties in terms of effects on cardiodynamics of perfluorocarbon emulsion (PFE) in ex vivo-induced ischemia–reperfusion injury of an isolated rat heart.

Methods

Experimental protocol

- All of the experimental procedures were carried out in accordance and with the permission of the Institutional Ethics Committee for Laboratory Animal Welfare.
- All animals were male, 10 weeks old, and weighed 200 ± 20 g on average.
- *Ex vivo* protocol using Langedorff apparatus

Perfluorocarbon emulsion

- 10% perfluoroemulsion (PFE) in different doses (8, 12, 16 ml/kg of body weight), and time (1, 10, or 20 h before ischemia as single administration)

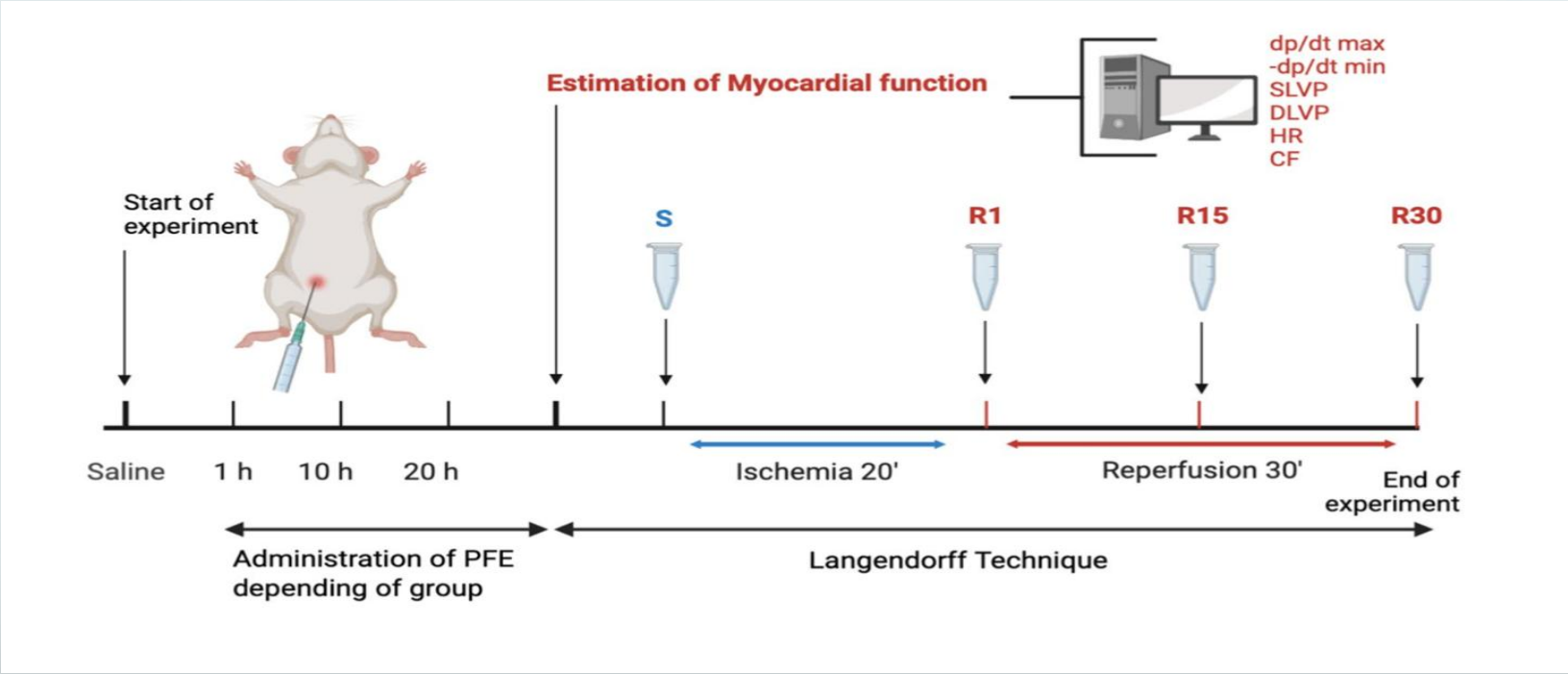
Table 1 Chemical composition of Perfluorocarbon emulsion

Name	Amount*
Perfluorodecalin	7 ml
Perfluoro-N-4- (methylcyclohexyl) -piperidine	3 ml
Proxanol-268	4,0 g
Sodium chloride (NaCl)	0,6 g
Potassium chloride (KCL)	0.039 g
Magnesium chloride ($MgCl_2$)	0.019 g
Sodium bicarbonate ($NaHCO_3$)	0.065 g
Sodium phosphate monobasic (NaH_2PO_4)	0.02 g
Glucose	0,2 g
Distilled water (H_2O)	100 ml
[F^-]	10 μM
Osmolality	280–310 mOsm/l
pH value	7.2–7.8
Viscosity	2.3 cPs
Average particle size	60–70 nm

*Total amount per 100 ml of PFE solution

Ex vivo ischemia/reperfusion protocol

- After the stabilization period, the Krebs–Henseleit solution flow was stopped for 20 min, thus subjecting the myocardium to global ischemia.
- After that, the flow and reperfusion (at 1, 5, 10, 15, 20, 25, and 30 min).



Results

The effects of a different regimen of 10% PFE at the same dose on cardiodynamics

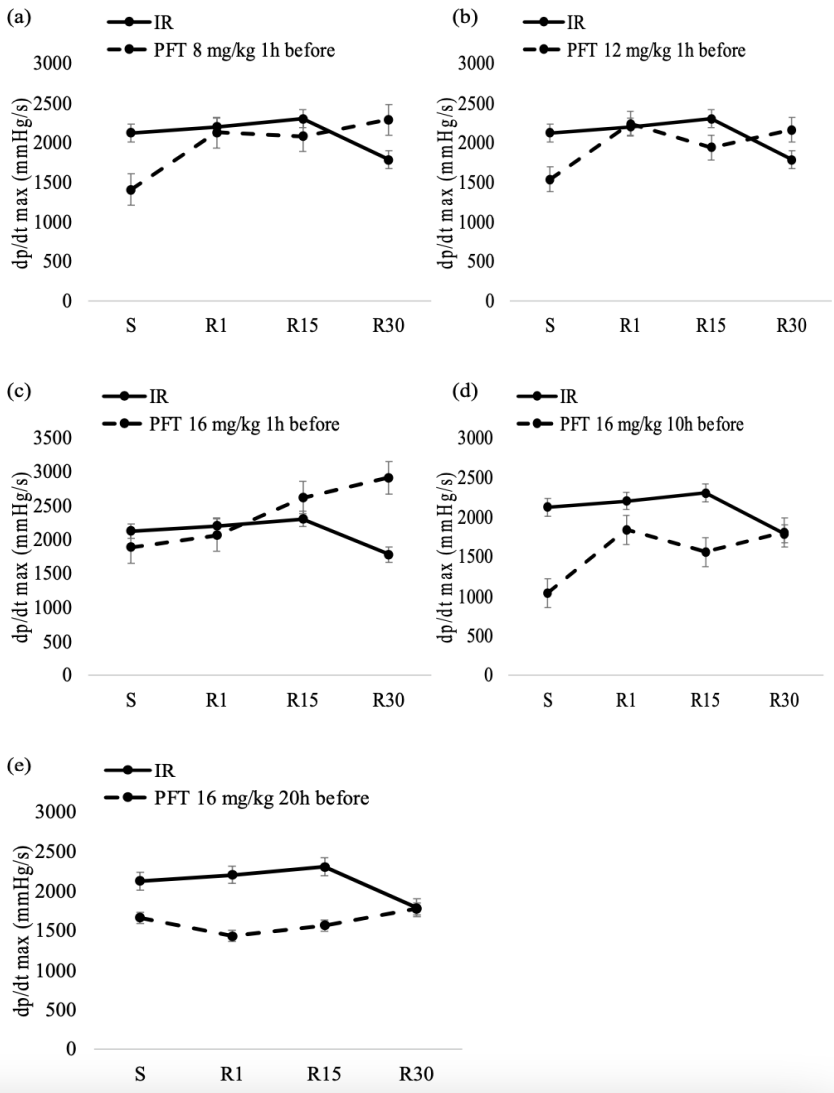
The effects of different doses of 10% PFE on cardiodynamics

Cardiodynamic parameters

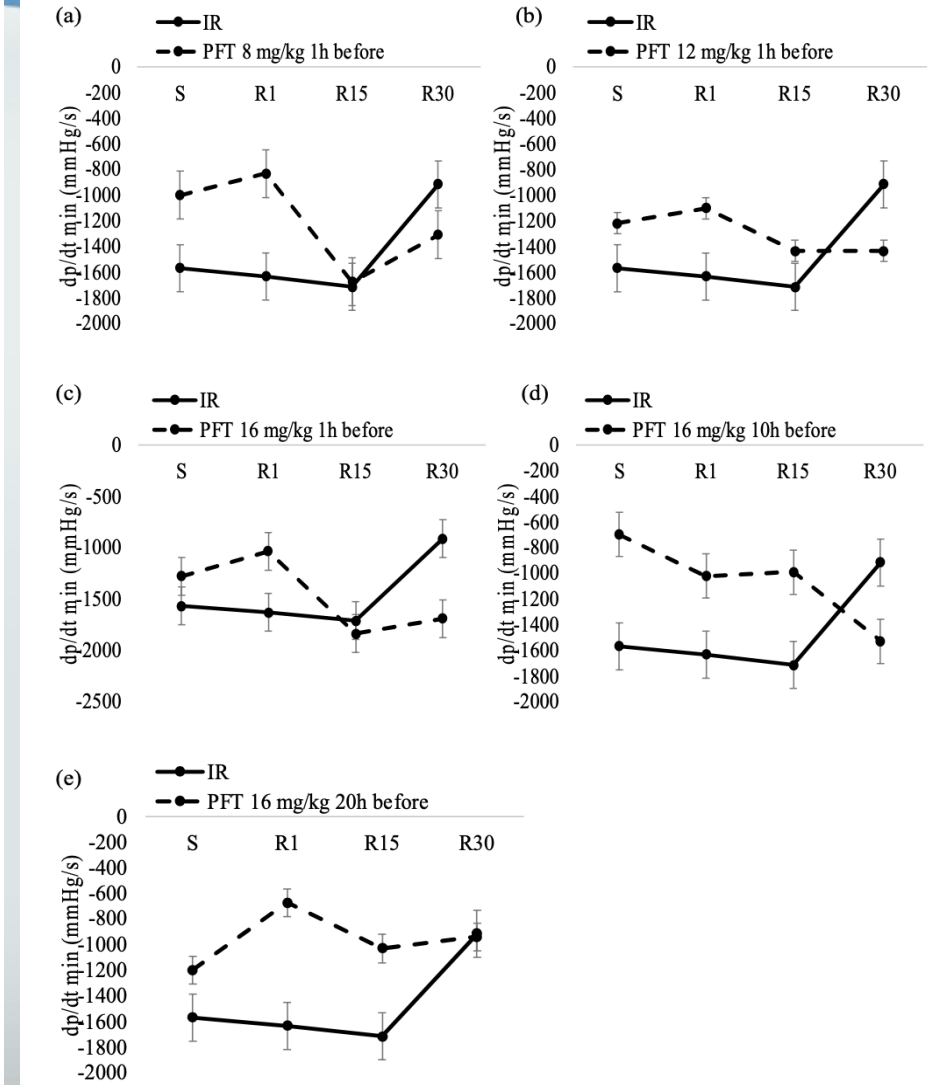
Table 2 The mean [SD] values of each parameter during pre-ischemia and post-ischemia among all groups

Values, mean [SD]		dp/dt max	dp/dt min	SLVP	DLVP	HR	CF
IR group	Pre-ischemic	2120.8 [211.1]	−1571.3 [−111.1]	72.3 [9.1]	1.5 [0.01]	289.2 [21.1]	8.6 [1.3]
	Post-ischemic	1780.7 [164.1]	−914.8 [−88.1]	47.2 [8.4]	1.1 [0.01]	267.1 [39.2]	5.6 [1.1]
PFT 8 mg/kg 1 h before	Pre-ischemic	1408.2 [194.1]	−1000.5 [−98.1]	36.2 [8.6]	2.5 [0.04]	299.5 [33.1]	10.6 [2.1]
	Post-ischemic	2289.5 [199.1]	−1310.5 [−132.1]	45.1 [5.4]	2.4 [0.01]	287.5 [45.1]	10.5 [1.9]
PFT 12 mg/kg 1 h before	Pre-ischemic	1536.7 [213.4]	−1220.3 [−122.9]	43.1 [2.5]	4.4 [0.05]	296.2 [50.8]	10.9 [2.3]
	Post-ischemic	2161.3 [202.1]	−1437.3 [−96.6]	44.6 [2.2]	3.5 [0.02]	295.2 [22.7]	12.1 [3.1]
PFT 16 mg/kg 1 h before	Pre-ischemic	1883.6 [155.8]	−1279.7 [−118.1]	55.4 [10.7]	2.9 [0.01]	302.7 [32.3]	15.5 [1.4]
	Post-ischemic	2909.1 [211.1]	−1691.3 [−88.3]	55.4 [11.1]	3.4 [0.02]	283.3 [43.2]	15.1 [2.3]
PFT 16 mg/kg 10 h before	Pre-ischemic	1040.7 [176.0]	−701. 1 [−112.1]	31.7 [7.9]	3.9 [0.02]	263.7 [28.1]	13.2 [2.4]
	Post-ischemic	1802.7 [182.2]	−1530.7 [−99.1]	57.3 [6.7]	2.1 [0.01]	257.3 [22.8]	10.1 [1.8]
PFT 16 mg/kg 20 h before	Pre-ischemic	1657.7 [166.0]	−1201.3 [−145.3]	43.2 [10.3]	3.2 [0.03]	261.3 [22.3]	10.9 [1.9]
	Post-ischemic	1769.7 [123.0]	−941.7 [−106.2]	36.6 [7.6]	1.6 [0.01]	279.7 [45.1]	11.5 [2.2]

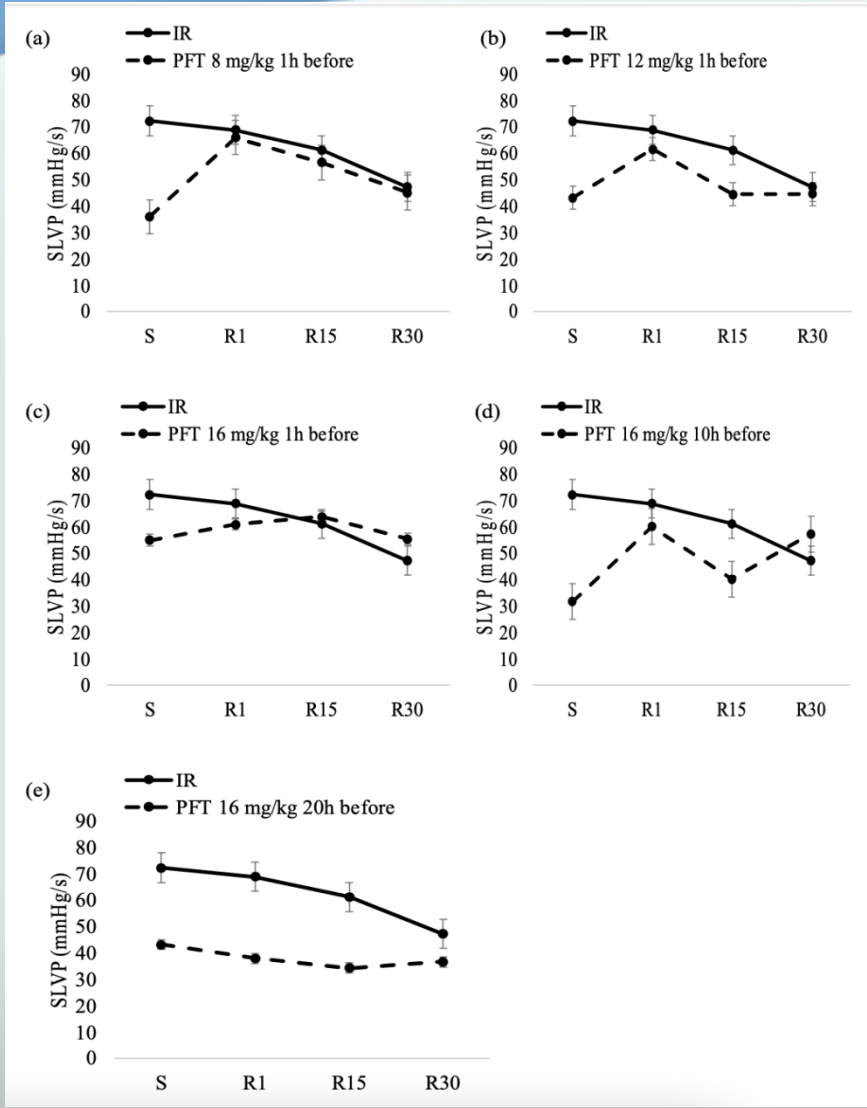
Dp/dt max



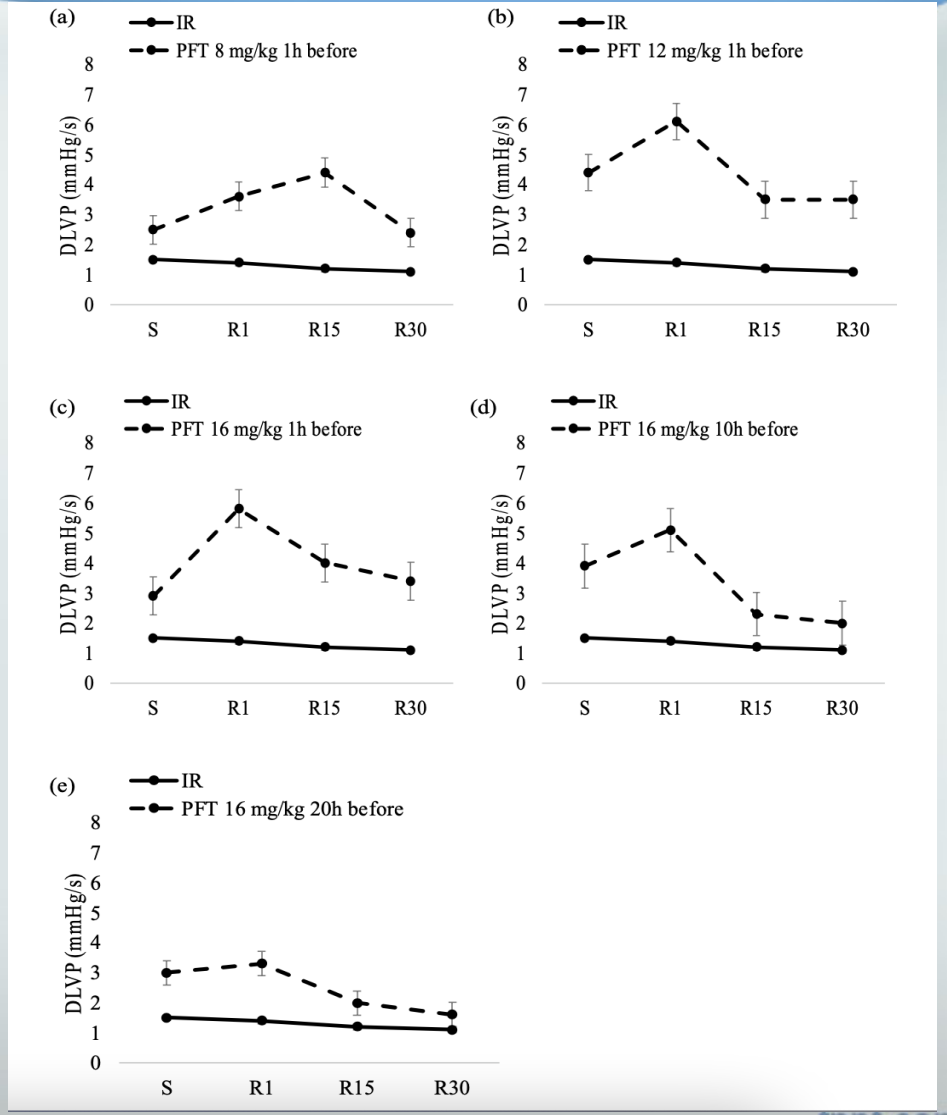
Dp/dt min



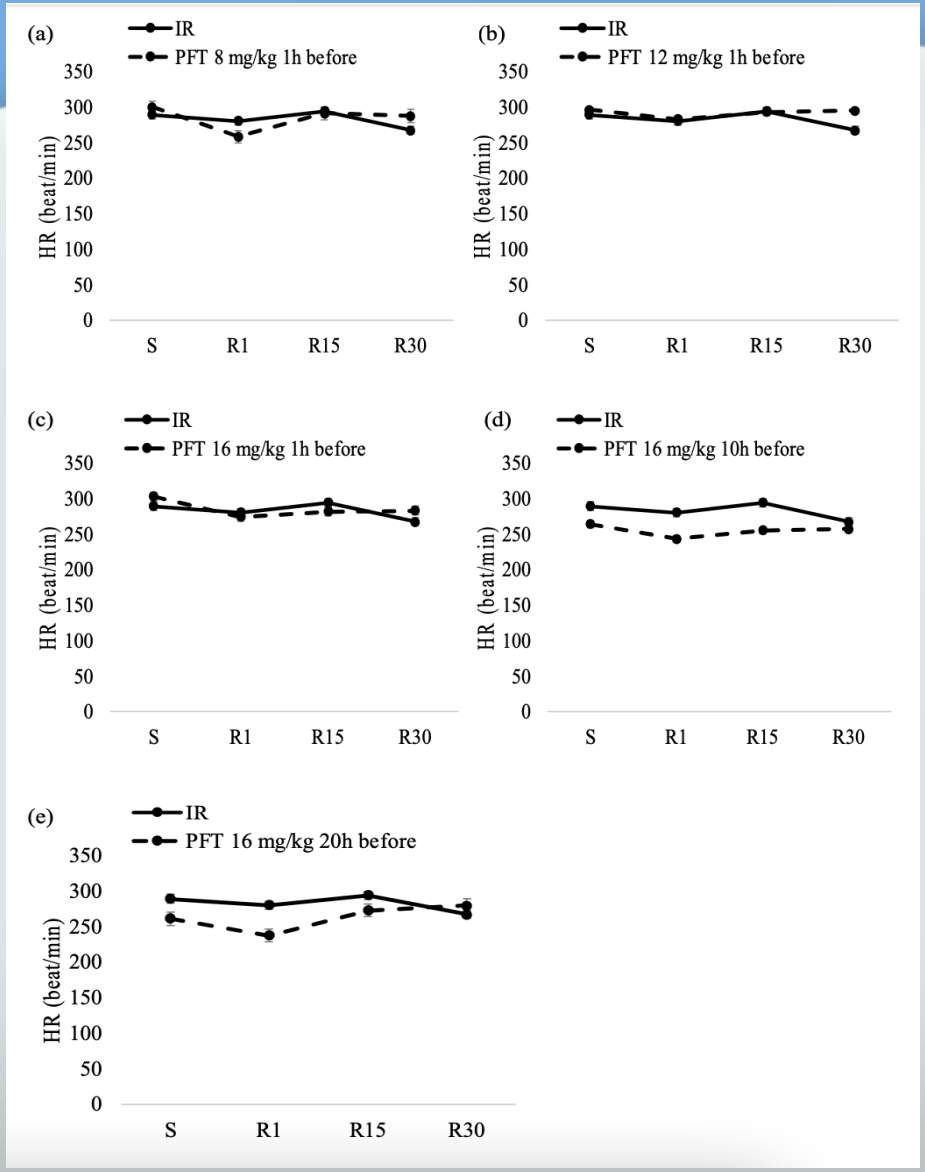
SLVP



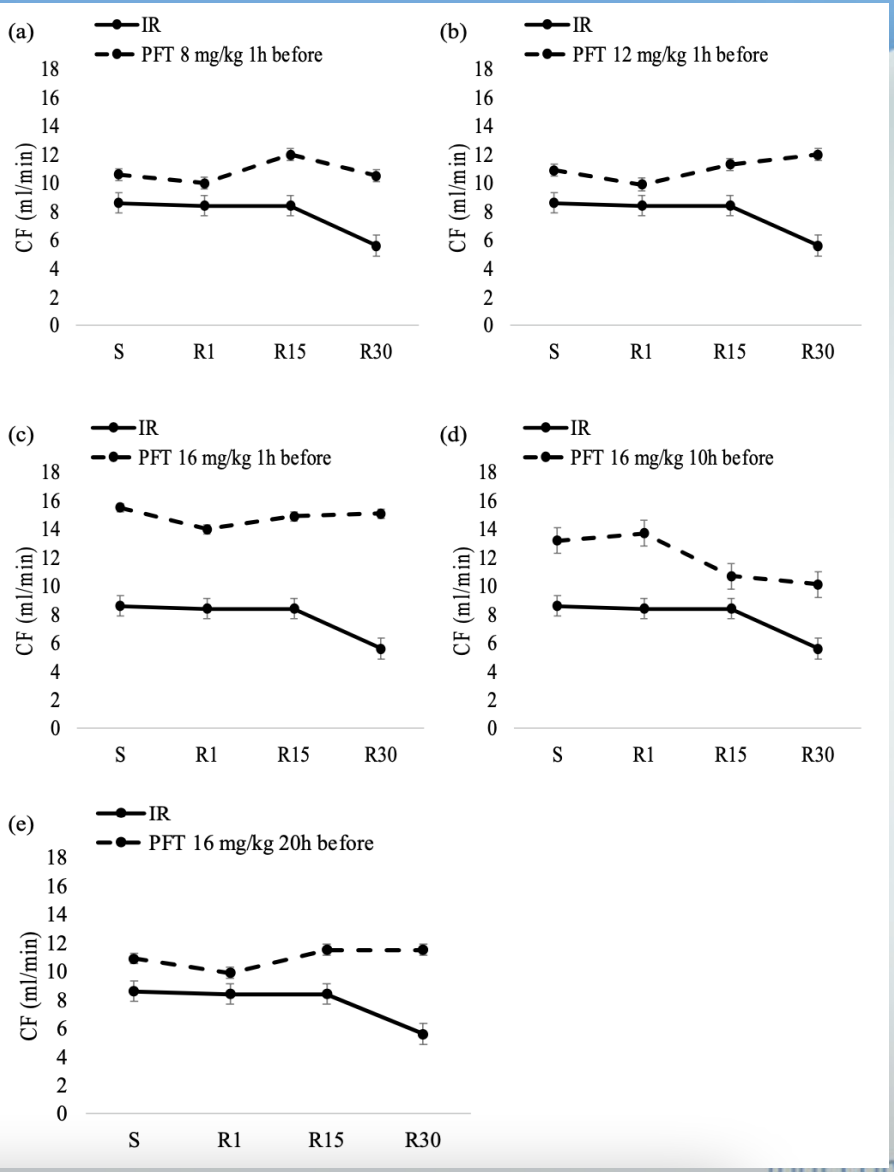
DLVP



HR



CF



Differences between the last point of reperfusion period (R30) and stabilization (S; pre-ischemic period) for all parameters and groups separately

Groups	Dp/Dt max R30-S [value] R30-S [%]	Dp/Dt min R30-S [value] R30-S [%]	SLVP R30-S [value] R30-S [%]	DLVP R30-S [value] R30-S [%]	HR R30-S [value] R30-S [%]	CF R30-S [value] R30-S [%]
IR	−340.1	656.5	−25.1	−0.4	−22.1	−3.0
	−16.0	41.8	−34.7	−26.7	−7.6	−34.9
PFT 8 mg/kg 1 h before	881.5	−310.0	9.1	−0.1	−12.0	−0.1
	62.6[a]	−31.0[a]	25.3[a]	−4.0[a]	−4.0	−0.9[a]
PFT 12 mg/kg 1 h before	624.6	−217.3	1.5	−0.9	−1.0	1.1
	40.6[b]	−17.8[b]	3.5[b]	−20.5	−0.3	10.1[b]
PFT 16 mg/kg 1 h before	1025.4	−411.6	0.4	0.5	−19.7	−0.4
	54.4[c]	−32.2[c]	0.7[c]	17.2[c]	−6.5	−2.6[c]
PFT 16 mg/kg 10 h before	762.0	−829.7	25.6	−1.9	−6.4	−3.1
	73.2[d]	−118.4[d]	80.8[d]	−48.7[d]	−2.4	−23.5
PFT 16 mg/kg 20 h before	112.0	259.3	−6.6	−1.4	18.4	0.6
	6.8	21.6[e]	−15.3	−46.7	7.0	5.5[e]

The letter means that $p < 0.05$ between:

[a]IR vs. PFT 8 mg

[b]IR vs. PFT 12 mg/kg 1 h

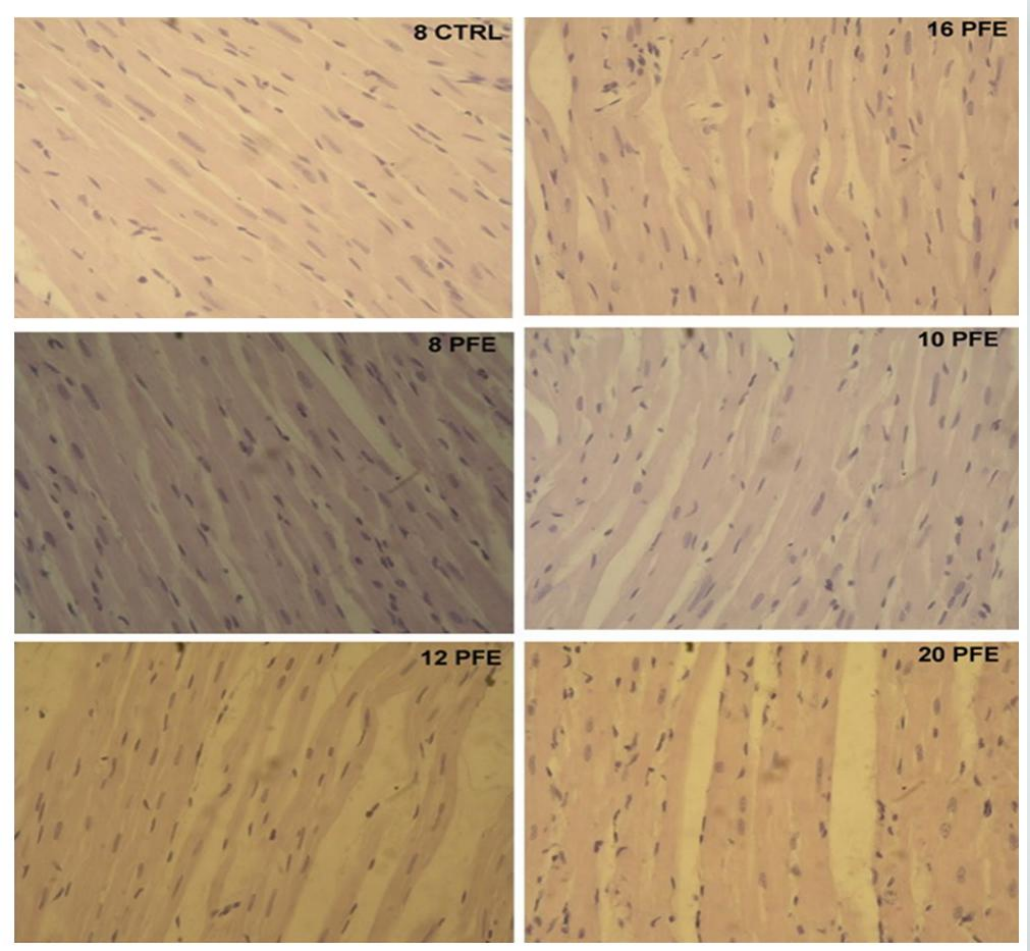
[c]IR vs. PFT 16 mg/kg 1 h

[d]IR vs. PFT 16 mg/kg 10 h

[e]IR vs. PFT 16 mg/kg 20 h

Representative Histopathological sections of heart tissues

- Only in groups treated with 16 mg/kg PFE 10 and 20 h, a partial loss of transverse striation of individual cells is observed (less prominent signs of ischemic changes).



Conclusion

- We can conclude that PFE administered before ischemia (1 h) has less positive effects on myocardial function in an isolated rat heart model compared to earlier administration (10 and 20 h).
- Also, the effects of 10% PFE are more pronounced if there is a longer period of time from application to ischemia, i.e., immediate application of PFE before ischemia (1 h) gave the weakest effects on the change of cardiodynamics of isolated rat heart.
- Therefore, the future of PFE use is in new indications and application methods, and PFE can also be referred to as anti-hypoxic and anti-ischemic blood substitute with mild membranotropic effects.

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Thank you for your attention!