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Aging, sex and NLRP3 inflammasome in cardiac ischaemic disease

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ABSTRACT

Experimentally, many strong cardioprotective treatments have been identified in different animal models of acute ischaemia/reperfusion injury (IRI) and coronary artery disease (CAD). However, the translation of these cardioprotective therapies for the benefit of the patients into the clinical scenario has been very disappointing. The reasons for this lack are certainly multiple. Indeed, many confounding factors we must deal in clinical reality, such as aging, sex and inflammatory processes are neglected in many experiments. Due to the pivotal role of aging, sex and inflammation in determining cardiac ischaemic disease, in this review, we take into account age as a modifier of tolerance to IRI in the two sexes, dissecting aging and myocardial reperfusion injury mechanisms and the sex differences in tolerance to IRI. Then we focus on the role of the gut microbiota and the NLRP3 inflammasome in myocardial IRI and on the possibility to consider NLRP3 inflammasome as a potential target in the treatment of CAD in relationship with age and sex. Finally, we consider the cardioprotective mechanisms and cardioprotective treatments during aging in the two sexes.

1. Introduction

Ischaemic heart disease in an aging population poses a heavy social burden. Early reperfusion by the primary percutaneous coronary intervention (PPCI) is undoubtedly necessary to improve patient outcomes. Nevertheless, the reperfusion adds more damage to the ischaemic injury and the prevention of long-term complications is still an unmet need. Therefore, current therapeutic approaches, which fail to completely prevent the cardiac injury induced by ischaemia/reperfusion (I/R), are associated with significant costs for health systems. To address these

Abbreviations: ACE2, angiotensin-converting enzyme 2; AGE, advanced glycation end-products; Akt, Protein kinase B; AMI, acute myocardial infarction; ASC, Apoptosis-associated speck-like protein containing a CARD; CAD, coronary artery disease; CVDs, cardiovascular diseases; DAMP, damage-associated molecular patterns; ECM, extracellular matrix; ECs, endothelial cells; EF, ejection fraction; eNOS, endothelial NO synthase; ER, oestrogen receptor; ERK1/2, extracellular signal-regulated kinase ½; ERβ, ER type β; EVs, extracellular vesicles; FS, fractional shortening; GSK3β, Glycogen synthase kinase 3 beta; HNX, H^+/Na^+ exchanger; HSP, heat shock proteins; ICAM-1, intercellular adhesion molecule (ICAM) 1; IFN-γ, interferon γ; IL, interleukin; IPostC, ischaemic post-conditioning; IPreC, ischaemic pre-conditioning; I/R, ischaemia/reperfusion; IRI, ischaemia-reperfusion injury; LV, left ventricle; LPS, Lipopolysaccharide; MDPs, Mitochondria-derived peptides; mitoKATP, mitochondrial KATP channel; MMPs, matrix metalloproteinases; MnSOD, manganese superoxide dismutase; mPTP, mitochondrial permeability transition pore; MRI, myocardial reperfusion injury; NCX, Na^+/Ca^{2+} exchanger; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLR, nucleotide oligomerization domain -like receptors; NO, nitric oxide; NOD-like receptor family pyrin domain containing 3, NLRP3 inflammasome; PAMP, Pathogen associated molecular patterns; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PMN, polymorphonuclear neutrophils; PPCI, primary percutaneous coronary intervention; PRR, pattern recognition receptors; RCS, respiratory chain super-complexes; RIC, remote ischaemic conditioning; RIPreC, remote ischaemic pre-conditioning; RIPerC, remote ischaemic per-conditioning; RIPostC, remote ischaemic post-conditioning; RIRR, ROS-induced ROS release; RISK, reperfusion injury salvage kinase pathway; RNS, reactive nitrogen species; ROS, reactive oxygen species; RyR, ryanodine receptor; SAFE, survivor activating factor enhancement; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SDF-1α, stromal derived factor 1α; SIRT1, sirtuin 1; SMCs, smooth muscle cells; SR, sarcoplasmic reticulum; STAT3, signal transducer and activator of transcription 3; TMAO, trimethylamine N-oxide; TNFα, tumour necrosis factor α; VDAC, voltage-dependent anion channel; βERKO, ERβ knockout.

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obstacles and improve patient outcomes, novel pharmacological and interventional approaches have been proposed, alone or in combination. However, the translation of cardioprotection beneficial results from the experimental bench to clinical application to the patient with acute coronary artery disease (CAD), or undergoing surgery, has been largely disappointing. The reasons behind this lack of translation of cardioprotection are attributable to various confounding factors, including comorbidities and co-medications [1]. Moreover, factors such as aging, sex and inflammatory mechanisms certainly play an important role [2,3]. Therefore, a better understanding of how aging, sex and inflammatory processes affect ischaemia-reperfusion injury (IRI) is needed.

Numerous papers report the significant differences in IRI in male and female subjects, where important differences in age and outcome of heart damage occur. These differences suggest a central role for sex hormones in the different animal models employed [2,3,4,5,6,7,8].

As a result of aging, at the cardiac level, there is a lower tolerance to stress, and lower contractile and mitochondrial function, while there is a significant increase in the susceptibility to apoptosis and necrosis, thus causing a loss of cardiomyocyte number. Reduced cardiac function is revealed by a decreased systolic and diastolic function of the left heart, decreased cardiac output, and decreased response to catecholamines [9,10]. During aging, a progressive impairment of metabolic pathways is observed in the organism linked to insulin resistance, oxidative stress, inflammation, and the alteration of cellular functions that regulate life and death.

The NOD-like receptor family pyrin domain containing 3 (NLRP3)-inflammasome is involved in many of these processes and is heavily involved in causing and exacerbating cardiovascular disease [11]. Intriguingly, several data suggest a close pathophysiological link between gut microbiota and NLRP3 in several conditions, including metabolic and cardiovascular diseases. Indeed, the term "Metaflammation" describes a systemic inflammatory state associated with metabolic diseases [11]. This chronic inflammatory state is also observed in the elderly population without comorbidities, hence, the term "Inflammaging" has been proposed. When metaflammation and inflammaging interact, they interfere with IRI and cardioprotection and may contribute to the failure of clinically relevant outcomes.

Recently, some data on NLRP3 role in physiological and pathological aging in the two sexes are emerging. Therefore, this article briefly reviews the current literature about the modulatory effects of aging and sex on IRI development and their potential effects on cardioprotective interventions. Then we consider the role of NLRP3 inflammasome in ischaemic diseases in the two sexes and the molecular mechanisms through which NLRP3-dependent inflammation can affect cardiovascular aging. Finally, we consider these aspects in modifying endogenous cardioprotection by conditioning.

2. Age as a modifier of tolerance to ischaemia/reperfusion injury in the two sexes

2.1. Myocardial reperfusion injury mechanisms

Cardiomyocyte death and myocardial damage attributable to reperfusion begin within minutes after reopening the culprit artery and usually progresses for at least 6–24 h after reperfusion of an ischaemic zone. This phenomenon is referred to as "myocardial reperfusion injury" (MRI) [2,10,12]. This damage is the principal cause of death for arrhythmias and stunned cardiomyocytes, and it is represented by more than half of the final infarct size. Therefore, following the reperfusion of the ischaemic myocardium, which certainly leads to a reduction in ischaemic damage, an appreciable death of myocardial cells can occur due to the mechanisms triggered by the reperfusion itself. These MRI occur as a result of a series of mechanisms that are activated at the time of the reintroduction of oxygen and the recovery of intracellular pH. The endpoint of acute IRI is an increase in the opening probability of the mitochondrial permeability transition pore (mPTP). The opening of mPTP leads to the loss of the potential of the inner mitochondrial membrane and triggers various modes of cell death (necrosis, apoptosis, necroptosis, and pyroptosis) and reduction of autophagy [13,14,15]. Besides pH recovery, several alterations of physiological mechanisms are responsible for the mPTP opening. Such alterations include the production of reactive oxygen and nitrogen species (ROS/RNS) and alterations of calcium homeostasis (calcium oscillations), which may sustain the so-called RIRR (ROS-induced ROS release) [16]. After ischaemia or hypoxia, cardiac cells show significant metabolic abnormalities, in particular an increase in anaerobic fermentation. In ischaemic conditions, the enhanced concentration of protons and the consequent intracellular pH decrease leads to the intracellular Na^+ increase through the H^+/Na^+ exchanger (HNX). The excessive intracellular Na^+ will promote a slowdown of the Na^+/Ca^{2+} exchanger (NCX), which subsequently will work in a reverse mode, leading in both cases to calcium overload [17]. Therefore, as a consequence of I/R, the intracellular calcium homeostasis becomes dysfunctional. Of note, during I/R an injury to the sarcolemmal membrane and the sarcoplasmic reticulum is also observed, with a consequent increase in cytosolic calcium. Calcium overload also occurs at the mitochondrial level where it also facilitates the opening of the mPTP. Following these cellular modifications, the activation of proteases (*e.g.* calpain) occurs with consequent damage to the myofibrils, hyper-contracture and necrosis [18,19] (Fig. 1). At the reperfusion time, ROS/RNS production, already altered as a result of ischaemia, is increased, thus contributing to the myocardium and endothelium damage [16]. Following MRI, inflammatory pathways capable of determining and increasing vascular permeability in the area subjected to ischaemia are triggered, thus favouring the migration of polymorphonuclear neutrophils (PMN) into this area upon reperfusion [20,21]. PMN play a critical role in expanding MRI for two reasons: early recruitment and their production of ROS. PMN activation is induced by an oxidative burst which produces several types of ROS. In fact, the ROS produced during the MRI induce a *vicious circle*: activation of PMN that consequently produces other ROS. The recruitment of PMN occurs in the early stages of reperfusion, their action is due to their adhesion characteristics with endothelial cells (ECs) through the interaction with different adhesion molecules (*e.g.* selectins, integrins) [22]. Studies conducted on experimental models have shown that the MRI due to the action of PMN depends on the exposure to damaged cardiac cells of adhesion molecules, such as ICAM-1, and on the activation of the integrin CD18 [22,23]. In *in vivo* I/R experiments, it was found that ROS are mainly released by adherent PMN, supporting the central role of PMN adhesion at the cardiomyocyte level in MRI [24]. The early production of ROS during reperfusion, within 2–10 min from the start of reperfusion, is mainly due to mitochondrial dysfunction. These ROS act as a trigger for chemotaxis against immune system cells such as PMN, by activating components of the *Complement cascade* and the production of pro-inflammatory cytokines by the NF-κB pathway [22]. Following the activation of the PMN, the release of proteolytic enzymes is observed, as well as ROS, with consequent tissue and endothelial damage. At the vascular level, neutrophils aggregates with platelets, causing vessels closure; furthermore, vasoconstrictor molecules released by platelet, neutrophil, or endothelial cause prolonged vasoconstriction of the coronary microcirculation determining the phenomenon of "*no-reflow*". The latter further damages the myocardium, and despite the reperfusion which restores the flow in the epicardial circulation, the distal flow in the small arterial vessels and microvessels is insignificant [19]. Therefore, IRI are due to both cardiomyocytes damage and alterations of the coronary microvasculature. Platelets and PMN mediating inflammation also play synergic, self-fuelling roles in determining the final entity of MRI.

2.2. Aging and myocardial reperfusion injury

Despite being a physiological process, the progressive alterations due to aging determine important modifications at different levels, from cell

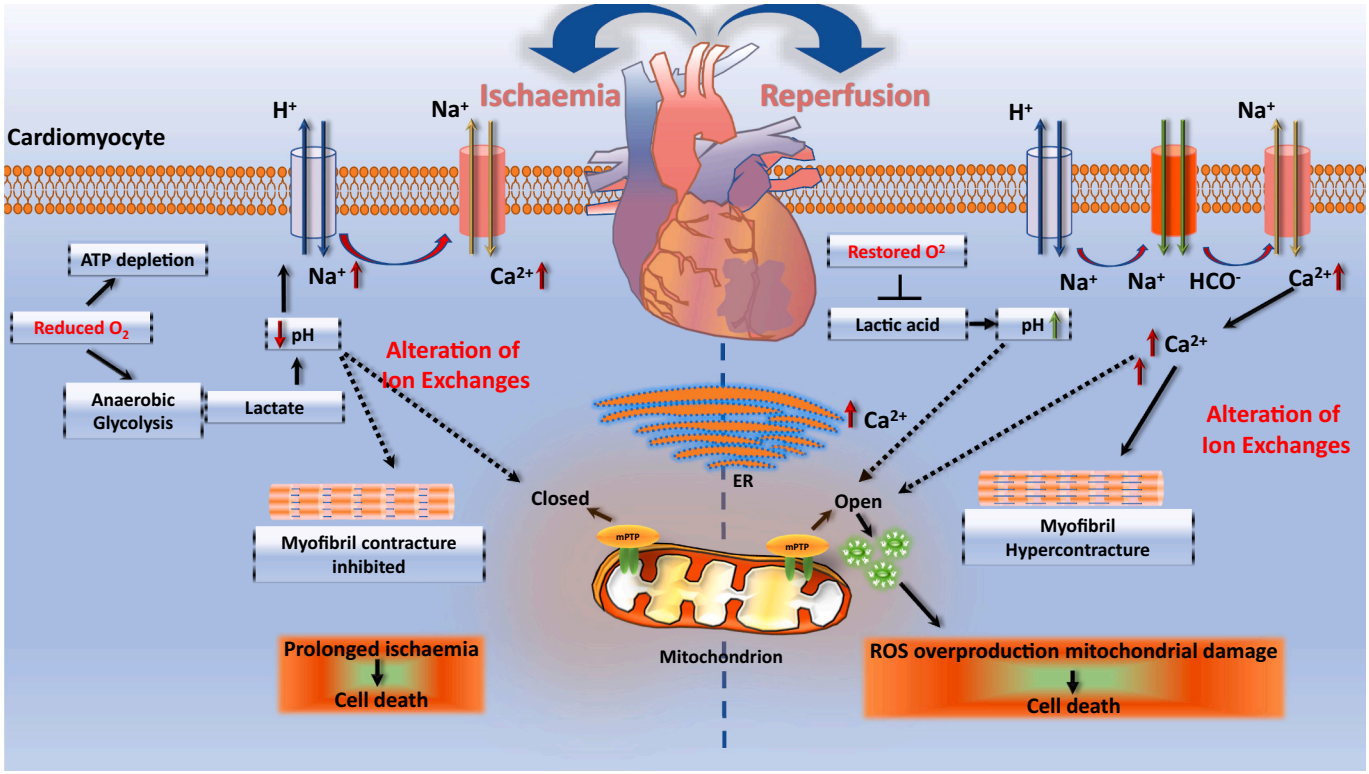

Fig. 1. Cellular events leading to ischaemia-reperfusion injury in the heart. Reduced oxygen supply during ischaemia induces inhibition of Na^+/K^+ ATPase due to depletion of high-energy metabolites such as ATP and produces lactate which leads to intracellular acidification. To restore the intracellular pH, it deactivates the Na^+/H^+ exchanger which increases the intracellular concentration of Na^+, further activating the Na^+/Ca^{2+} exchanger, resulting in calcium overload. During myocardial ischaemia, the mitochondrial permeability transition pore (mPTP) remains closed and opens within the first few minutes after myocardial reperfusion in response to mitochondrial Ca^{2+} overload, oxidative stress, restoration of a physiologic pH, and ATP depletion. Reperfusion and reactivation of the Na^+/H^+ exchanger result in washout of lactic acid, resulting in the rapid restoration of physiological pH, which releases the inhibitory effect on mPTP opening and cardiomyocyte contracture. Prolonged cardiomyocyte ischaemia or high ROS production in reperfusion leads to cell death.

metabolism to the individual organ [25]. The reduction of cardiac function following aging is significantly more evident under stress conditions (*e.g.* during I/R), characterized by reductions of both the ejection fraction (EF) and the fractional shortening (FS) [25,26]. The larger extension of the infarct area and the decrease of contractile recovery in the clinical setting is predictive of lower survival in elderly patients [27].

In the lab scenario, it should be noted that the definition of young or aged varies considerably in the various animal models used for cardiovascular and aging studies (Table 1). Strain differences may exist. For instance, the various cardioprotection studies tend to consider male Wistar rats aged 3, 12 and 18 months as representative of humans in adolescence and of about 30 or 45 years old, respectively [2,9,28,29].

The reduced tolerance to IRI during aging involves intracellular cardiomyocyte alterations, as well as changes in extracellular matrix and ECs, platelets, and inflammatory cells function (for reviews, see [2,30,31]).

Intracellular cardiomyocyte alterations: the main alterations occurring in aged cardiomyocytes resemble those observed in cardiac failure, involving *mitochondrial function, calcium handling, metabolism, and signalling pathways* [32]. In physiological conditions, ATP production is finely tuned to the cell energy demand by Ca^{2+} ions transferred from the sarcoplasmic reticulum (SR) to mitochondria. In particular, the adequate mitochondria-SR communication depends on the tight alignment (50–100 nm distance) between the SR ryanodine receptors and the mitochondrial voltage-dependent anion channel (VDAC) [33]. The partial detachment of SR from mitochondria observed in aged hearts can alter calcium exchange and promote oxidative stress at their interface [34]. Of note, the gating properties of ryanodine receptor 2 (RyR2), the major cardiac RyR isoform expressed in myocardial cells [35], have been shown to be altered in the aged heart, despite no changes in the protein expression [36]. Recently, RyR2s have been identified as prominent targets of glycation in cardiac cells from both aged mice and elderly (≥75 years) patients. RyR2 glycation promotes calcium leak in cardiomyocytes and SR vesicles of aged mice, leading to increased calcium in interfibrillar mitochondria directly exposed to SR calcium release [37]. Specifically, it has been suggested that RyR2 glycation during aging may lead to disruption of RyR-VDAC bridges, resulting in SR -dependent calcium leak and an increase in calcium precipitation in the mitochondrial matrix [37]. This makes mitochondria from aged cardiomyocytes more prone to undergo mitochondrial permeabilization during I/R. Indeed, it has been shown that the contribution of mPTP in cell death enhances with age [38,39]. In addition, the reduced ability of aged cardiomyocytes' mitochondria to actively buffer cytosolic calcium aggravates calcium overload, hyper-contracture, and contraction band necrosis, leading to cell death independently of mPTP [39,40].

Besides calcium handling, aging may also affect some components of the *mitochondrial interactosome*, a protein supercomplex present in the contact sites between outer and inner mitochondrial membranes, responsible to deliver ATP in accordance with the energy demand [41].

Table 1
Classification of laboratory animals used in cardiovascular experiments as young or aged.

Species	Young	Aged	References
Mice	2–4 months old	13–28 months old	2,9,29
Rats	3–6 months old	12–24 months old	2,9,29
Rabbits	12–24 months old	30–48 months old	2,9,29
Sheep	6–12 months old	60–84 months old	2,9,29

Among the different components of interactosome, VDAC is able to regulate the permeability of mitochondrial outer membrane [42]. The VDAC permeability depends on both age and sex: in rats, an initial increase is observed only in middle-aged females, followed by a parallel increase occurring in both sexes during aging. In addition, both mitochondrial oxidative phosphorylation [43] and intracellular energy transfer *via* the phosphocreatine/creatine kinase network decrease significantly as a result of aging [42]. It has been suggested that the enhanced contribution of glycolysis to ATP production occurring during aging [43] may counterbalance the reduced mitochondrial oxidative phosphorylation, though whether this change has cardioprotective effects in elderly patients remains unknown.

Aging may also affect the *respiratory chain supercomplexes* (RCS) activity. In particular, it has been suggested that RSC alteration and lack of complex IV play an important role in the impairment of mitochondrial function with age [44]. Interestingly, preconditioning may modulate RCS by altering the lipid composition of mitochondrial membranes in aged animals [45].

Recent studies suggest that the age-related *Mitochondria-derived peptides* (MDPs) decline is involved in the heart's increased susceptibility to aging. MDPs are small peptides with cytoprotective properties, such as the ability to maintain cell viability and mitochondrial function under stress, particularly in response to inflammation and oxidative stress. MDPs are involved in chronic inflammation and many senescence and aging-associated diseases. Within the cardiovascular system, MDPs exert a protective effect by improving mitochondrial function, reducing the consequences of oxidative stress damage and high ROS levels. In particular, the anti-inflammation and anti-atherosclerosis effects may reduce endothelial dysfunction, while the anti-apoptotic effect represents a protective mechanism against myocardial IRI [46].

Following aging, higher ROS levels are observed in elderly patients with acute myocardial infarction (AMI) [47]. Proteomic analysis showed that ROS production is lower at the mitochondrial level, thus making older animals protected and increasing their life expectancy. Under stressful conditions, such as IR, the uncontrolled production of ROS causes oxidative damage to various structures, such as proteins, cell membranes, and mitochondria, leading to greater cell death [47].

The alterations of extracellular matrix (ECM): aging alterations in ECM may contribute to worsening IRI. Among the different collagen subtypes present in the heart, the most abundant are collagen I and III, which cause stiffness and elasticity to ECM, respectively. In old male individuals, collagen type I fibers were increased in number and thickness [48]. While changes in the ECM are limited in healthy aging, they become marked as a consequence of the remodelling of the ECM matrix due to the interaction between age and age-related diseases [49,50]. Such changes can be observed in the case of hypertension and in the presence of reduced influence of the gonadal hormone [51]. Other collagen subtypes present in the T-tubules have been suggested to be responsible for the age-related alterations of T-tubules structure and function in male mice [52,53].

A recent study on the heart-tube of *Drosophila melanogaster* suggests that also the thickening of the basement membrane may participate in contractility reduction observed with aging [54].

Among matrix metalloproteinases (MMPs), MMP-9 seems to be particularly important in age-related ECM alterations. The increased expression of MMP-9 with age leads to a progressive replacement of elastin with collagen and increases the stiffness of the vessel wall. Indeed, MMP-9 null male and female mice were partially protected against age-dependent diastolic dysfunction, and vascular permeability and inflammation increase [55].

Due to the slow turnover of ECM components, a further contribution increasing matrix stiffness derives from products of degradation and advanced glycation end-products (AGE) in the interstitial space of the heart and in the vessel wall with aging [56].

Altogether, the alterations of ECM caused by aging may worsen IRI by hindering the oxygen diffusion from the capillaries to cardiac cells, altering integrin signalling and T-tubule function in cardiomyocytes, and finally limiting the possibility to adapt coronary blood flow to oxygen demand [24,48,49].

Blood cells and endothelium alterations: besides the alterations of cardiac cells and ECM, the reduced I/R tolerance in aging may also involve blood cells and endothelium. The enhanced *platelet* activity observed in both sexes with aging, in association with altered monocyte phenotype and function, results in increased platelet-monocyte interaction and activation of thrombo-inflammatory pathways. Platelet "priming" in the elderly may generate excessive responses and may increase the propensity for adverse clinical outcomes in patients undergoing I/R [57]. In addition, aging alters the amount of circulating and vessel wall-associated factors, increasing the procoagulants/anticoagulants ratio and consequently favouring a prothrombotic state. Among these factors, the von Willebrand factor has been reported to increase with age in both animal models and the human population. In addition, circulating levels of von Willebrand factor are influenced by various pathologies associated with aging and cardiovascular risk factors such as insulin resistance and obesity. Although clinical studies suggest that the trend of increasing von Willebrand factor with age is similar in women and men, middle-aged women (41–50 years) have a higher rate of increase, likely due to changes in hormone levels during pregnancy, menstrual cycle, hormonal contraceptives, and menopause [58].

Chronic low-grade inflammation in the elderly, due to an increase in circulating pro-inflammatory cytokines and ROS - produced by damaged ECs and/or adjacent immune cells - reduces the bioavailability of nitric oxide (NO) and triggers a *vicious circle* with further endothelial damage [59]. Notably, a decrease in both acetylcholine (ACh)-mediated and flow-mediated vasodilation (endothelium-dependent vasodilation) with age has been observed in men and women, although they are not affected in women until the onset of menopause [60]. Indeed, both oestrogen and testosterone are considered among the major modulators of endothelium-dependent vasodilation, as they activate eNOS [60,61]. Indeed, endothelium-dependent vasodilation is attenuated in premenopausal women treated with a gonadotropin-releasing hormone antagonist [62] and in young men in whom endogenous oestrogen production has been blocked by an aromatase inhibitor [63]. In addition, both testosterone and oestrogen possess antioxidant and anti-inflammatory properties that are lost in hormone-deficient states [64,65]. The role of endothelin-1 in endothelial function with age and between the sexes is currently less clear. Endothelin-1 (ET) acts on the receptor subtypes ETA and ETB present on vascular smooth muscle cells (SMCs) and exerts a potent vasoconstrictor effect. On the other hand, stimulation of ETB receptors located on the endothelium results in vasodilation [66]. The differential response to endothelin may be sex- and age-dependent, as suggested by the preferential binding of endothelin-1 to ETB receptors in women [67] and the reduction in ETB-mediated vasodilation in postmenopausal women [68].

The effects of aging on Ca^{2+} signalling in vascular SMCs and ECs were recently reviewed by Harraz and Jensen [69]. Alteration in vascular tone has been associated with abnormal expression or activity of voltage-gated Ca^{2+} channels, Ca^{2+}-activated K^{+} channels, or TRPC6 (Transient Receptor Potential Cation Channel Subfamily C Member 6), often accompanied by changes in caveolae density, microRNA expression, or 20-hydroxyeicosatetraenoic acid (20-HETE) content. Defective Ca^{2+} handling by intracellular stores that accompanies aging has been explained by decreased expression of intracellular Ca^{2+} release channels and Ca^{2+} reuptake or efflux pumps. These changes are often associated with increased mitochondrial Ca^{2+} uptake and oxidative stress. Aging may also strongly influence spontaneous and evoked Ca^{2+} transients in ECs as well as structural changes at the EC-SMC interface [70]. Although the elderly showed a diminished response to the endothelium-dependent vasodilator ACh [71], muscarinic receptor-mediated Ca^{2+} signalling in arterial ECs was minimally altered [72] or even enhanced during aging [73]. In carotid arteries, EC sensitivity to ACh increased with age, although the latency of Ca^{2+} responses increased significantly

[74]. In addition, aging has been shown to impair intercellular electrical conduction in vessels due to increased current loss (leaky conduction) caused by increased activation of the small- and intermediate-conductance Ca^{2+}-activated K^+ channel [75]. Currently, further studies are needed to explore the possible changes in Ca^{2+} influx pathways, such as TRP channels, during aging. For instance, altered EC TRPV4 (Transient Receptor Potential Cation Channel Subfamily V Member 4)-mediated Ca^{2+} signalling likely contributes to endothelial dysfunction and vascular complications with aging [76].

Taken together, the alterations of Ca^{2+} signalling on myogenic tone, endothelium-dependent vasodilatation, and vascular structure are likely to contribute to blood pressure dysregulation and blood flow distribution deficits.

The endothelial progenitor cells dysfunction, due to oxidative stress, may also explain the impaired angiogenic function and the more severe ischaemic tissue injury observed in aging hearts [77]. Inflammaging is associated with a senescence-associated secretory phenotype (SASP), which is characterized by the activation of a pro-inflammatory transcriptional program in which an important role is played by the NLRP3 inflammasome. This activation causes a chronic secretion of pro-inflammatory cytokines by peripheral blood cells and resident fibroblasts. (see below).

2.3. Sex differences in tolerance to IRI: Biological mechanisms

Sex hormones exert important effects on cardiovascular function, both in health and disease conditions. It is well known that exogenous administration of the corresponding sex hormone reduced I/R injury in both sexes in animals subjected to gonadectomy [2,4,78].

The major damage caused by acute myocardial I/R in young male mice and rats compared to females, includes neutrophil infiltration, adverse left-ventricular (LV) remodelling, cardiac rupture, and higher mortality [79,80]. The reduced tolerance to I/R in aged female animals strongly suggests an important protective role of oestradiol [2,8].

Actually, the beneficial effects of exogenous oestradiol treatment have been reported in experimental gonad-intact and gonadectomised animals of both sexes [81]. Nevertheless, the efficacy of menopausal hormone administration in postmenopausal women is less clear [81]. Similar conflicting results have been obtained when testosterone therapy was applied to counteract the decline in testosterone levels in aged men [82].

Mechanisms: besides steroid sex hormones, the reduced susceptibility to acute myocardial I/R in females, compared to males, has been explained by the presence of improved intracellular calcium homeostasis and NO signalling [83], reduced apoptosis [84], reduced ROS levels [84,85] and more positive interaction between ROS and the expression/activation of some kinase-dependent survival pathways [84,85,86,87]. Of note, the steroid sex hormones, oestrogens, progesterone, and androgens, exert beneficial effects against IRI, particularly at pharmacological concentrations. These effects mainly depend on the activation on non-genomic cellular responses [88]. In particular, non-nuclear G-protein coupled oestrogen receptors, are present on both male and female cardiac cells [89]. Pre-treatment of both male and female hearts with oestradiol, exerts a cardioprotective effect either through PI3-K pathway activation, converging on GSK3β and leading to mitochondrial protection, or by upregulation of NO signalling and S-nitrosylated proteins [89,90]. Two important protective pathways activated by oestrogens and involved in the IRI are Gp/PI3K/mitochondrial KATP channel (mitoKATP) and the endothelial NO synthase (eNOS)/S-nitrosylation pathway of mitochondrial molecules [7,89,91].

Experiments performed on specific oestrogen receptor (ER) knockout mice showed a more severe I/R injury in ERβ knockout (βERKO) females than WT females, while conflicting results have been obtained in αERKO mice [89].

Progesterone has been shown to reduce myofilament calcium sensitivity and cardiac contraction in female but not in male mouse hearts [92]. Interestingly, the main androgen hormone testosterone, is able to give cardioprotection. The presence of aromatase, the enzyme responsible for converting androgens to oestrogens, in both sexes hearts suggests that the protective effect of testosterone may be oestrogen mediated.

Modifiers: many factors can modify the response to I/R in females. Besides age (see next paragraphs), different pathophysiological alterations may limit the reduced susceptibility to acute myocardial I/R in females [93]. For instance, sex-dependent differences in functional recovery after I/R, disappear in hearts made fibrotic after treatment with angiotensin II. Depending on the length of the study and the timing of the gonadectomy (*i.e.*, young or adult animals), the loss of gonadal function resulted in increased severity of IRI in both sexes [94].

A cardioprotective activity has been also described for the insulin-like peptide, relaxin, and oxytocin; both hormones are produced in increased amounts during pregnancy [95,96].

3. Age as a modifier of cardioprotective mechanisms in the two sexes

3.1. Cardioprotective mechanisms

Aging is a modifier of cardioprotective mechanisms in both sexes. Age is known to limit the cardioprotective efficacy of many protective procedures, including mechanical and pharmacological pre-, post-and per-conditioning, as well as mild hypothermia and exercise-induced cardioprotection. Before analysing the main mechanisms of cardioprotection let's define the conditioning procedures.

Mechanical conditioning procedures involve the intermittent occlusions of an arterial vessel; if they are performed before ischaemia in the same organ that undergoes I/R protocol (target organ) it is defined as ischaemic pre-conditioning (IPreC), whereas if occlusions are performed in the same tissue that undergoes I/R protocol immediately after ischaemia it is called ischaemic post-conditioning (IPostC). If the intermittent occlusions are performed distant from the target organ it is called remote ischaemic conditioning (RIC). RIC can be performed before, during, and/or after the I/R protocol in the target organ. They are called remote ischaemic pre-conditioning (RIPreC), remote ischaemic per-conditioning (RIPerC) and remote ischaemic post-conditioning (RIPostC), respectively (Fig. 2).

The main mechanisms of cardioprotection: Reperfusion Injury Salvage Kinase (RISK) pathway, including PI3K/Akt/eNOS and Survivor Activating Factor Enhancement (SAFE) pathway, including JAK-STAT3, are the main protective signalling cascades activated within the myocardium by both conditioning and exercise-induced protection [97,98,99,100]. Alterations of the RISK and SAFE signalling pathways in the aging models have been suggested to explain the failure of the aged heart to be protected [101,102,103]. The alteration includes post-translational modifications, different subcellular localization and expression of key proteins involved in signalling transduction cascades, such as many elements of RISK/SAFE cascades (Akt, ERK1/2, PKC, STAT3) and mitochondrial connexin-43 [9]. Several other factors protect human myocardium from IRI, including the Stromal derived factor 1alpha (SDF-1α) [104]. Indeed, SDF-1α/CXCR4 signalling axis is also involved in RIC [105,106]. The molecules and pathway involved in the cardioprotection are numerous and often the molecular pathways have common points [107,108,109,110].

3.2. Aging in cardioprotection

As mentioned above, the increased susceptibility of IRI during aging is caused by several mechanisms involved in post-transductional modifications and altered expression of the kinases involved in cardioprotection, such as Akt, PKC, STAT3, ERK1/2, and connexin-43. Therefore, the RISK/SAFE signalling cascades, classically involved in cardioprotection, are attenuated or defective during aging [111].

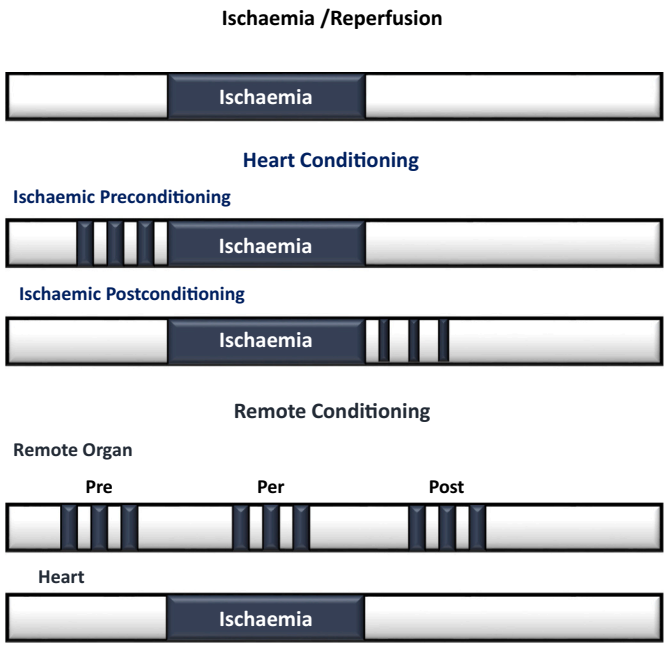

Fig. 2. Schematic representation showing the time-course of typical protocols of ischaemic cardioprotection according to the moment in which the maneuvers are performed. The parts signed in blue indicate periods of ischaemia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Several others responsible factors with detrimental effects are described. These include mitochondrial dysfunction, excessive ROS production, impaired calcium homeostasis. Glycoxidation or proinflammatory pathways, are also exacerbated.

During exposure to H_2O_2, resistance arteries from female mice are more resistant to cell death than those from male mice [112]. Moreover, H_2O_2-induced apoptosis has been shown to be greater in vascular SMCs than in ECs. Consistent with their greater death, the increase in $[Ca^{2+}]_i$ in SMCs during H_2O_2 exposure exceeds that observed in ECs. Interestingly, conditions associated with chronic oxidative stress (advanced age, Western diet) protect SMCs during H_2O_2 exposure, as does female sex [112]. Possible mechanisms contributing to the differential susceptibility of different cell types include dependence on glycolysis *vs* oxidative phosphorylation, mitochondrial structure and function, coupling between intracellular organelles, intracellular Ca^{2+} handling, and respective roles in modulating apoptosis. These variables may also contribute to greater cellular resistance in female vessels compared to male vessels [113].

Is the NLRP3 inflammasome activation exacerbated in aging? Deletion of NLRP3 in elderly mice has been shown to increase muscle endurance and strength, and can also stop the increase in the number of myopathic fibers [114]. In addition to motor performance, the genetic deletion of NLRP3 in mice improves the wellness duration by attenuating multiple age-related degenerative modifications, such as glycaemic dysregulation, bone loss, and impaired cognitive function [93,115]. Despite these evidence and those concerning the involvement of the inflammasome in many other conditions, few studies consider the role of NLRP3 in the exacerbation of myocardial IRI observed in elderly animals and humans (see below).

3.2.1. *Effect of age on cardioprotective treatments*

3.2.1.1. Ischaemic conditioning. As previously underlined, several studies indicate that the effectiveness of myocardial conditioning gradually wears off in middle-aged or elderly animals (reviewed by [2,9,116].

For instance, reduced protection by IPreC against IRI has been shown both *in vitro/ex vivo* in 18 months old mouse [117] and 12–18-months-old rat hearts, and *in vivo* [118,119], as well as in mice older than 13 months [29]. Yet, other *in vitro* and *in vivo* studies indicate IPreC is still able to exert a protective effect in 17-months-old rat hearts [120] or in 20-months-old rat hearts [121].

Conflicting data on the cardioprotective effects of ischaemic postconditioning (IPostC) with aging are also reported. These may be due to the IPostC protocol and/or the age of the animals. Indeed, although the protection afforded by IPostC is lost in middle-aged (12 months) and aged (20 months) mice *in vitro/ex vivo* [122], in middle-aged mouse hearts *in vivo* (older 13 months) it depends on the strength of the IPostC maneuver [103]. The reduced effectiveness of IPostC reported in aged mice has been confirmed in rats where cardioprotection observed *in vivo* in (16–18 months old hearts [123], is lost in 24 months old hearts *in vitro/ex vivo* [124].

Similar to the other conditioning maneuvers, the effectiveness of RIPreC is abolished in 22–24 months old rats [125].

The decline of cardioprotection with aging has been explained by the reduced expression and activation of proteins, such as mitochondrial connexin-43, STAT3 and those constituting the RISK pathway, together with alterations of mitochondrial function [9,101,108,126]. All these functions must be kept active to preserve the cardioprotection of the aged myocardium.

3.2.1.2. Pharmacological conditioning. The limited cardioprotective efficacy of pharmacological preconditioning with the volatile anesthetics, isoflurane, and sevoflurane, in 20–24 months old rat hearts, has been explained with the reduced Akt pathway activation, ROS generation, and mPTP opening inhibition [127,128]. Anesthetic-induced preconditioning with isoflurane reduces cell death and preserves mitochondrial respiratory function to a greater degree in middle-aged patients with human atrial myocytes subjected to simulated IRI than in aged (54 ± 7 *vs* 74 ± 6 years) patients [129].

The lack of activation of the RISK pathway and inhibition of the mPTP opening may explain the reduced protective effect of pharmacologic PostC with sevoflurane or isoflurane in the myocardium of 20–24 months old rats, in comparison with younger (3–5 months old) rats [130]. A similar lack of effects has been observed in 20–24 months old rats after pharmacological inhibition of glycogen synthase kinase 3 beta (GSK3β) to inhibit mPTP opening and reduce IS [126]. On the other hand, PostC with sphingosine was unaffected in 27 months old rat hearts, suggesting that the signalling cascade involving the protein kinase G (PKG) or protein kinase A (PKA) pathway is not affected by aging [131].

3.3. *Effect of sex on cardioprotective treatments*

Several experimental animal studies indicate that sex may influence the effectiveness of all cardioprotective maneuvers.

It has been hypothesized that oestrogen, physiologically acting on the female heart, masks the protective effect of conditioning, thus explaining the fact that the positive effects of IPreC observed in male were absent in female hearts. This hypothesis is supported by the different expressions of cardioprotective mediators involved in IPreC in the two sexes. Indeed, in female hearts, KATP channels and heat shock proteins (HSP) are constitutively more activated [89,132], while the ability to control increasing ROS [133] and maintain PI3-K/NOS pathway activation through Notch1 and GPR30 are improved [7]. Contrasting results have been recently obtained in rat hearts, showing no sex-dependent significant difference in infarct size after IPreC [134]. This discrepancy can be explained by the different animal models or experimental protocols. For instance, it has been reported that, in females, IPreC can induce cardioprotection only in presence of a marked ischaemic insult, capable to overcome the existing oestrogen protection

[135,136]. In addition, it should be taken into account the confounding effect of age: probably due to incomplete sexual maturation, IPreC is ineffective on young females, while male animals show cardioprotection after IPreC at any age [137].

Accordingly to IPreC, significant differences for the ability to exert cardioprotective effects in different sexes have been reported also for IPostC [138]. Only male rat hearts take advantage of PostC, and the improved post-ischaemic recovery of function is accompanied by increased MnSOD expression, while superoxide production, Bax/Bcl-2 ratio, and caspase-3 activation are reduced. In addition, while in male hearts IPostC enhanced the expression in p-Akt, p-GSK3β and p-PKC+, no changes were observed in female hearts [86].

Also, it has been reported that in male rat hearts IPostC activates the cGMP/PKG pathway, which may contribute to IPostC protection by delaying normalization of intracellular pH during reperfusion, likely *via* PKG-dependent inhibition of HNX [139].

As in the case of IPreC, the ineffectiveness of IPostC in female hearts could be related to the activation of pathways already physiologically stimulated by oestrogens; this may explain why PostC is able to exert protective effect only when female hearts have been exposed to a more pronounced injury [6,140].

A very limited number of studies have been devoted to investigating the influence of sex on RIC. Recent findings suggest that some of the humoral factors released after RIC are age- and sex-dependent. RIC plasma of young male volunteers reduced infarct size in both young and, to a lesser extent, aged rat hearts, while RIC plasma of aged male volunteers had no protective effect. Neither the plasma of young nor old female volunteers induced cardioprotection. The protective activity of humoral factors derived from young males after RIPreC has been related to the phosphorylation of GSK3β, which promote mPTP inhibition through the RISK pathway [141]. In contrast with these findings, a recent study by Lieder et al., [134] indicates that sex is no determinant of RIPreC-induced cardioprotection in isolated rat hearts. Yet, in this study, RIPreC-induced cardioprotection resulted greater with a greater mass of I/R peripheral tissue.

Recently, it has been suggested that *extracellular vesicles* (EVs) play a central role in the protective effects of RIC, in particular, due to the anti-apoptotic effect induced by the release of EVs containing miR-24 [142,143]. Furthermore, it has been reported that short-term exercise can affect the release of EVs into the bloodstream in a tissue- and sex-dependent manner, suggesting that the benefits of exercise may depend on an interplay of endocrine and metabolic factors in various tissues, modulating the formation and content of EVs [98,144,145]. Therefore, monitoring of EVs as sex-specific biomarkers may be useful to better understand the beneficial effects of physical activity in patients.

A limited number of studies in humans investigated the different responses to cardioprotective maneuvers in the two sexes. Unfortunately, none of these studies yielded definitive results. In some human clinical trials, PostC has been proven to be ineffective, although the different sex response was not considered in these studies [146,147], and able to reduce infarct size without sex differences [148]. A recent clinical study evaluating the impact of sex differences on IPostC during the PPCI showed a higher rate of major adverse cardiac events at 1 year in women compared to men [149].

Similar controversial results have been reported for RIC, showing no cardioprotective effects [150], or cardioprotection without sex differences [146,151,152,153]. In conclusion, the limited number of studies and the presence of oestrogens as possible confounding factors are responsible for the current lack of definitive results on the differences between sexes after cardioprotective maneuvers [154,155].

4. Role of NLRP3 inflammasome in myocardial ischaemia/reperfusion

A plethora of stimuli activates NLRP3, including cholesterol, fatty acids, silica, and amyloid deposits, to name only a few. Several experimental results indicate that the activation of NLRP3 inflammasome after reperfusion may concur to the progression of cardiac IRI, promoting cardiomyocyte loss by inducing pyroptosis, and favouring adverse remodelling by inducing the release of IL-1β and IL-18 [156,157,158]. Of note, the activation of the NLRP3 inflammasome and caspase-1 and the subsequent pyroptosis can occur especially when the myocardium has previously been "primed" by the presence of comorbidities [3].

The NLRP3 complex is a pattern recognition receptors (PRR) molecule belonging to the Nod (nucleotide oligomerization domain) -like receptors (NLR), capable of recognizing both molecular patterns associated with pathogens (PAMP) and damage-associated molecular patterns (DAMP), with consequent downstream stimulation of pathways leading to the systemic immune response [159].

This complex consists of multiple proteins: a sensor (NLRP3), an Apoptosis-associated speck-like protein containing a CARD (ASC) adapter protein, and a precursor of the protease cysteine procaspase-1 [159]. The interaction of activated NLRP3 with ASC leads to the conversion of procaspase-1 in active caspase-1. In turn, caspase-1 leads to the activation of the inflammatory cytokines IL-1β and IL-18. These cytokines increase the release of various inflammatory molecules (*e.g.* TNFα and IL-6, IFN-γ, IL-13, IL-4, IL-8), thus contributing to the inflammatory immune responses [159].

Recently, numerous studies were focalized on the role of the inflammasome in the IRI [159,160,161], in particular showing a reduced infarct size and improved cardiac function after deficiency or blockade of one of the components of the NLRP3 inflammasome [162,163,164], or using several NLRP3 inhibitors and gene silencing (Fig. 3). The ineffectiveness of the ischaemic preconditioning maneuvers in NLRP3 KO mice of both sexes suggests a complex role of NLRP3 and the possible presence of compensatory mechanisms [165]. The study of inhibitors of the NLRP3 complex also involves other key components of the inflammasome, such as ASC, whose inhibition appears to reduce myocardial damage at 24 h [3,159]. These different responses have been related on the one side to the lower levels of IL-6 and on the other hand to the phosphorylation of STAT3, and involvement of the SAFE pathway [3,159].

Also, regarding NLRP3 we would be faced with a dual role, already highlighted by the ROS [166,167,168], which, despite the well-known dangerous activity in reperfusion, turn out to be fundamental in triggering protective pathways during IPostC maneuvers. Similarly, NLRP3 could be involved in triggering IPreC protection by allowing the preservation of myocardium viability and on the other hand by inducing pyroptosis with the consequent increase in the infarct area and remodelling by the release of IL-1β and IL-18 in ischaemia/reperfusion [169]. The preclinical studies have provided excellent results in terms of reduction of infarct size when the inhibitors used were not aimed at reducing the expression of NLRP3, but at blocking the aggregation of NLRP3 with the other components of the complex (*e.g.* ASC) by inhibiting the ATPase or NF-κB action [169].

4.1. NLRP3-microbiota interaction in myocardial injury

Inflammasome represents a major downstream inflammatory pathway of PRR activation to eliminate host-microbial invasion. Yet, several pieces of evidence suggest a mechanistic link between gut microbiota and NLRP3 in several conditions, including CVDs.

Indeed, gut microbiota influences human physiology by interacting with the host to control nutrient uptake and metabolism, as well as immune signalling pathways and acts as an endocrine organ by regulating a variety of neurotransmitters. Therefore, changes in microbiota composition may shape the immune and inflammatory responses far from the gut. For instance, gut-brain axis and gut-lung axis alterations have been associated with the pathogenesis of neurodegenerative and lung inflammatory diseases [170,171], including the progression of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in which

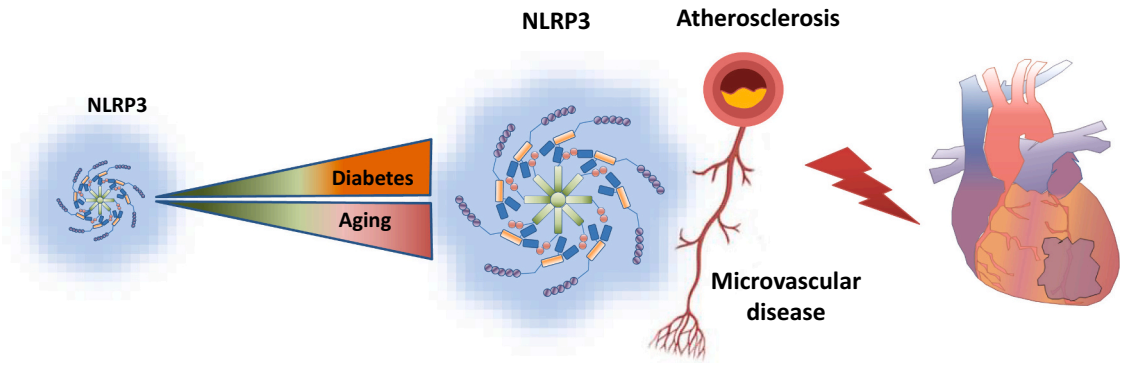

Fig. 3. Progressive alteration of NLRP3 in aging and diabetes. Aging and diabetes induce NLRP3 inflammasome activation and the consequent production of pro-inflammatory cytokines. NLRP3 activation leads to cell damage and related diseases such as CVDs, namely vascular damage, hypertension, atherosclerosis, and metabolic syndrome.

the expression of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 receptor, was hypothesized to be the bridge between the lung and the gut. Intriguingly, meta-analysis detected gene clusters associated with SARS-CoV-2 cytokine storm and identified promising genes, which could be targeted by probiotics action on ACE2-mediated virus entry, TLR-mediated innate immune response and NLRP3 inflammasome complex response attenuation [172].

The above data are in line with the accumulating evidence linking the alterations in the gut microbiota with CVDs [173,174]. Actually, a correlation between microbiota-derived aromatic amino acid metabolites and the degree of IRI has been reported in rat hearts [175]. In this study, probiotic treatments have been proposed to potentially shift the intestinal microbial population towards a cardioprotective phenotype. In fact, the administration of gut commensal *Lactobacillus plantarum* decreased myocardial infarct and improved post-ischaemic mechanical function. *Lactobacillus rhamnosus GR-1* administration also attenuated left ventricular hypertrophy and heart failure after experimental AMI in rats [176]. Supplementation with L. *plantarum* 299v (Lp299v) reduced infarct size in male hypertensive rats [177] and improved vascular endothelial function in men with stable CAD, who were supplemented daily with Lp299v for six weeks [178]. The authors demonstrated a reduced *ex vivo* arteriolar endothelial response to ACh, which was validated by *in vivo* assessment of flow-mediated dilation of the brachial artery in the dominant arm. The observed NO increase and decrease in systemic inflammation associated with IL -8, IL −12 and the decrease in leptin suggested the important role of probiotics or even their gut-derived metabolites such as short-chain fatty acids in enhancing endothelium-dependent vasodilation in CAD men [178]. However, the microbial diversity in the gut of men and women, which is related to sex hormones but also to changes in dietary habits, might influence the response in CVD [179].

High circulating levels of *trimethylamine N-oxide* (TMAO), a choline metabolite of gut microbiota, on the contrary, are associated to the progression of atherosclerosis and other CVDs and are present in the circulatory system of heart failure patients [180]. TMAO contributes to endothelial injury by inducing NLRP3 inflammasome formation and activation with increased IL-1β production [180]. Heart failure severity was significantly enhanced in mice on choline or TMAO-supplemented diet [181]. Moreover, the recent observation that TMAO modifies the purinergic response by affecting the intracellular ATP-induced calcium increase, the release of NO, and $eNOS^{Ser1179}$ suggests a possible involvement of TMAO in the impairment of the endothelium-dependent vasodilator mechanism [182].

Lipopolysaccharide (LPS), which is known to be involved in NLRP3 activation, increased together with d-lactate gut translocation and correlated with systemic inflammation and adverse cardiovascular events prediction, in a metagenomic analysis characterizing systemic bacteria pools in patients with stable CAD or STEMI (ST-elevation MI) [183]. Actually, LPS induced cardiac injury, inflammation, apoptosis, and pyroptosis by activating NLRP3 in mice cardiomyocytes. The production of ROS caused by LPS was necessary to induce the NLRP3 translocation to the cytoplasm from the nucleus [184,185]. Therefore, gut microbiota disturbances may occur during AMI leading to LPS release that, in turn, contributes to myocardial NLRP3 activation during AMI. The attenuation of AMI by decreasing the TLR4 and NLRP3 signalling pathways triggered by LPS can represent an important target of clinical relevance [186]. Long-term administration of different *probiotic Lactobacillus strains* to spontaneously hypertensive rats improved endothelial function, increased eNOS phosphorylation, and abolished the ROS increase in the aorta by downregulating TLR4 mRNA levels and NADPH oxidase activity [187].

Overall, these observations support the concept that the targeted intake of probiotic supplements may have a beneficial effect on the vasculature and thus may be an effective method to reduce cardiovascular risk.

4.2. NLRP3 inflammasome as a potential target in the treatment of ischaemic diseases in relationship with age and sex

Several studies suggest that aging can cause the accumulation of dysfunctional mitochondria, ROS formation, and consequent activation of the NLRP3 inflammasome, producing pro-inflammatory cytokines accompanied by a decline in autophagy capacity. These factors impair cellular cleaning and expose cells to a greater risk for many age-related diseases, such as atherosclerosis and type 2 diabetes, which are predisposing factors to cardiovascular disease. However, there are very few studies on the role of NLRP3 in ischaemic myocardial disease and cardioprotection in the aging heart of both sexes.

While the involvement of NLRP3-inflammasome in cardiovascular diseases has been clearly proven [11,156,157,158,159], its role in physiological and pathological cardiac aging is largely unknown. The aging-related NLRP3 alterations are linked to the increasing impairment of metabolic pathways related to insulin resistance, inflammation, and autophagy dysfunction. Therefore, it is likely that NLRP3 plays an important role in ischaemic disease in aging. Indeed, as reported above, genetic deletion of NLRP3 in mice can attenuate multiple age-related degenerative changes, such as bone loss, glycaemic dysregulation, and cognitive function alterations, thus improving muscle performance and health lifespan [112,113].

A recent study by Marín-Aguilar et al. [188] shows that NLRP3 suppression may prevent several age-related metabolic alterations, preserving cardiac function and increasing the lifespan of aged male mice. In particular, ablation of NLRP3-inflammasome attenuated alterations in cardiac structure and electrical activity, reducing the age-dependent prolongation of the PR interval, which is often linked to atrial fibrillation. In addition, compared with old wild mice, old NLRP3

KO mice showed a PI3K/AKT/mTOR pathway inhibition and autophagy improvement and preserved Nampt-mediated NAD^+ levels with increased SIRT1 protein expression [188].

Evidence as to whether the NLRP3 inflammasome is regulated differently in men and women in the literature is still scarce and should be integrated, but some insights are gathered below. It has been demonstrated that oestrogen is able to drive the M2 phenotype polarization of macrophages [189]. The M1 phenotype is predominant in males, along with a greater expression of NLRP3 and IL-1β. Cowie et al. [190] demonstrated that after a surgical incision the production of NLRP3 mRNA was similarly increased in both males and females; despite this, following the deletion of NLRP3, the level of IL-1β was decreased only in males, suggesting that females have an NLRP3-independent mechanism of IL-1β production [189].

NLRP3 has been found to be regulated by oestrogen during allergic airway inflammation, against which oestrogen seems to have a protective effect [189]. In the work of Cheng et al. [191], ovariectomized mice sensitized with ovalbumin to establish airway inflammation, showed higher expression of the NLRP3 inflammasome and downstream effectors caspase-1 and IL-1β. The long-term administration of 17β-oestradiol on the same mice markedly reduced ovalbumin-induced airway inflammation and led to a suppression of both mRNA and protein expression of NLRP3, caspase-1, and IL-1β [191]. Further studies are needed to elucidate the role of oestrogen and to find the molecular pathways involved in the regulation of NLRP3 capable of reducing inflammasome formation and inflammatory pathologies, including CVDs.

The NLRP3 inflammasome seems to play a pivotal role in the progression of many CVDs, specifically in the pathologies that are more affected by chronic inflammation and vascular damage such as hypertension, metabolic syndrome, and atherosclerosis [3]. For example, in rodent atherosclerosis models, the deletion of NLRP3 or other players of the inflammasome such as caspase-1 led to a significant reduction in atherosclerotic plaques [192]. These results suggest a central and different role for NLRP3 in coronary artery disease in the two sexes and aging that deserves to be further investigated. Intriguingly, sodium-glucose cotransporter-2 inhibitors (SGLT2-I), such as *Dapagliflozin and Empagliflozin,* a novel class of anti-diabetic drugs, attenuate NLRP3 inflammasome activation, which may explain, at least in part, their cardioprotective effects. For instance, SGLT2-I may have therapeutic potential for diabetic atherosclerosis (one of the major determinants of coronary artery disease) as well as against doxorubicin-induced cardiovascular injury, and these benefits may depend on the inhibitory effect on IL-1β secretion *via* the ROS-NLRP3-caspase-1 pathway and on the related anti-inflammatory effects [193–195].

Some drugs have been proposed to block inflammatory cascades at different levels, in preclinical and clinical settings, with conflicting results. A recent review summarizes the most relevant clinical trials with anti-inflammatory therapies in cardiovascular diseases [196]. Disappointing results in clinical trials with anti-inflammatory therapies are reported, with the only exceptions of trials targeting elements of the NLRP3 inflammasome.

5. Conclusions

Aging, sex, and NLRP3 inflammasome alter myocardial metabolism and play important roles in myocardial ischaemia/reperfusion injury modulation. Some initial evidence shows involvement of NLRP3 inflammasome in the pathophysiology of aging and in the exacerbation of IRI in the elderly. Less evidence supports the role of sex and hormones in the modulation of NLRP3 inflammasome and for its involvement in myocardial damage extension in the two sexes.

A relation between the sex, host status, and gut microbiota in cardiovascular disorders is evidenced. Indeed, host statuses, such as obesity, diabetes, and aging, may alter gut microbiota profile. The imbalance of gut microbiota alters both metabolite and cytokine pool of the body, which consequently impairs the host homeostasis, NLRP3 activation, and increased the occurrence of cardiovascular diseases.

Intriguingly, the “anti-diabetic” and “anti-aging” drug, metformin [197], exerts protective effects by regulating myocardial IRI-induced inflammatory response [160]. These effects were largely dependent on the activation of the AMPK pathway, which in turn suppressed the NLRP3 inflammasome activation. We have recently shown that Ticagrelor, an antiplatelet drug, induces conditioning effects, despite these are not additive to those obtained by directly inhibiting NLRP3 [109]. Yet, additive effects were observed combining Ticagrelor and an inhibitor of Caspase-1, a downstream target of NLRP3 [198]. These findings and many others reported in this review suggest that there is a complicated crosstalk among intracellular pathways and that there is much more to study in these intricate processes and pathways, especially in relation to aging and sex. Importantly, regarding clinical translation into treatments for patients with acute coronary artery disease, who generally are elderly, have comorbidities and receive various drugs, the alleviation of short- and long-term inflammatory processes, including inflammasome activation, must also be considered to improve clinical outcomes, in aging subjects of the two sexes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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