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Review Article

Significance of the nitrosative-oxidative stress disequilibrium on endothelial dysfunction during cardiac development

Maqsood M. Elahi[1], Bashir M. Matata[2]

[1]Division of Cardiothoracic Surgery, Department of Surgery, Texas A&M Health Science Center at Scott & White Memorial Hospital, Temple, TX, United States
[2]Department of Clinical Research, The Liverpool Heart & Chest Hospital NHS Foundation Trust, Liverpool, United Kingdom

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Corresponding Author
Bashir M. Matata
Liverpool Heart & Chest Hospital
NHS Foundation Trust,
Thomas Drive,
Liverpool, L14 3PE, UK.
matata_bashir@hotmail.com

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Abstract
Endothelial dysfunction as a consequence of a variety of common cardiovascular disease risk factors is thought to be associated with increased reactive oxygen species (ROS) and the subsequent decrease in vascular bioavailability of nitric oxide (NO). In this article we give a detailed discussion of evidence of the impact of oxidative-nitrosative stress during maternal pregnancy on fetal development in animal models and also the association with the onset of cardiovascular conditions in adult humans. We highlighted specifically the presence of ROS in circulating blood as the key intermediary related to vascular injury and organ dysfunction, the evidence that red blood cells regulate the arteriolar microcirculation, coupling oxygen delivery with blood flow, and highlighting their role in NO bioavailability. The unique nature of relationship between cell-signalling, transcriptional mechanisms and oxidative-nitrosative stress in the progression of coronary heart disease has also been discussed in greater detail. We have also discussed the emerging concepts that pharmacological prevention of cardiovascular events in the future might consists of the control of classical risk factors with specific interventions targeting oxidative stress while simultaneously improving NO production.

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INTRODUCTION

Experimental studies have so far reported the significance of the vascular endothelium in the regulation of homeostasis and myocardial wellbeing [1-5] through the participation of different metabolic, synthetic, and regulatory pathways within our body. We now know that a normal endothelial function is needed to maintain the control of antithrombotic and thrombolytic activity, vascular architecture and permeability, leukocyte interactions with the vessel wall, and regulation of vascular tone. In this context we and others have suggested the particular importance of endothelium derived nitric oxide (NO) bioavailability within blood [6-10]. We further suggested that the modulation of the bioavailability of NO and its precursors might play a key role in the oxidative-nitrosative disequilibrium phenomenon that might be related to the development of atherosclerotic lesions [11].

Nitric oxide, as we know today, is a free radical species that diffuses and concentrates in the hydrophobic core of low-density lipoprotein (LDL) and serves as a potent antioxidant [12]. Peroxynitrite ($ONOO^-$), the product of the diffusion-limited reaction between NO and superoxide anion, as well as lipoxygenase, represent relevant mediators of oxidative modifications in LDL. Previously we suggested the interactions between NO, peroxynitrite and lipoxygenase during LDL oxidation, were relevant in the development of early steps of myocardial remodelling in the disease phase as well as progression of atherosclerosis [13]. We also suggested recently the role of NO in redirecting peroxynitrite reactivity in LDL, the lipophilic antioxidant sparing actions of NO and the effects of novel potential

pharmacological strategies against such process [14]. Thus the knowledge of reduced NO bioavailability in human circulation is of prime interest to comprehend fascinating issues regarding cardiovascular disease process and heart-failure pathophysiology. Attempting to understand these issues requires insight into the pharmacologic and biologic underpinnings of mechanisms that enhance NO availability.

Here we summarize the present understanding of NO metabolism in blood and its availability towards signalling mechanism responsible for heart-endothelial cells interaction in maintaining homeostasis. We also discuss in detail the emerging concepts that pharmacological prevention of cardiovascular events in the future might consists of the control of classical risk factors with specific interventions targeting oxidative stress while simultaneously improving NO production.

BIOMECHANICS OF NITRIC OXIDE

Nitric oxide is a ubiquitous signalling molecule that influences cardiovascular functioning by modifying post-translational effectors of cysteine residues [15]. This process is called *S*-nitrosylation that regulates key processes in both the heart and the vascular tree, and thus, can affect both cardiac performance and vascular tone [16]. This is a highly versatile signalling mechanism, facilitated by superoxide ($O_2^{\bullet-}$) in dual faceted moieties in a concentration dependent manner [15] (Fig.1).

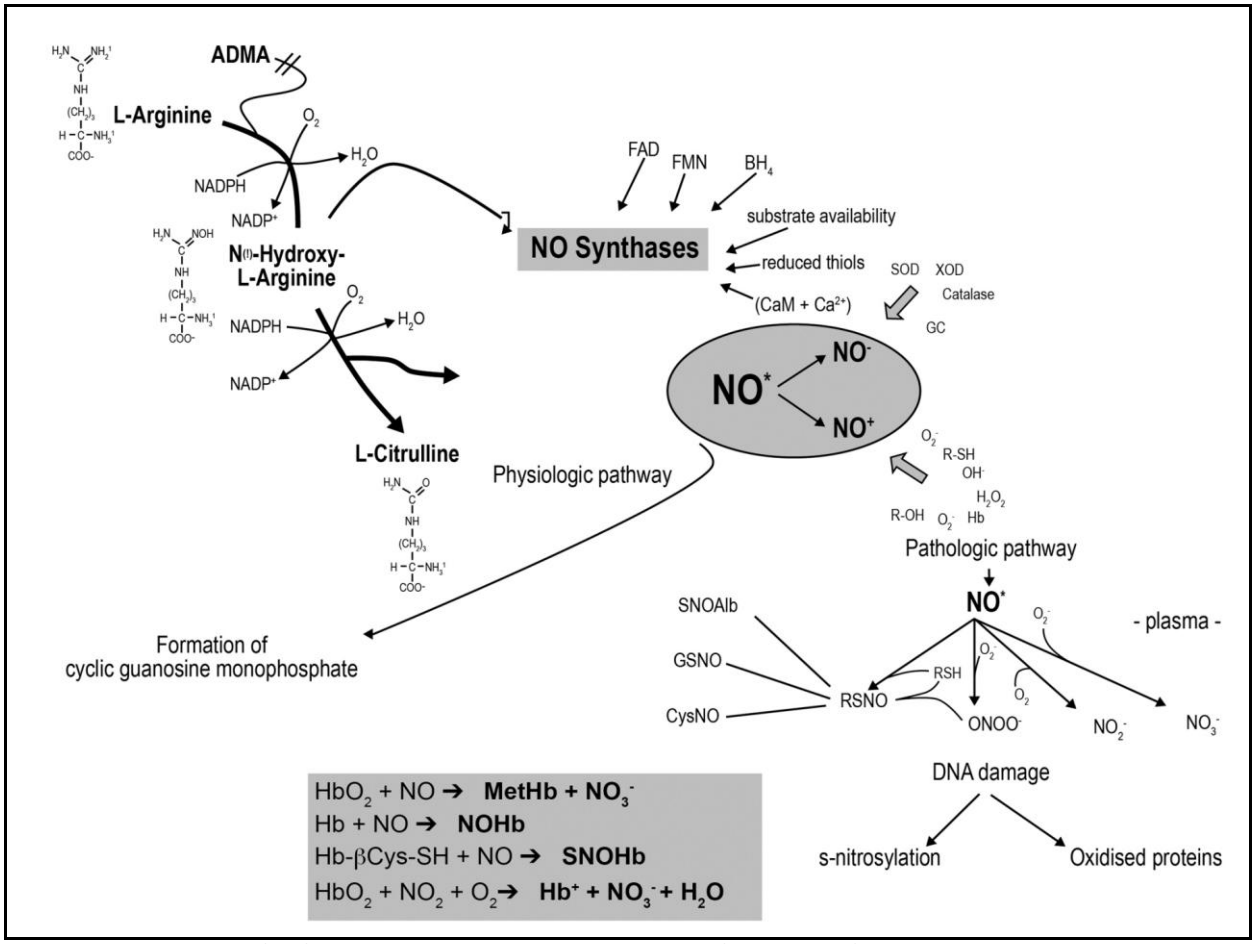

Figure 1. Adopted and modified from Lauer *et al* [17]. Cartoon representation of the activity of nitric oxide (NO) synthesis and its potential decomposition. NO synthases (NOS) catalyze the oxidation of L-arginine to NO and L-citrulline with the intermediate N^{ω}-hydroxy-L-arginine (NOHA). NO synthesis is critically influenced by various cofactors, like tetrahydrobiopterin (BH_4), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), the presence of reduced thiols, endogenous NOS inhibitor asymmetric dimethylarginine (ADMA), and substrate availability. Additionally, NOS I and III are dependent on calmodulin (CaM) and Ca^{2+}. The subsequent mode and rate of NO elimination depends on its concentration, its diffusibility, and the concentration of other bioreactants. In plasma, NO may react with molecular oxygen to form nitrite (NO_2^-) or with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($OONO^-$), which subsequently decomposes to yield nitrate (NO_3^-). Alternatively, the nitrosonium moiety of NO may react with thiols to form nitrosothiols (RSNO). Furthermore, NO may reach the erythrocytes (RBCs) to react with either oxyhemoglobin to form methemoglobin (metHb) and NO_2^-, with deoxyhemoglobin to form nitrosylhemoglobin (NOHb), or with the Cys93 residue of the β subunit to form *S*-nitrosohemoglobin (SNOHb). In addition, plasma NO_2^- could be taken up by RBCs, where it is oxidized in a Hb-dependent manner to NO_3^-. SOD, superoxide dismutase; XOD, xanthine oxidase; GC, guanylate cyclase; R-SH, sulfhydryl group; R-OH, hydroxyl group; Hb, hemoglobin; SNOAlb, *S*-nitrosoalbumin; GSNO, *S*-nitrosoglutathione; CysNO, *S*-nitrosocysteine, RSH, sulfhydryl group.

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Excess $O_2^{\bullet -}$ also reacts directly with NO, disrupting its physiologic signalling and potentially leading to the production of other toxic and reactive molecules, notably $ONOO^-$ [16]. Thus, a central pathophysiological consequence of oxidative stress is the disruption of NO signalling causing nitrosative oxidative redox imbalance in the myocardium. In medium-to-large size conductance vessels, NO acts as the prototypical endothelium-derived relaxing factor by activating guanylyl cyclase to produce cyclic guanosine monophosphate. Finally, in the microcirculation, NO carried by *S*-nitrosohemoglobin (SNOHb) regulates blood flow [18]. It is important to note that NO is not produced by endothelial cells in the microcirculation but, rather, is carried there by hemoglobin itself [19, 20]. Thus, to help guide the reader through these various reactions, it is useful to elaborate reactive indices of NO bioavailability that contribute to cardiovascular disruptive signalling.

Nitrosation chemistry and nitrosative-oxidative disequilibrium *in vivo*

A better knowledge of the fate of NO is an important prerequisite for a proper understanding of its physiology in blood. It is well opinionated that a continuous production and release of endothelial NO plays an important role in vascular homeostasis and cardiac function [21]. The supposedly rapid conversion of NO to biologically inactive metabolites in human blood formed the rationale for utilisation of inhaled NO therapy, where the rationale is that the short half-life of NO should confine its effect to the pulmonary circulation [22]. To address this issue, we recently studied the impact of inspired NO gas on physiological function and also determined markers of inflammation-oxidative stress for coronary artery by-pass graft surgery patients. Outcomes from subjects that received 5 ppm and 20 ppm of inspired NO were compared to those not given NO gas. Breath-to-breath measurement com-menced at the start of intubation and continued up to 4 h later. Indices of cardiovascular function, alveolar-capillary gas exchange and hematological parameters were not significantly different in outcomes for the inspired NO groups as compared with control. We observed a reduction in mean systemic arterial pressure in all subjects at 30 min and 4 h after by-pass when compared with pre by-pass values. Markers of systemic inflammatory response and oxidative stress increased during cardiopulmonary by-pass particularly at 4 h and 24 h after the initiation of by-pass. In contrast, we observed a reduction in expired NO, at 24 h after surgery in the groups given inspired NO. In addition, there was also a significant reduction in oxidative stress markers in blood at 24 h after surgery for the groups given inspired NO as compared with the control group. In contrast, cytokines response remained similar in all the three groups at all-time points. The results suggested that inspired NO gas has an antioxidant property that reduces the levels of cell death, and is not associated with significantly worse-off physiological outcomes [13].

In circulation, red blood cells (RBCs) are believed to be a major sink for NO by virtue of the rapid co-oxidation reaction of NO with oxyhemoglobin to form methemoglobin (metHb) and nitrate [1]. Alternatively, NO may react with hemoglobin (Hb) to form either nitrosylhemoglobin (NOHb) or SNOHb as discussed above. In addition to its reaction with RBCs, NO has to interact at some stage with plasma constituents, especially in view of the existence of an RBC-free zone close to the vessel wall. Recently, we provided evidence that how ROS in blood augment the cell signalling processes involved in the pathogenesis of coronary heart disease [5-7, 11, 14]. In particular, ROS is an important component of the cross-talk between blood and elements of the vasculature during the initial and latter stages of vascular injury and development of atherosclerotic lesions [5-6]. Currently, the thinking prevails although inflammatory processes may be prompted by different etiological factors from that of coronary heart disease, the presence of ROS in circulating blood is the key intermediary related to vascular injury and organ dysfunction. We reviewed, the clinical and experimental data of the mechanisms involved, and evaluated the wider implications of this concept (results discussed in the next section).

In an elegant series of experiments carried out by Jonathan Stamler's group [23] and recently confirmed by others, red cells were shown to regulate the arteriolar microcirculation, coupling oxygen delivery with blood flow [24-27]. A disruption in nitrosative-oxidative redox signalling clearly has the potential to contribute to events related to myocardial injury. At an enzymatic level, oxidant-producing enzymes are up-regulated and the level or spatial locations of nitric oxide-producing enzymes, namely nitric oxide synthases, are altered within cells [28]. In addition, a deficiency of NO-synthase actually increases the activity of oxidases, since NO may be a physiologic down-regulator of superoxide production [16].

On the other hand, levels of specific vascular NADPH oxidases have been reported to increase in the failing circulatory system, at least partly in response to increased levels of angiotensin II. This suggests a link between neurohormonal activation and a nitrosative-oxidative imbalance [29]. Along with this, the levels and activity of xanthine oxidase produced in myocardium, circulates via the blood throughout the cardiovascular system and contributes to vasoconstriction and depressed myocardial function [30-31]. Datta and colleagues recently demonstrated that the delivery of *S*-nitrosohemoglobin is impaired in the presence of both myocardial failure and one of the

major risk factors for myocardial failure, diabetes [32]. Disruption of nitric oxide delivery to the microcirculation almost certainly contributes to the vasoconstriction and uncoupling of oxygen delivery in skeletal muscle that are characteristic of myocardial failure. Thus, although the sources of oxidative stress may differ and several different enzymatic and biochemical mechanisms can disrupt NO signalling, a central problem in the failing myocardial circulation appears to be a shift in the nitrosative-oxidative imbalance away from physiologic *S*-nitrosylation to one of oxidative stress [33-35].

Cellular damage in this situation is often potentiated due to the stimuli leading to formation of inducible NOS (iNOS) capable of up-regulating oxidases, elevation in NO and concomitant the formation of peroxynitrite with the help of superoxide. A prolonged nitrosative-oxidative imbalance thus leads to the consequences more traditionally ascribed to oxidative stress-cell damage as a result of the oxidation of nucleic acids and proteins, cell loss owing to apoptosis, and phenotypic alteration as a result of the activation of abnormal gene programs (the fetal gene program and resultant cardiac hypertrophy are a prime example of this phenomenon) [36]. The results of upcoming work from our laboratory and others have shown regimens with the potential to restore such imbalance that leads to myocardial remodelling [36-38].

Evidence of involvement of nitrosative-oxidative stress disequilibrium in the pathophysiology of adults with cardiac disease

Nitrosative stress modulated factors are suggested to be elevated in the aging heart (unstable angina and heart failure). However whether such an increase is sufficient to elicit a biological response and whether these findings can be extrapolated to the *in vivo* situation in blood with RBCs being present as a potentially important intravascular sink for NO remains unclear. Keeping this in view, we carried out prospectively designed experiments where blood and myocardial biopsies (right atrium and left ventricular) were obtained from 3 groups of patients (n = 20 per group) undergoing elective coronary artery by-pass graft surgery before the surgical correction: The groups are *(i)* stable angina (SA), *(ii)* unstable angina (UA), *(iii)* and stable angina with severely impaired left ventricular function (ILVF), EF ≤ 30% (Tables 1&2). Our results demonstrated that peripheral blood mononuclear cells subsets CD14-positive cells were significantly greater in UA patients as compared with SA, and these cells produced significantly greater superoxide in UA and ILVF patients (Fig.2).

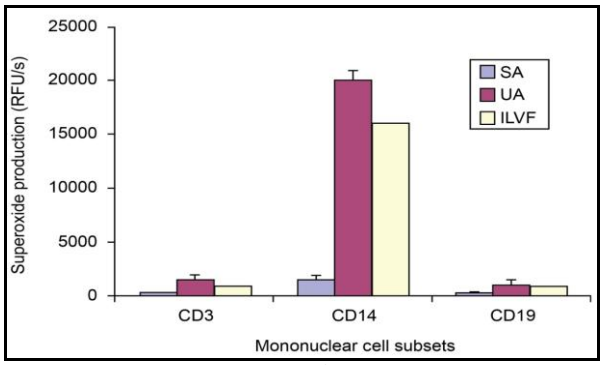

Figure 2. Superoxide production of peripheral blood mononuclear cells (PMBC) subsets. This demonstrates that PMBC subsets CD14-positive cells were significantly greater in UA patients as compared with SA, and these cells produced significantly greater superoxide in UA and ILVF patients.

Table 1. Preoperative clinical characteristics, results presented as mean ± standard error of the mean (SEM)

	Stable angina	Unstable angina	Impaired LV function
Number of patients	20	20	20
Age (years)	62 ± 1.8	65 ± 1.6	62.8 ± 2.9
Male:female	16:4	17:3	18:2
Diseased vessels (3:2:1)	9:3:8	10:2:8	8:4:8
Angina class (Canadian Cardiovascular Society, CCS)	2.2 ± 0.2	3.4 ± 0.1*****	2.6 ± 0.3
Dyspnea class (New York Heart Association, NYHA)	1.6 ± 0.2	2.4 ± 0.1*****	2.2 ± 0.3*****
Left ventricle (LV) ejection fraction (%)	48 ± 2.9	50 ± 3.9	16.5 ± 1.4*****
Hypertension	9	10	8
Hypercholesterolemia	9	7	8
Previous myocardial infarction (MI)	5	3	12*****
Atrial fibrillation	0	1	1
Active smokers	1	2	2
Plasma tumor necrosis factor (TNF)-alpha (pg/ml)	20 ± 3	12 ± 2*****	11 ± 3*****
Soluble TNF-R1 (TNF Receptor 1) (ng/ml)	1.5 ± 0.1	1.8 ± 0.2*****	1.8 ± 0.3*****
Infections	0	0	0
C-reactive protein (CRP) (mg/dl)	1.6 ± 0.2	10.3 ± 1.4*****	8.2 ± 3.4*****

*****$P < 0.05$ compared with the other group(s) (Mann Whitney test)

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Table 2. Preoperative medication and peripheral blood mononuclear cell subsets

	Stable angina (n = 20)	Unstable angina (n = 20)	Impaired LV Function (n = 20)
Medical treatment			
Beta-blockers	17	16	13
Organic nitrates	9	12	9
Ca^{2+} antagonists	7	8	8
Angiotensin converting enzyme (ACE) inhibitors	11	14	12
Aspirin	17	16	14
Statins	15	13	13
CD3 (x 10^6) cells	17.3 ± 5.6	18 ± 1.2	18.4 ± 6.1
CD14 (x 10^6) cells	5.9 ± 0.9	8.7 ± 0.8*****	4.9 ± 0.9
CD19 (x 10^6) cells	1.8 ± 0.4*****	4.0 ± 0.5	3.6 ± 1.1

*****P < 0.05 compared with the other group(s) (Mann Whitney test)

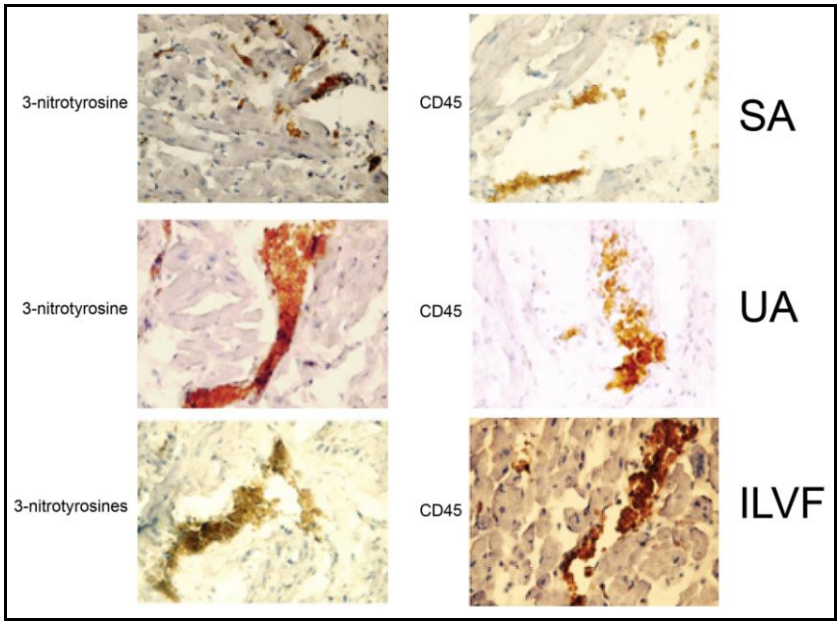

Figure 3. Immunohistochemical staining for protein 3-nitrotyrosine, NF-κB subunit p65, and iNOS was greater in the myocardium from UA and ILVF patients, and staining for CD45 followed an identical pattern. Interestingly, apoptosis in the right atrium biopsies of the ILVF group was significantly greater compared with the SA group, but the greatest values were observed in the UA group (P < 0.001).

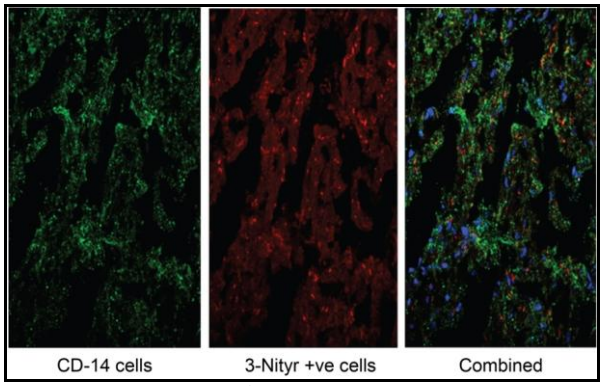

Figure 4. Co-localization of CD14 and 3-nitrotyrosine positive cells in unstable angina ventricular myocardium.

ELISA assays demonstrated soluble tumor necrosis factor (TNF)-alpha in circulation to be greatest for SA patients, whereas in contrast tissue TNF-alpha was greatest in myocardial extracts for UA and ILVF groups. Immunohistochemical staining for protein 3-nitrotyrosine, NF-κB subunit p65, and iNOS was greater in the myocardium from UA and ILVF patients, and staining for CD45 followed an identical pattern. Interestingly, apoptosis in the right atrium (Fig.3) and left ventricle (Fig.4) biopsies of the ILVF group was significantly greater compared with the SA group, but the greatest values were observed in the UA group.

Protein nitration and lipid hydroperoxides were significantly elevated in mononuclear lysates and plasma from UA and ILVF when compared with the myocardial extracts. Immunoblots and citrulline-conversion assays also showed that the iNOS content and activities were greater in mononuclear cell extracts than in the myocardium of UA and ILVF groups. Furthermore, NF-κB activities were significantly greater in the UA and ILVF groups than in the SA

group in both myocardial tissue and mononuclear cell extracts. The results of our this work clearly proposed that excessive oxidative/nitrosative stress induced by activated circulating leukocytes may be responsible for the elevated transcriptional activities and the induction of apoptosis observed in the myocardium of patients with unstable angina and severely impaired LV function, a process that may involve an increase in iNOS activity.

In that study, we demonstrate for the first time that release of NO via iNOS activity exerts systemic hemodynamic effects as judged by NF-κB activity found to be greater in UA and ILVF groups than in the SA group in both myocardial tissue and mononuclear cell extracts. In a separate set of experiments, we reported the mechanism of release of pro-inflammatory cytokines by blood granulocytes via NO-dependent pathways and modulation of nitrosation bioavailability [39]. Taken together, these results provide unequivocal evidence for the occurrence of nitrosation chemistry in the human myocardium and possible hemodynamic consequences.

Cross talk between nitric oxide bioavailability, oxidative stress and cardiac developmental dysfunction

In view of the evidence discussed above, we believe that ROS are implicated in the initiation and progression of cardiovascular disease even at the fetal developmental stage. It has already been observed in several studies [40-42] that ROS can oxidize lipoproteins, limit the bioavailability of NO, and promote translational expression of cytokines and adhesion molecules in dying cardiomyocytes. In addition, Nox proteins of the NADPH oxidase family are prominent sources of this, and Nox protein-dependent ROS production has been linked to this pathophysiology [40, 41]. Together with the phagocyte NADPH oxidase itself NOX2/gp91^{phox} gets upregulated at the mRNA and protein level [41]. In addition to NF-κB, activator protein 1 is an important transcription factor that may mediate pathogenic effects due to an increased pro-inflammatory state with direct effect on Forkhead O (FOXO) transcription factors as a direct target of phosphatidylinositol-3 kinase/Akt signalling in skeletal and smooth muscle and regulate the expression of the Cip/Kip family of cyclin kinase inhibitors in other cell types [42]. The desensitization of protein kinase B/Akt kinase activity and Akt-dependent phosphorylation may downregulate the translocation of p21 into the cytoplasm which in turn may lead to the promotion of Rho-kinases, a contributor to cardiomyocytes dysfunction [41, 42]. This leads to increased muscle loss or increased load, with time, progressive ventricular dilatation, increasing interstitial fibrosis and arrhythmia, and a decline in ejection fraction [40-42].

Therefore, inhibition of ROS production, inhibition of NF-κB and inflammatory protein production, and improvement in NO bioactivity may have additive beneficial effects on endothelial function, and overall cardiovascular pathophysiology. To test this hypothesis we investigated using animal models, whether feeding dams with a high fat (HF) diet during pregnancy and/or lactation can result impaired cardiac development and hypertension in their offspring [29]. Our findings suggested that maternal hypercholesterolemia increases ROS-mediated inflammation and inhibits endothelial progenitor cells (EPCs) differentiation, survival and function in the cardiomyocytes. It therefore affects key components of angiogenesis and endothelial repair in these offspring [43]. Interestingly, treatment of hypercholesterolemic dams with statins improved the number of circulating EPCs and reduced ROS levels in the adult offspring and may prevent the risk of later cardiovascular pathophysiology [44]. We further elucidated that the ability of maternal hypercholesterolemia to reduce EPC survival and differentiation may represent an important mechanism in the developmental origins of cardiovascular disease [45-46]. However, the impact of HF feeding during pregnancy and lactation on EPC biology and CRP levels in the offspring remains to be determined.

OXIDATIVE STRESS HYPOTHESIS AND THE EVOLUTION OF CARDIAC DEVELOPMENT DYSFUNCTION

Cardiac developmental dysfunction characterised by progressive left ventricular (LV) systolic impairment is believed to be modulated by the ROS. Evidence of this comes from studies that have investigated the impact of excess free-radical generation from a variety of sources in animal and human models, such as vascular nicotinamide adenine dinucleotide oxidases, [47] xanthine oxidases, autooxidation of catecholamines, [48] NOS activation, [49] or mitochondrial leakage [50]. The evidence also suggested that besides excessive ROS generation, myocardial antioxidant defences are also impaired [51]. These observations have prompted the formulation of an oxidative stress hypothesis of cardiac dysfunction. We hypothesized that nitrosative oxidative disequilibrium is characterized by generalized and cardiac-specific oxidative stress, and that chronic oxidant injury contributes to impairment of myocardial function and ultimately clinical progression into heart failure state.

We now know that oxidative-nitrosative stress is a common feature of many commonly described risk factors of or conditions associated with adverse (poor or excessive) fetal growth and/or preterm birth, such as preeclampsia, diabetes, smoking, malnutrition or excessive nutrition, infection or inflammation [52-54]. Plausibly, oxidative stress may be the key link

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underlying this pathophysiology. Oxidative stress insults may directly link or accompany many genetic, nutritional and environmental risk factors in association with the elevated risks of adverse cardiac dysfunctional growth indirectly through increasing gestational morbidities such as gestational hypertension and gestational diabetes (Fig.5)

Oxidative stress programming may operate either directly through the modulation of gene expression or indirectly through the adverse effects of oxidized lipids or other molecules at critical developmental windows and therefore resetting/programming the susceptibility to the cardiovascular developmental disorders [55]. The susceptibility of biological systems due to oxidative insults is likely dependent on its resilience and maturity stage at the time of insult. There could be different critical time windows (prenatal or even postnatal) in "programming" different diseases. Plausibly, prenatal and early postnatal periods are the most critical "windows" for oxidative stress programming.

Many studies on cells and tissues have demonstrated the deleterious effects of oxidized LDL (oxLDL) contributing to cellular toxicity, inflammation, vascular apoptosis and endothelial cell dysfunction [56]. Some F2-isoprostanes, such as F2a-8-isoprostanes (8-iso-PGF2a), are associated with potent vasoconstriction and the modulation of platelet functions (pro-aggregation) [57-58]. These or other yet unknown oxidized molecules may be involved in adverse programming by modulating tissue blood supply and growth through affecting vascular physiology or other mechanisms.

Although relatively little is known about the role of oxidative stress in cardiac growth, experimental studies have already demonstrated the role of redox imbalance in modulating gene expression and cell signal cascades [59-60]. ROS is capable of modifying the structure and functions of cellular proteins in defined ways (conformational changes by oxidation of cysteine residues to form disulfide and cyclic sulfonamide covalent bonds) to regulate signal transduction pathways and gene expression [61]. Recent studies in animal models observed that manipulating antioxidant-oxidant balance in pregnancy could alter blood pressure and vascular reactivity in rat offspring [62]. Furthermore, in mitochondria the cellular organelles responsible for regulating energy metabolism and monitoring of blood sugar, the DNA is much more vulnerable to ROS damage than nuclear DNAs [63] which may offer an additional venue of programming the risk of cardiovascular dysfunction as subtle damages which may be magnified with advancing age. It can be envisioned that the effects of oxidative insults on myocytes proliferation, function or energy metabolism at critical developmental windows in early life will have the maximal effects that will be further magnified or aggregated with additional oxidative insults in later life with the eventual manifestations of the heart failure.

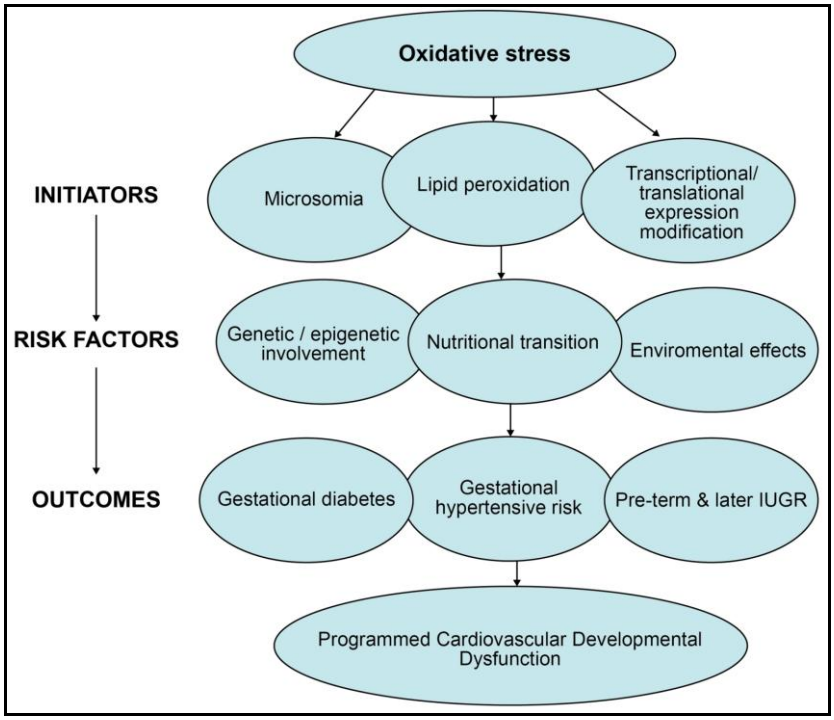

Figure 5.
Oxidative stress may be the key link between adverse insults and cardiovascular developmental programming through directly modulating gene expression and/or the indirect effects of oxidized lipids or other molecules.
IUGR= Intrauterine growth retardation

CONCLUSIONS

Oxidative-nitrosative stress is an important therapeutic target as it represents a common mechanism associated with a multitude of cardiovascular disease risk factors and also during cardiac development. There is extensive evidence from *in vitro* research and from experimental *in vivo* animal models in support of the hypothesis that oxidative stress has a negative impact on cardiac development. Excess of ROS generation or impaired antioxidant function adversely affects several cardiac myocytes' functions such as depression of myocardial contractility, myocardial tissue injury, and induction of myocyte apoptosis. However, the significance of oxidative injury to cardiac function; causes myocardial stunning and injury due to reperfusion after a period of ischemia thus confirming the role of oxidative stress. In addition, myocardial oxidative stress through the targeting of redox sensitive proteins and enzymes is now known to be an important regulator of cardiac developmental dysfunction; and also a regulator of the extracellular matrix deposition and degradation that contributes to ventricular remodelling and LV dilatation. In agreement with others, we propose here that the manipulation of ROS production and the simultaneous increase in NO production could be the basis for future novel therapeutic strategies for halting cardiovascular dysfunction particularly during fetal development.

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