Antioxidants, inflammation and cardiovascular disease

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Abstract
Multiple factors are involved in the etiology of cardiovascular disease (CVD). Pathological changes occur in a variety of cell types long before symptoms become apparent and diagnosis is made. Dysregulation of physiological functions are associated with the activation of immune cells, leading to local and finally systemic inflammation that is characterized by production of high levels of reactive oxygen species (ROS). Patients suffering from inflammatory diseases often present with diminished levels of antioxidants either due to insufficient dietary intake or, and even more likely, due to increased demand in situations of overwhelming ROS production by activated immune effector cells like macrophages. Antioxidants are suggested to beneficially interfere with diseases-related oxidative stress, however the interplay of endogenous and exogenous antioxidants with the overall redox system is complex. Moreover, molecular mechanisms underlying oxidative stress in CVD are not fully elucidated. Metabolic dybalances are suggested to play a major role in disease onset and progression. Several central signaling pathways involved in the regulation of immunological, metabolic and endothelial function are regulated in a redox-sensitive manner. During cellular immune response, interferon γ-dependent pathways are activated such as tryptophan breakdown by the enzyme indoleamine 2,3-dioxygenase (IDO) in monocyte-derived macrophages, fibroblasts, endothelial and epithelial cells. Neopterin, a marker of oxidative stress and immune activation is produced by GTP-cyclohydrolase I in macrophages and dendritic cells. Nitric oxide synthase (NOS) is induced in several cell types to generate nitric oxide (NO). NO, despite its low reactivity, is a potent antioxidant involved in the regulation of the vasomotor tone and of immunomodulatory signaling pathways. NO inhibits the expression and function of IDO. Function of NOS requires the cofactor tetrahydrobiopterin (BH4), which is produced in humans primarily by fibroblasts and endothelial cells. Highly toxic peroxynitrite (ONOO−) is formed solely in the presence of superoxide anion (O2−). Neopterin and kynurenine to tryptophan ratio (Kyn/Trp), as an estimate of IDO enzyme activity, are robust markers of immune activation in vitro and in vivo. Both these diagnostic parameters are able to predict cardiovascular and overall mortality in patients at risk. Likewise, a significant association exists between increase of neopterin concentrations and Kyn/Trp ratio values and the lowering of plasma levels of vitamin-C, -E and -B. Vitamin-B deficiency is usually accompanied by increased plasma homocysteine. Additional determination of NO metabolites, BH4 and plasma antioxidants in patients with CVD and related clinical settings can be helpful to improve the understanding of redox-regulation in health and disease and might provide a rationale for potential antioxidant therapies in CVD.

Key words: Atherogenesis; Cardiovascular disease; Neopterin; Nitric oxide; Tetrahydrobiopterin; Tryptophan; Oxidative stress; Homocysteine; Vitamins; Antioxidative therapy

Core tip: Crosstalk between a number of pathways in-
IMMUNOBIOLUMINARY PATHWAYS ACTIVATED IN ATHEROGENESIS


INTRODUCTION

Despite the availability of successful treatment strategies for dyslipidemia and hypertension, cardiovascular diseases (CVD) account for one third of all deaths worldwide, and prevalence still increases[1,2].

CVD comprise a class of diseases that involve heart and systemic blood vessels[3]. In coronary heart disease, cerebrovascular disease or peripheral arterial disease, impaired blood vessel function leads to an inadequate blood supply of organs. Deep vein thrombosis and pulmonary embolism are usually caused by blood clots in the leg veins.

Avoiding risk factors such as smoking, obesogenic lifestyle, e.g., unhealthy diet, physical inactivity, high blood pressure, diabetes and dyslipidemia, is strongly recommended for disease prevention. Nevertheless, beside lifestyle, genetic, epigenetic and environmental factors may essentially influence the risk of CVD.

The multifactorial background makes it difficult to unravel initial pathological events, which are suggested to occur in a very early phase of disease, where symptoms are subclinical. Inflammation is considered to play a key role in both disease initiation and progression[4]. Chronic inflammatory conditions attenuate endogenous antioxidant capacities due to continuous production of high levels of reactive oxygen species (ROS). Patients often represent with low blood levels of antioxidants[5] and enhanced oxidative stress markers[6]. This is usually due to increased demand in situations of overwhelming ROS production by activated immune effector cells like macrophages. Also insufficient nutritional intake may play a role. Uptake of exogenous antioxidants is suggested to beneficially interfere with diseases-related oxidative stress, however the interplay of endogenous and exogenous antioxidants with the overall redox system is complex.

The object of this review is to give an overview on immunobiochemical pathways activated in atherogenesis, which lead to oxidative stress-related pathological consequences. Understanding of the mechanisms will be helpful in the establishment of new preventive and therapeutic strategies.

MAIN FEATURES OF ATHEROGENESIS

Atherosclerosis is the most common pathological process that leads to CVD including myocardial infarction (MI), heart failure, stroke and claudication. A central event is the development of atherosclerotic plaques in the inner lining of arteries. Irritative inflammatory stimuli, hypertension, hyperglycemia and dyslipidemia cause endothelial stress leading to expression of adhesion molecules and recruitment of leukocytes[7].

Atherosclerotic plaques are characterized by necrotic cores, calcification, accumulation of modified lipids and foam cells, but also other cell types such as smooth muscle cells, vascular dendritic cells, T cells and endothelial cells are involved in lesion formation[8]. The “oxidative modification hypothesis” of atherogenesis implies that low-density lipoprotein (LDL) oxidation is an early event in atherosclerosis[9]. Cholesterol-containing LDL particles are retained in the artery wall and biochemically modified components of these particles in turn induce leukocyte adhesion but also intracellular cholesterol accumulation in invaded macrophages[10]. Chronic inflammatory conditions are maintained due to the production of pro-inflammatory mediators through immune competent cells in the lesions[11]. Activation of macrophages is a key factor in atherosclerotic plaque formation, fibrous cap disruption and thrombus formation.

While in the past atherosclerosis was viewed primarily as passive process of cholesterol accumulation, recent evidence indicates that it is a highly active process involving components of the vascular, immune, metabolic and endocrine system[12]. Initial pathological changes occur in a variety of cell types long before symptoms become apparent and diagnosis is made[13,14]. Of note, also in a large sample of cardiovascular disease-free adults, Chrysohoou et al[5] revealed an association of pre-hypertension with reduced serum antioxidant capacity and increased oxidized LDL, probably indicating initial pathological changes.

Atherosclerotic plaque composition, endothelial erosion, intraplaque hemorrhage, adventitial and intraplaque neovascularization, rather than the percentage of stenosis, turned out to be critical predictors for both risk of plaque rupture and subsequent thrombogenicity[15,16,17]. Disruption of a vulnerable or unstable plaque may lead to a complete occlusion, to plaque progression or result in an acute coronary syndrome, i.e., acute MI (AMI), unstable angina and sudden cardiac death or stroke in case of carotid plaque destabilization.

OXIDATIVE STRESS AND IMMUNE ACTIVATION

Although substantial efforts have been made to dissect...
molecular details of atherogenesis, a full understanding of the underlying mechanisms is still missing. However, activation of immune competent cells, leading to local and finally systemic inflammatory phenomena and the associated status of heightened oxidative stress are central events\(^4\).

Monocyte/macrophage accumulation at the lesion is a key factor in the pathology and involves several steps, such as monocyte recruitment by expression of adhesion molecules and chemotactic factors, induction of activation and differentiation processes as well as proliferation, and immobilization of macrophages in the inflamed plaque\(^5\). Current data indicate that due to the presence of variable differentiation stimuli, different macrophage populations reside in the atherosclerotic plaque\(^6\). Both M1 and M2 macrophages are present in atherosclerotic regions. Macrophage colony-stimulating factor (M-CSF), which is continuously present in circulation, induces predominantly M2-type macrophages with increased phagocytic activity, characterized by expression of interleukin (IL)-10, scavenger receptor A and mannose receptor\(^6\). Granulocyte-macrophage CSF (GM-CSF) induces M1-polarized cells with antigen presentation capacities, which express tumor necrosis factor alpha (TNF\(\alpha\)) and pro-inflammatory cytokines such as IL-1\(\beta\), IL-6, IL-8 and IL-12 upon stimulation with interferon gamma (IFN-\(\gamma\)) or lipopolysaccharides (LPS)\(^1,2,3\). While M1 macrophages were found to predominate in rupture-prone plaque regions, M2-type are located in the vascular adventitial tissue\(^21\). However, also other macrophage phenotypes are suggested to contribute to the inflammatory process in atherosclerosis, such as the recently described platelet chemokine CXCL4-induced M4 cells\(^22\).

Immune reactions in atherosclerotic lesions are mainly T helper (Th1) type responses, as indicated by the dominance of pro-inflammatory and macrophage-stimulating cytokines found in advanced plaques\(^11,12,24\). During Th1-type response, IFN-\(\gamma\) is probably the most important trigger for high ROS production in macrophages\(^23\) by phagocytic NADPH oxidase (NOX)\(^26\). Main reactive species are hydrogen peroxide (H\(2\)O\(2\)), superoxide anion (O\(2\)\(-\)), but also reactive nitrogen species such as peroxynitrite (ONOO\(-\)), nitric oxide and nitrite\(^27\). IFN-\(\gamma\) signaling initiates a variety of cellular defense mechanisms such as pro-inflammatory cytokite production via nuclear factor kappa B (NF-kB) signaling, enhancement of antigen presentation\(^28\) and other important pathways, e.g., neopterin formation via guanosine triphosphate (GTP)-cyclohydrolase 1 (GTP-CH-1) and indoleamine 2,3-dioxigenase (IDO)-mediated tryptophan breakdown\(^29\) (Figure 1).

Under normal conditions, low levels of ROS are mainly byproducts from electron transport chain reactions in the mitochondria\(^30\). They are important regulators of several redox-sensitive pathways involved in the maintenance of cellular homeostasis\(^30\), and act by modifying molecules, enzymes and transcription factors as well as by interfering with the endogenous antioxidant pool\(^27,31,32\). Depletion of endogenous redox buffer systems in conditions with overwhelming oxidative stress is critical, not only due to triggering of immune responses but also through leading to endothelial and smooth muscle dysfunction, and thus to the progression of atherosclerosis\(^33,34\).

**ROLE OF LIPOPROTEINS IN ATHEROSCLEROSIS**

Proatherogenic oxidized LDL (oxLDL) accumulates in the vascular wall and contributes to the pathogenesis of vascular dysfunction early in the development of atherosclerosis. After incorporation via scavenger receptors of macrophages, oxLDL leads to their transformation into foam cells\(^35\). Foam cells accumulate a variety of lipids in droplets in the cytoplasm and secrete extracellular matrix proteins that further support the retention of lipoproteins and attraction of immune cells, thus leading to an enlargement of the lesion\(^36\).

Oxidation of LDL is considered to occur primarily in the vascular wall\(^36\), but also circulating oxLDL was detected in CVD patients\(^37\). Although the amount of circulating oxLDL is small compared to oxLDL present in vessels\(^36\), enhanced serum levels of oxLDL are predictive for endothelial dysfunction and coronary heart dis-
The role of oxLDL as a relevant pro-atherogenic marker is further supported by the study of Meisinger et al.28, who found elevated oxLDL to be predictive for future coronary heart disease events in apparently healthy men. Oxidation of LDL contributes to the prooxidant environment in atherosclerotic lesions. OxLDL is a potent stimulus of vascular ROS formation, mainly through activation of NOX and uncoupling of endothelial nitric oxide (NO)-synthase (NOS) that promotes endothelial dysfunction.29

High-density lipoprotein (HDL) is another potential biomarker with anti-atherogenic properties due to its function in the reverse cholesterol transport and in decreasing lipoprotein oxidation.30 HDL is involved in several biological processes that counteract inflammation and oxidative stress, by beneficially influencing, e.g., pancreatic beta-cell function, endothelial vasoreactivity, endothelial apoptosis, restorative processes and monocytic activation as well as adhesion molecules expression, thus being highly vasculoprotective.31 Paraoxonase-1, a calcium dependent enzyme, is located at the surface of HDL particles and contributes to the antioxidant and anti-inflammatory role of HDL.32 In particular, HDL-associated paraoxonase was shown to inhibit the formation of “minimally oxidized” LDL.33 Nevertheless, other mechanisms are suggested to be involved in HDL-associated inhibition of LDL oxidation.34

Plasma HDL cholesterol (HDL-C) levels are inversely associated with CVD risk in preclinical and large epidemiologic studies. Low HDL-C level was identified as a robust predictor of lipid peroxidation irrespective of gender, age, obesity and inflammatory or metabolic biomarkers in the Syrián Juvenile Obesity/ Early DeteC-tion of Atherosclerosis study employing 797 participants aged from 5 to 50 years.35 However, HDL is highly heterogeneous and the atheroprotective functions of the different HDL subpopulations are not completely understood. Furthermore, current data indicate that therapeutically increased HDL-C levels per se do not always correlate with enhanced HDL functions in vitro.36,37

Of note, accumulation of free, unesterified cholesterol can lead to crystal formation both in vitro and in vivo.38 Crystallized cholesterol in atherosclerotic plaques was shown to activate the NLR family, pyrin domain containing 3 (NLRP3) inflammasome complex by employing the complement system, thereby leading to the release of proinflammatory cytokine IL-1β.39,40 Cholesterol crystals were mainly found in advanced plaques, however the inflammatory responses caused by NLRP3 inflammasome activation might represent an important trigger in disease progression and could thus represent an important pharmacological target.41

**NEOPTERIN FORMATION**

Neopterin, a marker of immune system activation, is produced by GTP-CH I in macrophages and dendritic cells (DC)42,43 and has emerged as an important independent and predictive marker in cardiovascular risk assessment.44 IFN-γ is the major stimulus for neopterin formation. Other cytokines have only limited stimulatory potential in vitro but some, e.g., TNFα, can indirectly enhance IFN-γ induced neopterin formation.45 Of note, also pro-inflammatory compounds like LPS can elevate neopterin levels.46 The amount of neopterin secreted by human macrophages correlates with their ROS-generation capacity in vitro and neopterin concentration in body fluids is considered as an indicator for immune activation-associated oxidative stress.

GTP-CH-I catalyzes the conversion of guanosine triphosphate (GTP) to 7,8-dihydronopterin triphosphate finally leading to the formation of neopterin, 7,8-dihydronopterin and 5,6,7,8-tetrahydriopterin (BH4).47 Human monocyte-derived macrophages and DCs are the most important source of neopterin and its partially reduced derivative 7,8-dihydronopterin, both present in relative constant ratio in human serum, but not of BH4 due to the relative deficiency of pyruvoyl-tetrahydropor- terin synthase in this cell types.48 Figure 1). In contrast, cells from other animal species and other human cell types such as endothelial cells or fibroblasts preferentially produce BH4, which is needed as a cofactor by several monooxygenases including NOS, phenylalanine hydroxylase or tyrosine hydroxylase.49

Elevated neopterin concentrations were reported to be associated with chronic immune activation in several diseases such as viral, bacterial and parasite infections, autoimmune or malignant tumor diseases and during rejection episodes in allograft recipients.50,51 Also patients with CVD present with increased neopterin concentrations, supporting the crucial involvement of chronic immune activation, in particular of macrophages, in atherogenesis. Several studies (Table 1) strengthened the impact of neopterin as an independent marker for CVD, with predictive value for coronary artery disease (CAD) progression.52

Of note, neopterin-positive macrophages were found in coronary atherectomy specimens from patients with stable angina pectoris and to a lesser extent in those with unstable angina pectoris, and the number of those macrophages correlated with T cell and neutrophil count in the lesions.53 Furthermore, neopterin was shown to induce an atherothrombotic phenotype in human coronary endothelial cells in vitro by promoting cellular adhesion molecules (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) and tissue factor (TF) expression mediated by activation of NF-κB54. These data suggest that neopterin is not only associated with the systemic inflammation process in atherosclerosis, but might also be of importance for the inflammatory process within the plaque and thus for plaque destabilisation.55

Neopterin concentrations correlate with IFN-γ-induced ROS production.56 In addition, neopterin has pro-oxidant properties itself by intensifying the effects of ROS as well as of reactive chlorine and nitrogen species.57 Of note, Herpfer et al.57 showed that neopterin is able to enhance ONOO− as well as Cu(II)-mediated LDL oxidation, whereas 7,8-dihydronopterin may protect...
Chronic stable angina pectoris

Increased urinary neopterin

Critical limb ischemia, 1-yr follow-up

Elevated plasma neopterin in about 50% of hospitalized patients undergoing conservative or surgical therapy, higher neopterin levels were overrepresented in patients with higher Frederickson type

Different clinical stages of atherosclerosis

Serum neopterin correlated with the extent of carotid atherosclerosis

Cross-sectional community-based screening study (Ischemic Heart Disease and Stroke Prevention Study, Bruneck, Italy)

Increased neopterin was associated with increased risk of death and CAD progression correlated with increased neopterin and high-sensitivity C-reactive protein as well endothelial activation markers

Increased neopterin may also enhance the effects of ONOO− in the processes of protein nitration. This pro-oxidant property of neopterin indicates a potential involvement in the antimicrobial and antitumoral action of macrophages. The property of neopterin to interfere with and enhance the effects of various ROS might be of central relevance also in atherogenesis.

Inflammation-associated oxidative stress may lead to a rapid consumption of circulating antioxidants. In patients with CAD, higher neopterin concentrations were associated with a decline in levels of several antioxidant compounds and vitamins such as ascorbic acid, α-tocopherol, lycopene, lutein and zeaxanthin.

**TRYPPTOPHAN BREAKDOWN**

In parallel to neopterin formation, other IFN-γ-dependent pathways are activated during cellular immune response such as tryptophan breakdown by IDO. IDO catalyzes the rate-limiting step in the conversion of tryptophan (Trp) and other indole derivatives to kynurenine (Kyn) and is induced in monocyte-derived macrophages but also in fibroblast, endothelial and epithelial cells (Figure 1). Both expression and activity of the haeme-containing enzyme IDO is sensible to redox-regulation and IDO enzyme itself can exert antioxidant activity by scavenging of O2−.[60,61] The estimation of Kyn to Trp ratio (Kyn/Trp), expressed as μmol kynurenine per mmol tryptophan, can be used as measure of IDO enzyme activity both in vitro and in vivo.[60,68] Simultaneous measurement of immune activation markers such as neopterin, IFN-γ or soluble interleukin receptors, allow to relate circulating Trp levels with inflammation-induced IDO activity, as also hepatic tryptophan 2,3-dioxigenase (TDO) could degrade Trp. TDO, however, is regulated via tryptophan content and steroid hormones such as glucocorticoids[89,90], while IDO is strongly induced in response to several pro-inflammatory stimuli such as IFN-γ, TNFα or LPS.[85,86] Depletion of the essential amino acid Trp contributes to the development of an antiproliferative environment and represents an effective antimicrobial and antitumoral strategy.[81] Also T cell proliferation depends on Trp availability, thus IDO activation is a metabolic checkpoint of immunoregulation.[75] IDO activity is crucially involved in the control of T cell proliferation and in the generation of regulatory T cells, and thus in the suppression of autoimmune responses and promotion of tolerance.[82,83]
Metabolic control by reduction of Trp levels may slow down hematopoiesis in addition to other proinflammatory stimuli by affecting the growth and differentiation of erythroid progenitor cells. In line with this, in patients with inflammation-induced anemia, Kyn/Trp was found to inversely correlate with haemoglobin levels\[94,95\].

Accelerated Trp breakdown was reported in patients with coronary heart disease\[96\] and IDO activity correlated significantly with several risk factors for atherosclerosis in the Cardiovascular Risk in Young Finns Study\[97\]. Niinisto et al\[98\] reported that IDO activity positively correlated with coronary artery intima/ media thickness, an early marker of atherosclerosis, although this association did not remain significant after adjustment with classical risk factors in this patient group.

In inflammatory diseases including CVD, a concurrent increase of neopterin production and tryptophan degradation is usually observed. The prognostic ability of neopterin is likely to relate to the association with IFN-γ in the atherogenic process\[8\]. IDO-mediated tryptophan breakdown is suggested to be responsible for several additional aspects observed during disease progression\[29\], e.g., the development of depression. Because tryptophan is a precursor for the biosynthesis of serotonin, the lowered tryptophan availability under inflammatory conditions may limit serotonin formation and thus enhance the susceptibility for lowered mood and depression\[99\]. Of note, development of depressive symptoms have been associated with increased CAD risk and poor prognosis\[100\]. Estimation of Trp breakdown rate could contribute to a better understanding of the interplay between inflammation, metabolic syndrome, mood disturbance, and anemia, all previously described as significant predictors of an unfavorable outcome in patients with CVD\[101\].

**NITRIC OXIDE**

NOS converts L-arginine into citrulline, thereby synthesizing NO. Free NO can migrate through cell membranes by diffusion, and although it relatively low reactivity, NO is a potent antioxidant molecule that can protect from ROS damage\[102\]. However, NO is a free radical, and can undergo oxidation to nitrite and nitrate, react with O₂: to form ONOO−, or bind to transition metals\[103\]. NO signaling is strongly concentration dependent and although endogenous NO is essentially involved in many physiological processes and beneficial in a variety of circumstances, its reaction products may mediate nitrosative and oxidative stress. However, NO products can have also protective effects. In plasma, NO circulates primarily complexed in S-nitrosothiol species\[104\] that are suggested to be a transport and buffer system that controls intercellular NO exchange. S-nitrosylation of the proteome is a unique form of posttranslational modification that can have significant consequences for protein function and cell phenotype. In particular in the cardiovascular system, S-nitrosothiols were shown to exert many actions, including promoting vasodilation, inhibiting platelet aggregation, and regulating Ca(2+) channel function of myocytes\[105\]. The impact of S-nitroso but also N-nitroso protein formation on the reduction of free NO under inflammatory conditions in vitro has still to be investigated\[106,107\].

Endothelial and neuronal NOS are constitutively expressed and produce NO at low concentrations, while inducible NOS is activated, e.g., in macrophages of several species in response to pro-inflammatory stimuli giving rise to higher NO output\[108\]. Endothelial dysfunction, e.g., vasodilation and/or platelet inhibition, a key feature of early atherosclerosis, is associated with the reduced availability of endothelium-derived NO\[109\]. Defects in NO production, metabolism and response have been described to be responsible mechanisms.

In the presence of O₂, ONOO− formation may be a factor that limits NO bioavailability. Beside being strongly vasoconstrictory, ONOO− has been shown to oxidize the NOS cofactor BH₄, thereby leading to eNOS uncoupling and O₂: production\[110\], thus starting a vicious cycle (Figure 1). Reduced vascular BH₄ levels were found in rat and mice models of atherosclerosis and diabetes\[111\].

High NO output and generation of reactive nitrogen species via iNOS contribute to cytocidal defense strategies in inflammation. However, although this has been reported for several species, including mice, until now, large output of NO by iNOS could not be equally demonstrated in human macrophages\[112,113\]. Human macrophages produce neopterin at the expense of BH₄ and low BH₄ leads to NO enzyme uncoupling. Furthermore, the pro-oxidant properties of neopterin may compensate for deficient NO and ONOO− production\[114\].

Of note, NO inhibits IDO expression and function by reversibly binding to the active site heme\[115\]. Induction of IDO and NOS in IFN-γ-mediated inflammatory response is suggested to be functionally cross-regulated\[116\]. The absence of NO-mediated immunoregulation may support enhanced IDO activity at the site of inflammation.

**POTENTIAL ROLE OF TH2 RESPONSES IN CVD**

Th1 responses are in general proinflammatory and known to be proatherogenic, while Th2 cells are usually involved in helminthic and allergic responses. The role of Th2 cells in atherosclerosis seems to be very complex and even contradictory. A potential protective role of Th2 response is discussed in few studies\[117,118\], while Ait-Oufella et al\[26\] assume a potential proatherogenic function of Th2 cells within the complex interaction theater of CD4+ T cell subsets in atherosclerosis. Thus, the exact role of the Th2 response remains to be elucidated based on an improved understanding of the complex interplay between Th1, Th2, and other T cell populations such as Th17 and Tregs within the atherosclerotic scenario\[118,119\]. Overall, Th cell subset polarization in atherosclerosis is less distinct in humans compared to mice\[119\].

High cholesterol diet of ApoE(-/-) mice with differ-
ent T cell subset polarization resulted in increased development of atherosclerosis in the aortic root and abdominal aorta in mice with predominantly Th1-like immune responses [ApoE(-/-) BL/6 mice] in comparison to animals with Th2 predominance [ApoE(-/-) BALB/c] [129]. A potential of IL-4 to limit Th1 cell responses and reducing lesion size was observed in several murine atherosclerotic models [123,122].

Only recently, Engelbertsen et al. [128] reported an association between Th2 immunity and reduced risk of MI, as high numbers of Th2 cells were associated with decreased mean common carotid intima-media thickness, reduced risk of AMI in women and IL-4 was independently associated with reduced risk of CVD. Although some limitations, as, e.g., differences in lymphocyte number between healthy man and women or the use of long-term cryo-conserved cells, this study provides first hints for the clinical importance of an improved understanding of Th2-type responses in CVD. However, again in contrast to these positive, protective attributes, Shimizu et al. [126] suggested a role for Th2 cells and cytokines in the promotion of arterial aneurysm formation.

**ANTIOXIDANTS IN CVD THERAPY**

Oxidative stress triggers inflammation and endothelial disruption in atherosclerosis. A number of studies showed that exogenous antioxidants can modulate endothelium-dependent vasodilation responses, endothelium-leukocyte interactions as well as balance between pro- and anti-thrombotic properties [129]. Accordingly, antioxidant therapy was suggested to beneficially interfere with development and progression of atherosclerosis. Th1/Th2 balance is crucially dependent on redox-events; while Th1 responses prevail at oxidative conditions, Th2 responses were shown to be supported by “antioxidative stress” [128]. Thus, disequilibrium of Th1/Th2 cytokines may be involved in CVD as a mechanism of immunotoxicity. As Th1 and Th2 reactions crossregulate each other to balance immune responses [127], suppression of Th1-type response by antioxidants would favour Th2-type reactions. Of note, several types of antioxidant were shown to reduce IFN-γ-stimulated tryptophan degradation and neopterin in peripheral blood mononuclear cells *in vitro* [87,128].

A number of studies reported an inverse relationship between plasma antioxidants, or total antioxidant capacity and cardiovascular diseases [8,130]. Low intake of antioxidants, in particular of vitamins, was suggested to be associated with an increased risk of CVD [128,131]. Thus, the finding of an inverse correlation between concentrations of antioxidant compounds and vitamins and disease risk could relate to an increased requirement for antioxidant molecules during inflammatory diseases and insufficient supply with these compounds may further accelerate disease process. However, this assumption has not been conclusively proven in clinical trials and is still controversially discussed in the literature [131-134]. Likewise, equivocal effects of antioxidant supplementation with vitamin E, beta-carotene, alpha lipoic acid, coenzyme Q10, alone or in combination, on cardiovascular health were reported [135].

Major effects were expected from vitamin E therapy. Due to its fat-solubility, vitamin E is part of cell membranes and lipoprotein particles, where it counteracts oxidation events. Vitamin E-mediated protection from oxidative stress and atherosclerotic plaque formation has been shown both *in vitro* and in mouse models. However, while in several clinical trials vitamin E supplementation lead to a reduction of risk of fatal and nonfatal AMI, others reported even a slight increase of mortality upon high dose vitamin E treatment [136]. Thus, no final suggestion can be made about the impact of vitamin E supplementation and even recent metanalysis including a large trial number lead to inconsistent results [137].

So far, although a general association of low vitamin levels and oxidative stress related conditions is established, no clear evidence for a beneficial effect of vitamin supplementation exists. The association between vitamin deficiency in patients and disease symptoms is suggested to result mainly from the inflammation-associated consumption of oxidation-sensitive vitamins [29,132,138], which may lead to a variety of secondary effects.

Apart from being part of the antioxidant defense system, some vitamins act as enzyme cofactors. Low B vitamin availability (B6, B12 and folic acid) leads to impaired remethylation of homocysteine to methionine and thus to homocysteine accumulation, as they are essential cofactors in homocysteine-methionine metabolism. Increased homocysteine levels were found to be associated with arteriosclerotic outcomes and risk of stroke in elderly individuals [139], and are considered as an independent risk marker for CVD [140]. However, lowering homocysteine levels by B-vitamin supplementation failed to demonstrate beneficial effects in CVD [140]. Also, in open-label study with demented patients on B vitamins, a decline of homocysteine has been observed, while neopterin levels were not affected [142]. Recent data indicate that homocysteine accumulates secondarily due to heightened oxidative stress associated with immune activation [143-145]. Thus, also the impact of the selected marker has to be critically evaluated when assessing the effect of vitamin supplementation.

A broader understanding of antioxidant action is clearly warranted. Beside their direct effects in the prevention of biomolecule oxidation by being oxidized themselves, several antioxidants mediate a variety of effects that are of longer duration, as they may induce signaling changes in the biological system [146]. However, a variety of drugs may act also as antioxidants, thus antioxidant vitamins could interfere with pharmaco-relevant signaling pathways. This is of particular relevance for multi-target drugs such commonly used statins.
Statins are inhibitors of 3-hydroxy-3-methylglutaryl-co-enzyme A (HMG-CoA) reductase, and their lipid-lowering effects are suggested to reduce the risk of coronary heart disease\[149\], although therapeutic efficacy is controversially discussed\[150\].

The primary mechanism of statin action is suggested to be the reduction of LDL cholesterol, but several clinical trials indicate that statins exert pleiotropic effect that contribute to therapeutic efficacy. Statins act as effective antioxidants by inhibiting generation of ROS, but also by interfering with NOX and NOS, antioxidant enzymes, lipid peroxidation and LDL cholesterol oxidation\[151\]. In \textit{in vitro} studies with vascular smooth muscle and mononuclear cells, treatment with atorvastatin could reduce NF-κB activation and expression of pro-inflammatory cytokines and chemokines\[152\]. In human peripheral blood mononuclear cells and in monocyte cell lines, atorvastatin was shown to suppresses stimulation-induced neopterin formation and tryptophan degradation, suggesting that both immunoreactivity of T cells and of monocyte-derived macrophages are down-regulated by this statin\[153\]. Treatment with several statins could promote Th2 polarization of CD4+ T cells primed \textit{in vitro} with anti-CD3 antibody and splenic antigen-presenting cells\[154\]. These findings strongly suggest that statins contribute to the regulation of Th1/Th2 cell balance also \textit{in vivo}. In endothelial cells, statins were shown to be involved in restorative processes by increasing NO-bioavailability and promoting re-endothelialization\[155\]. Of note, lovastatin was able to prevent neopterin-induced activation of human coronary artery endothelial cells \textit{in vitro} by interfering with NF-κB activation and decreasing expression of cellular adhesion molecules and TF\[77\]. Furthermore, lovastatin reduced C-reactive protein -induced NF-κB activation in human umbilical vein endothelial cells\[156\]. Beside NF-κB, activation of inflammatory transcription factors activator protein 1 and hypoxia-inducible factor 1 alpha were shown to be down-regulated in human endothelial and vascular smooth muscle cells upon treatment with HMG-CoA reductase inhibitors\[157\]. In line with the reported antioxidant and anti-inflammatory properties, statin use has been associated with lower neopterin levels in patients\[158,159\].

The influence on redox-balance and Th1-type signalling pathways such as neopterin formation and tryptophan breakdown has been described for a variety of (potentially) cardioprotective antioxidant drugs and vitamins, e.g., aspirin\[160\], atorvastatin\[155\], vitamins C and E\[151\] and seems to be a common mechanism at least \textit{in vitro}. Furthermore, circulating vitamin E was shown to increase upon statin therapy\[162,163\]. Thus, due to inferences with common pathways, therapeutic efficacy might change when combining several antioxidant drugs and supplements.

Furthermore, antioxidant composition may differ between patients, and estimation of antioxidant profiles before therapy could be useful to select candidate patients that would profit from antioxidant therapies\[164,165\] and to avoid overdosage. Excessive antioxidant consumption may lead to adverse reactions ranging from favoring of Th2-type responses such as allergy and asthma to an increase of mortality\[166-168\]. So far, for patients who respond well to statin/niacin therapy, additional supplementation might only be advantageous when nutritional deficiencies are still detectable, however this hypothesis has to be investigated in more detail.

Another question is, if moderate vitamin deficiencies cannot be better (and safer) regulated by changes of lifestyle factors, e.g., by increasing the consumption of antioxidant-rich food.

**NUTRITION, ANTIOXIDANTS AND CVD**

The strong relationship between redox-status, immune response and metabolism is supported by the close association of metabolic diseases such as diabetes, obesity and metabolic syndrome with CVD\[169\]. Tissues that are important in metabolism are suggested to have an evolutionary potential to mediate inflammatory responses\[170\]. Metabolic and immune response pathways are closely cross-regulated to respond to the energetic demands necessary during immune activation. Several metabolic and immune cells show similarities on genetic and functional level, e.g., pre-adipocytes can transdifferentiate into macrophages\[171\] and activate similar transcriptional responses\[172\].

In contrast to classical activation of the immune system, e.g., by infection or tissue injury, inflammation may also be induced by metabolic triggers. So called metaflammation or para-inflammation is crucially involved in the development of chronic diseases such as diabetes, fatty liver disease and CVD\[172,173\].

A variety of dietary factors are able to produce cardiometabolic imprints that predispose to disease development. E.g., increased consumption of trans fatty acids (TFA) is supposed to activate pathways that are linked to insulin resistance syndrome. High TFA intake was found to be associated with harmful changes in serum lipids, systemic inflammation, endothelial function, and prospective observational studies demonstrated strong positive associations with the risk of MI, coronary heart disease death, and sudden death\[174\]. Changes of traditional nutrition patterns, as it is the case, e.g., in India, where “Westernization” led to an increase in uptake of sugar, salt, high fat dairy products, and TFA-rich food, are suggested to be at least partially responsible for an about 3-fold increase in the prevalence of CVD and diabetes in the latter part of the 20th century\[175\].

But also excessive intake of antioxidants is a burden of modern life due to the omnipresence of preservatives, food colorants and vitamin supplements in the “Western diet”. Although still nutritional deficiencies...
may exist for some specific vitamins or other antioxidants, overall antioxidant stress may favour a Th2 environment by suppressing Th1 responses (Figure 2). In combination with high caloric diet and low physical activity, this may contribute to the development of obesity. Food additives such as sodium benzoate, propionic acid, sodium sulphite, sorbic acid and curcumin were shown to suppress Th1-type immune response in vitro. Antioxidant food additives also interfere with satiety saturation circuits, as they have shown to inhibit leptin release in cultured lipopolysaccharide-stimulated murine adipocytes in a dose- and time-dependent manner. Lowering the amount of circulating leptin is suggested to contribute to a obesogenic environment, as the reduced satiety effect in turn could lead to compensatory antioxidant craving and thus even more food intake. Leptin is considered as a proinflammatory cytokine with proatherogenic features, as it increases monocyte chemoattractant protein-1 and endothelin-1 secretion by endothelial cells, enhances oxidative stress, promotes migration and proliferation of smooth muscle cells and increases platelet aggregation, thus facilitating thrombosis. In the initial phase of obesity-related inflammation, leptin is predictively associated with interleukin 6 plasma levels in juveniles. However, leptin resistance, which later develops during obesity, does also favor atherogenesis.

Obesity-related immune mediated systemic inflammation was found to be associated with the development of the metabolic syndrome and altered Trp metabolism. However, across lifespan from juvenility to adulthood, differences in the Trp breakdown rate were observed. While juvenile overweight/obese individuals showed a decreased to unaltered Kyn/Trp ratio in comparison to normal weight controls, obese adults had significantly elevated Kyn serum levels and an increased Kyn/Trp ratio. Thus, while in younger patients Th2-type responses might be favored, potentially due to the high antioxidant intake, overwhelming inflammation with Th1-type cytokines may predispose for the development of atherosclerosis in adult age.

Epidemiological observations suggest that consumption of certain foods rich in bioactive compounds, e.g., vitamins E and C, polyphenols and carotenoids such as lycopene and beta-carotene, and coenzyme Q10, is associated with decrease of atherosclerotic risk and such antioxidant-rich diet is supposed to be particularly effective in the early stages of atherosclerosis by preventing LDL oxidation and the oxidative lesion of endothelium. However, a balanced diet cannot always be translated into clinical benefit, despite its beneficial impact on human health.

There is accumulating evidence about the importance of maternal diet and early nutrition on different epigenetic mechanisms that promote the susceptibility to the development of metabolic diseases in adulthood, such as metabolic syndrome, insulin resistance, type 2 diabetes, obesity, dyslipidemia, hypertension, and also CVD. Of note, both under- and overnutrition have been associated with adverse responses. Several studies indicate that impaired foetal growth, and/or in utero exposure to risk factors, especially maternal hypercholesterolaemia, may be relevant for the early onset of cardiovascular damage. Translational studies support this hypothesis; however, a direct causality in humans has not been ascertained.

The influence of epigenetic mechanisms on the developmental induction of chronic diseases raises the possibility that nutritional or pharmaceutical interventions may be used to modify long-term cardio-metabolic disease risk and combat this rapid rise in chronic non-communicable diseases.

CONCLUSION

Adaptive and innate immune responses are centrally involved in the chronic inflammatory process, which leads to destabilization of atherosclerotic lesions, these processes are tightly connected to metabolic factors, which are essentially influenced by life style and also the genetic/epigenetic frame. Inflammation-induced oxidative modifications contribute to all important clinical manifestations of CVD such as endothelial dysfunction and plaque disruption. However, due the poor performance of antioxidant strategies in limiting atherosclerosis and cardiovascular events, it remains to be answered if oxidative modification is causal for the initiation or is an injurious response to atherogenesis. Disease underlying interactions are too complex and the understanding is too fragmentary that clear, reliable therapeutic recommendations can be given.

The strong interconnection of metabolic and inflammatory pathways suggests that metabolically induced inflammatory processes should be considered as early,
or even primary events. Many data support that there is a large time span between initial pathological changes and the onset of clinical manifestations. This time frame could be used for preventive strategies, however a better understanding of disease development and more sensitive detection methods would be a prerequisite.

A detailed knowledge on inflammatory and redox-regulated processes would also allow a better adaption of treatment regimes. Stable biochemical markers are necessary to control disease courses and treatment efficacy. In this context, e.g., neopterin is a useful indicator of the immune activation status and oxidative stress and Kyn/Trp ratio accounts for aspects of immunoregulation via IDO and represents an important metabolic checkpoint. Normalization of tryptophan metabolism represents an important goal to improve the outcome of patients suffering from CVD, whereby treatments with IDO inhibitors such as 1-methyl tryptophan could be considered. However, IDO is well known for its immunosuppressive properties, and its inhibition by medications may also lead to adverse effects.

Also several antioxidant drugs, botanical extracts, phytocompounds and vitamins but also food-contained preservatives and colorants have been shown to negatively interfere with IDO. Both inhibition of enzymatic activity as well as downregulation of activatory signals may lead to a normalization of tryptophan breakdown ratio. Thus, nutrition might be considered as a major factor that influences tryptophan metabolism and underlying inflammation in a more gentle and balanced manner than medication.

Measurement of tryptophan and kynurenine concentrations, and calculation of the Kyn/Trp ratio are important predictors of an unfavourable outcome in patients with CVD. It will be important to investigate if these parameters can provide a basis for more successful and precise biologically grounded therapeutic protocols to further reduce cardiovascular morbidity and mortality. Combined measurements of multiple markers, such as additional determination of lipoproteins, NO metabolites, BH4 and plasma antioxidants, will also be helpful to understand redox-regulation in health and disease and may allow to discriminate best between different clinical diagnostic categories and to evaluate treatment strategies.

In summary, a general evaluation of the effect of an “antioxidant therapy” is not possible at the moment. While vitamin supplementation might be beneficial under certain circumstances, a variety of studies indicate no or even adverse effects when administered alone and even more when used in combination with lipid-lowering agents. However, also for statin and niacin treatment a panel of adverse effects has been described. Although antioxidant supplementation may have some benefit to counteract secondary symptoms, their role in CAD seems to be of moderate importance. Surveillance of the antioxidant status before and during therapy would allow seek out patients that could benefit from vitamin supplementation. Impact of lifestyle factors such as nutrition and physical exercise, however, has turned out as a major factor in CVD prevention and also in influencing treatment efficacy.

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