Age associated endothelial dysfunction: Role of oxidative stress, inflammation and Western Diet

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Abstract. Aging is a major risk factor in the development of cardiovascular diseases attributed to the development of vascular endothelial dysfunction. Reduced bioavailability of endothelial- nitric oxide (NO) synthase (eNOS) and its product NO is considered the key mechanism mediating reduced endothelial function with aging. Vascular oxidative stress and inflammation increase with age and Western diets rich in fat, specifically saturated fat, refined carbohydrates and limited plant foods increase oxidative stress and inflammation. In aged endothelium, Western diets can augment endothelial dysfunction because functional changes have already likely begun. This review discusses possible mechanisms responsible for endothelial dysfunction in aging and the links between endothelial aging and Western diets.

1. Introduction

Aging is a visible phenomenon reflecting a cascade of mostly invisible patho-physiological changes in the human body. The cardiovascular system is subject to life-style induced changes as well as natural deterioration due to the aging process. During the last 50 years, the number of people over age 60 years has dramatically increased and is expected to continue to increase [1]. Despite the advances in science and medicine, many older people are suffering from age-associated cardiovascular diseases. The vascular endothelium is a critical regulator of vascular homeostasis and endothelial dysfunction contributes to the pathogenesis and clinical expression of cardiovascular diseases. Aging of the endothelium is suggested to contribute to progressive losses in its function. A dysfunctional endothelium results in blood pressure dysregulation, increased vessel atherogenicity and instability and increased thromboembolic disease risk [2]. Several studies have shown age-related declines in endothelial function [3–5] and age is a strong univariate and multivariate predictor of endothelium-dependent vasodilation [6].

In addition to age, emerging evidence suggests that dietary factors have a significant role in modulating endothelial function. Western type diets characterized by high energy, high intake of fats, especially saturated fat, refined carbohydrates and limited plant food are associated with increased markers of endothelial dysfunction [7, 8]. In an aged endothelium, Western diets can augment endothelial dysfunction. On the other hand, it is also possible to enhance endothelial function through dietary approaches that emphasize fewer calories from rapidly digestible carbohydrate and saturated fats and more calories from plant foods rich in polyphenols [9–12]. Therefore, the intention of this review is to provide an overview of age associated endothelial dysfunction specifically focusing on the role of oxidative stress, inflammation and Western dietary patterns.

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2. Assessing endothelial function and its association with aging

Endothelial function is clinically assessed by determining changes in blood flow or vessel diameter in response to various stimuli presented to the endothelium [2, 13]. The two most commonly used techniques to assess endothelial function are strain-gauge plethysmography and flow mediated dilation (FMD). FMD methodology is an ultrasound-based noninvasive, highly technical, valid method for the assessment of endothelial dependent relaxation (EDR). FMD is used as a surrogate marker of macrovascular endothelial function [14]. Peripheral arterial tonometry (PAT) is another relatively non-invasive technique that assesses peripheral microvascular endothelial function [15]. Pulse wave analysis (PWA) also provides useful information about endothelial function by assessing the mechanical properties of the arterial tree [16]. Laser Doppler flowmetry and imaging accompanied by cutaneous perfusion of iontophoresis of acetylcholine and sodium nitroprusside is another method used to measure endothelial function [17]. Endothelial function may also be assessed indirectly by measuring biomarkers of platelet and fibrinolytic function, leucocytes adhesion, and endothelium associated proteins such as von Willebrand factor, vascular-derived microparticle concentration, among others [18–20]. Elevation in these markers is suggestive of endothelial dysfunction. Overall, these tools and biomarkers are useful in the pursuit to understand endothelium's functional capacity, but provide limited mechanistic evidence.

Impaired endothelial function is a well-established response in people with cardiovascular risk factors such as hypertension, diabetes and elevated blood cholesterol concentrations [20–22]. Endothelial dysfunction was shown to be positively correlated with hypertension using intracoronary Doppler and intravascular ultrasound examination [20]. A double-blind cross-over trial of 12 patients diagnosed with type 2 diabetes indicated that treatment for insulin resistance significantly increased acetylcholine induced vasodilation (maximum forearm blood flow 12.8 ± 1.3 vs. control 8.8 ± 1.3 ml/100 ml; $P < 0.05$) [23], suggesting therapies targeted at improving insulin sensitivity will also impart endothelium benefits.

Endothelial dependent relaxation (EDR) declines progressively with age [6, 24, 25]. In a cohort study of 119 healthy subjects aged 19 to 69 years, EDR as measured by forearm blood flow declined steadily with increasing age [6]. EDR is predominately associated with endothelial NO synthase (eNOS) enzyme and availability of its product, NO. Several investigations reported that eNOS expression, activation (phosphorylation) and NO bioavailability declines with aging [25–27]. However, a few studies have observed increased expression of eNOS with aging, which may be an adaptive mechanism to increase the bioavailability of NO [28, 29]. The mechanism of reduced eNOS expression and activity in aging is not fully known; however, several factors have been suggested including changes in growth factors and hormones that decline with age. [30–32]. The most common factors involved in endothelial aging are thought to stem from oxidative stress and inflammation that impact mechanisms that alter NO bioavailability, foam cell formation, MMP expression, Nuclear Factor-KappaB (NFκB) signaling, adhesion molecule, cytokine and chemokine expression. From this perspective, the role of the Western diet in promoting endothelial dysfunction and possibly premature aging of the endothelium becomes apparent through augmentation of systemic oxidative and inflammatory imbalance.

3. Oxidative stress in aged endothelium

Oxidative stress is known to increase with advancing age; hence the hypothesis "free radical theory of aging" [33]. Oxidative stress is defined as an excessive accumulation of reactive oxygen species (ROS) in the body beyond the capacity of endogenous antioxidants to balance redox status [34–36]. The endothelium appears to be a tissue where high amounts of oxygen radicals are generated [37–42] (Fig. 1). Removal of the endothelium and inhibition of eNOS has been shown to reduce ROS generation in the aorta of aged rats [43]. Studies on aging in humans have shown that bioavailability of NO is diminished in parallel with increased levels of endothelium derived ROS [28, 40]. The sources of ROS in aged endothelium include excessive stimulation of NAD(P)H oxidase or from sources such as mitochondrial electron transport chain, xanthine oxidase (XO) and uncoupled eNOS [44–50].

4. ROS and the endothelium

Chemical reaction converting O_2^- to H_2O is not 100% efficient in biology. Residual O_2^- acts directly
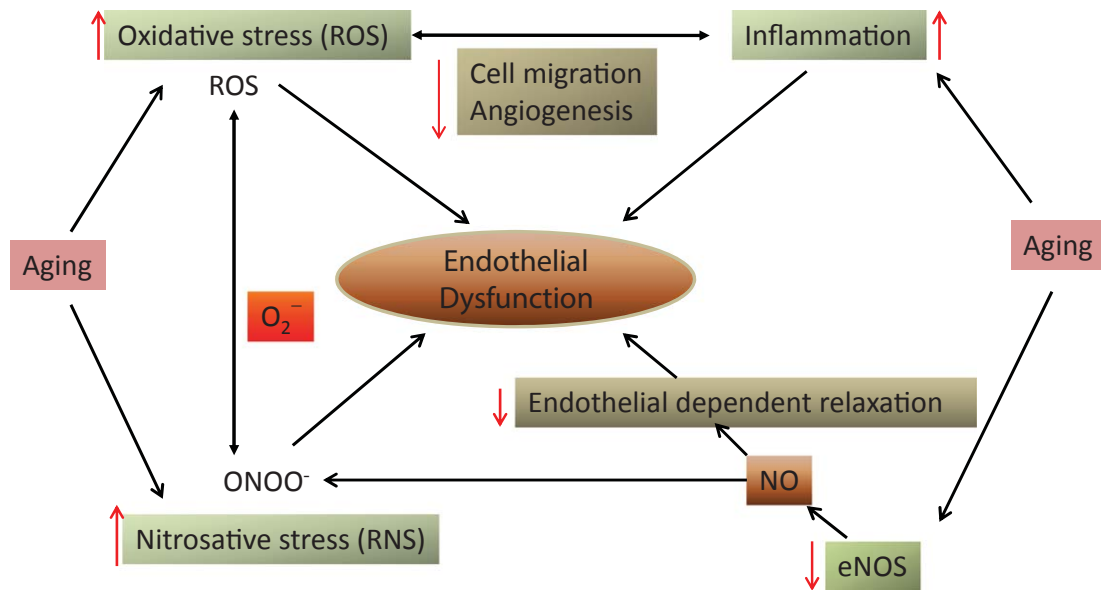
Fig. 1. Age associated endothelial dysfunction. Aging is associated with increased oxidative stress and oxidative damage. The endothelium appears to be a tissue where high amounts of oxygen radicals are generated. Rapid degradation of nitric oxide (NO) by oxygen radicals resulting in peroxynitrite (ONOO$^-$) formation is one of the most widely accepted mechanisms involved in the endothelial dysfunction. Furthermore, endothelial dysfunction with aging is associated with increased levels of inflammatory markers and closely associated with increased oxidative stress. Impaired expression/activity of endothelial NO synthase (eNOS) resulting in decreased NO production has been shown in aged endothelium. ROS-Reactive oxygen species.

as an oxidant or reacts immediately with NO to produce peroxynitrite (ONOO$^-$), a potent and harmful reactive nitrogen species (RNS) in NO-producing endothelial cells [51, 52]. Rapid degradation of NO by O$_2^-$ is one of the most widely accepted mechanisms involved in the alteration of the eNOS/NO signaling pathway resulting in impaired endothelial function [38]. Unlike O$_2^-$, ONOO$^-$ can easily penetrate into cells causing oxidative modifications of macromolecules, such as protein, DNA and lipids via direct oxidative reactions through the nitrosylation of tyrosine and cysteine residue causing impaired cellular signaling [38, 49, 53]. Increased cardiovascular ONOO$^-$ formation has been documented during the aging process [38, 54]. Furthermore, increased nitrosative stress has been demonstrated in arteries from aged animals [28]. Endothelial function as assessed by FMD has been shown to be inversely associated with nitrotyrosine levels in endothelial cells obtained from brachial artery from aged human subjects [44]. Additionally, in human models, age-dependent nitrotyrosine formation has been documented in mesenteric micro-vessels and endothelial dysfunction been partially restored by scavenging ONOO$^-$ [55]. H$_2$O$_2$ is another stable, yet

diffusible ROS that can be converted to hydroxyl radical (OH$^-$), which is not stable. Aging exacerbates H$_2$O$_2$-induced alteration of vascular reactivity in animal models [47, 56]. Collectively, the data strongly support that highly reactive free radical molecules are, at least partially, responsible for age related endothelial dysfunction.

The NAD(P)H oxidases are multi-subunit membrane-spanning proteins with NAD(P)H and FAD binding domains in their C-terminal tails that produce O$_2^-$ by transferring an electron from NAD(P)H to molecular oxygen [46, 57]. It has been demonstrated that increased superoxide generation from NAD(P)H oxidase results in impaired vasodilation in aged rat aorta and carotid arteries [43] and inhibition of NAD(P)H oxidases have been shown to increase endothelial function in rat arteries as measured by increased NO bioavailability [46, 58]. The mitochondrion is suggested to be a major source of ROS generation in aged endothelium [48, 59]. Up-regulation of NAD(P)H oxidase in mitochondria during aging supports the speculation that NAD(P)H oxidase plays a significant role in modulating ROS production during aging and the aging process in the

endothelium [35, 36]. Vascular endothelium is also rich in xanthine oxidase (XO) [60]. Circulating XO has been shown to increase ROS and impact vascular dysfunction in animal models [61–63]. Age related increase in XO also has been documented in both animal and human models [64]. Newas et al. (2006) indicated that XO, but not NAD(P)H oxidase, acts as the main source of oxidative stress in endothelium [63]. These data are supported by others suggesting a role of XO in vascular aging [63, 65, 66], however, some reports suggest otherwise [67, 68]. It appears that involvement of NAD(P)H oxidase and XO in age associated ROS generation is tissue/species dependent; nevertheless both are responsible for generating ROS.

Another possible source of O_2^- in vascular aging is uncoupling of eNOS [49]. Inadequate bioavailability of tetrahydrobiopterin (BH$_4$), an essential cofactor of eNOS, results in uncoupling of eNOS and is associated with increased levels of O_2^- [69]. Bioavailability and activity of BH$_4$ is decreased with aging and the mechanism is not clear [70]. BH$_4$ concentrations are reported to be either reduced [71, 72] or unchanged with aging [73, 74]. However, administration of BH$_4$ in older adults improved endothelial function compared to young people [74, 75]. Similarly, endothelial dependent vasodilation is increased in response to increased bioavailability of BH$_4$ in old rat arteries [71].

5. Role of inflammation in aged endothelium

Low-grade chronic inflammation is another issue linked with aging of the endothelium (Fig. 1). Several studies have demonstrated that impaired endothelial function with aging is associated with increased pro-inflammatory gene expression profiles even in the absence of risk factors for the development of atherosclerosis [76–78]. An activated endothelium is considered a component of endothelial dysfunction, which leads to recruitment of circulating inflammatory cells, such as monocytes and T lymphocytes [79]. A significant body of literature indicates that NFκB activation plays a role in age-related endothelial dysfunction [44, 80, 81]. NFκB activation can induce transcription of genes involved in vascular inflammation including several cytokines, chemokines and cellular adhesion molecules [81]. Among the most commonly measured inflammation markers/mediators

are C-reactive protein (CRP), tumor necrosis factor-alfa (TNF-α), Interleukin-6 (IL-6), inducible NOS (iNOS) and endothelial adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) [82–86]. In the Framingham heart study, FMD was shown to be inversely related to markers of inflammation including CRP, IL-6 and ICAM-1 in an older population [87]. However, after correcting for conventional CVD risk factors, the results were not significant. Nevertheless, inhibition of NFκB signaling has been shown to significantly improve in brachial artery FMD by 74% ($P < 0.0001$) in healthy overweight and obese middle age and older adults [88].

Age related up-regulation of TNF-α has been described in coronary arteries associated with impaired endothelial function [80]. Chronic inhibition of TNF-α in aged animals significantly improves FMD [89]. Furthermore, it has been demonstrated that TNF-α treatment increases iNOS and endothelial adhesion molecules concentrations, which links with increased NAD(P)H oxidases-derived ROS [28, 80]. Recent research indicated that iNOS expression was increased in vascular tissue of aged rats [90]. The iNOS iso-form generated higher amounts of NO than eNOS and therefore rapid reactions resulted in higher amounts of ONOO- [38, 90]. Selective inhibition of iNOS has been shown to improve age-dependent endothelial function [55]. The involvement of IL-6 in endothelial function is well documented [91, 92]. There is strong evidence that serum IL-6 concentrations increase with age [93–95]. However, the mechanism of increased concentrations of IL-6 in aged populations is not well understood. Oxidative stress may play a significant role in elevated IL-6 concentrations in older populations [96]. CRP, is stimulated by IL-6 and has also been shown to be increased in aged populations [78, 94]. CRP decreases eNOS activity in endothelial cells and inhibits endothelium-dependent NO-mediated vasodilation *in vivo* and *in vitro* [97–99]. It has been suggested that CRP inhibits endothelium-dependent NO-mediated dilation in coronary arterioles by producing superoxide from NAD(P)H oxidase mediated mechanism [100].

Endothelial adhesion molecules are pro-inflammatory proteins that play a significant role in cell–cell/cell–matrix interactions. E-selectin, P-selectin, VCAM-1, and ICAM-1 are the major players of endothelial and leukocyte cell adhesion. These molecules maintain low levels in normal physiological

conditions, but expression can be enhanced when the endothelium is activated in response to various stimuli such as pro-inflammatory cytokines, ROS, or RNS [85, 101]. The expressions of adhesion molecules are also up-regulated in aging [85, 101]. There is evidence that the altered expressions of adhesion molecules may be due to increased oxidative stress, which is characteristic of advanced age and may be prevented by anti-oxidative actions [101].

Modified low density lipoproteins (LDL) are also pro-inflammatory in the vascular cell wall [102]. Oxidatively modified LDL (OxLDL) have increased susceptibility to macrophage uptake via scavenger receptors resulting in foam cell and plaque formation, which promote atherosclerosis development [103]. Although high levels of OxLDL are associated with endothelial dysfunction and atherosclerosis, the data on the association between aging and OxLDL are limited. Recently, a study by Zuliani et al. [104] demonstrated that in an elderly population ($n = 1025$, mean age 76 ± 7 yrs; females 55%), LDL-C, triglycerides, and HDL-C were the most common determinants of OxLDL concentrations. However, no association was found between higher OxLDLs concentrations and 9 years CVD/cardiac mortality, suggesting that in advanced age the prognostic information added by OxLDLs on CVD/cardiac mortality might be negligible [104].

6. Diet and the endothelium: Pre-mature "endothelial" aging?

Several dietary factors have been identified to associate with endothelial dysfunction. Dietary factors associated with increased oxidative stress and inflammation have detrimental effects in older populations due to impaired endogenous protective mechanisms. Regular consumption of energy dense diets, high in fat, specifically saturated fat and refined carbohydrates promote increased oxidative stress and inflammation that can result in a host of inter-related metabolic abnormalities and endothelial dysfunction at any age [105–109]. Although the links between high fat and/or carbohydrate diets on endothelial aging have not been fully elucidated, diet forms an important basis for the deleterious perturbations in aging of the endothelium (Fig. 2). Dyslipidemia and impaired glucose tolerance are the most common metabolic abnormalities associated with chronic intake of high fat and/or carbohydrate

diets [110, 111] and both affect endothelial function [112–114]. In this section, the role of age- and diet-related metabolic disturbances that affect the function of the endothelium and possibly contribute to early-onset endothelial dysfunction will be discussed.

6.1. Dietary fat, lipemia and endothelial function

Endothelial function declines with age and dyslipidemia has been shown to augment this process by favoring the generation of ROS [115]. LDL cholesterol and triglyceride concentrations increase, and HDL cholesterol concentration decrease with age [116]. A study carried out by Heitmann BL, (1992) in Danish men and women aged 35–65 years indicated that all blood lipid concentrations were dependent on gender and age. However, they did not consider the contribution of dietary lipids [105]. Age-related disruption of lipid homeostasis/metabolism is suggested to be due to the gradual decline in fractional clearance of LDL, reduced LDL receptor activity, the progressively reduced ability to remove cholesterol through conversion to bile acids, and the decreased activity of the rate-limiting enzyme in bile acid biosynthesis, cholesterol 7 –hydroxylase [117]. Age dependent changes in cholesterol and lipoprotein metabolism has also been linked with the progressive decrease in growth hormones secretion, a characteristic feature of aging [118]. The low levels of testosterone and high free androgens index (testosterone (nmol/L)/SHBG(nmol/L)$\times$100) have been associated with the atherogenic lipid profile in men [119, 120].

Dyslipidemia occurs with poor dietary patterns and reversed with improvements in diet composition such as increased plant foods, fiber, unsaturated fat and reduced intake of saturated fats, red meats and refined grains [110, 111]. Quality and quantity of dietary fat not only influences the plasma lipid profile, but also endothelial function. Short-term and long-term intervention studies have consistently shown a relationship between higher intake of dietary fat and decreased FMD and increased blood markers of endothelial dysfunction [106, 121–123]. However, it is suggested that diets containing high saturated fat has a more significant role on endothelial dysfunction than total fat content; although both play a role. A study by Steer et al. [106] indicated that a diet providing 35% energy from fat (Western type meal) significantly decreased endothelial function as measured by forearm
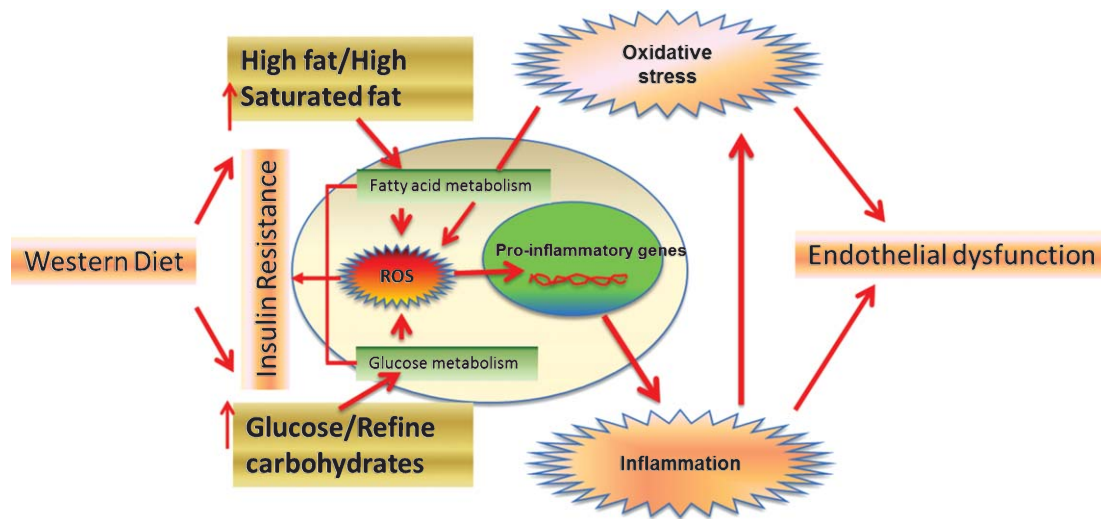
Fig. 2. Endothelial dysfunction and Western diet: Regular consumption of western type diets, which are energy dense and high in fat specifically saturated fat and refined carbohydrates, promote increased oxidative stress and inflammation resulting in a host of metabolic abnormalities such as insulin resistance. Increased oxidative stress and inflammation cause endothelial dysfunction. ROS-Reactive oxygen species.

blood flow after 1h consumption compared to meal containing 20% and 3% energy from fat in healthy men and women aged 20–30 years [106]. Further, a cross over study with isocaloric diets high in polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), or saturated fatty acids (SFA), containing at least 25 g of the relevant fat or a low-fat control diet showed that SFA impaired FMD compared with all other diets ($5.41 \pm 2.45\%$ versus $10.80 \pm 3.69\%$; $P = 0.01$) [107]. In contrast to diets high in saturated fats, the Mediterranean-style diet rich in unsaturated fats is associated with cardio-vascular risk factor reduction, including improved endothelial function. A randomized, single-blind trial conducted in people with metabolic syndrome sub-jects showed an improved endothelial function score in the Mediterranean diet intervention group ($+1.9 \pm 0.6$; $P < .001$) but remained stable in the control group ($+0.2 \pm 0.2$; $P = 0.33$) after 2 years [108]. Similarly, a study conducted in people with type 2 diabetes mel-litus showed that FMD measured before (fasting) and several times after a meal (2 h, 4 h and 6 h postprandial) increased after the MUFA rich meal by $5.2 \pm 2.5\%$ and decreased after the SFA-rich meal by $16.7 \pm 6.0\%$ ($\Delta = -11.5 \pm 6.4\%$; $P = 0.008$ – data expressed as incremental area under the 0–6 h curve) [109].

Elevated concentrations of free fatty acids (FFA) typically observed in insulin resistance decreases endothelial function. Mittermayer et al. [124] showed that infusion of triglyceride/heparin substantially increased FFA concentrations ($P < 0.001$) and reduced endothelium-dependent vasodilation by $38 \pm 17\%$ ($P = 0.024$) in healthy male subjects [124]. Cholesterol lowering, especially LDL cholesterol has been shown to improve endothelial function as assessed by FMD in human subjects [125, 126]. A significant improve-ment in FMD was observed in subjects with LDL cholesterol <80 mg/dL in response to lipid-lowering dietary advice and simvastatin treatment over 12 weeks (5.6 ± 1.7 vs. $13.6 \pm 2.6\%$, $P < 0.028$) compared to subjects with higher LDL cholesterol concentrations (4.4 ± 1.8 vs. $8.2 \pm 1.6\%$, $P = 0.173$) [126]. These data support continued efforts to help people achieve lower LDL cholesterol concentrations.

More than 80% of the CVD-associated death and disability is attributed to atherosclerosis, a product of excessive accumulation of lipids, inflammatory cells, and connective tissue in the vessel wall [127, 128]. Dysfunction of the endothelium is considered one of the first steps in the atherosclerotic process [129]. The described observations link higher dietary intake of total fat, but particularly saturated fat with endothelial dysfunction along with higher concentrations of circu-lating lipids and their metabolic products on endothe-lium dysfunction. Hence, the diet is a critical point of intervention to maintain a healthy endothelium.

6.2. Glycemia and endothelial function

Hyperglycemia is commonly observed in people with diabetes mellitus (both type I and II) and plays an important role in the development and progression of CVD. The glycemic index (GI) is a value used as an indicator of carbohydrate quality reflecting the effect of a food on blood glucose. The glycemic load (GL) is an indicator of both carbohydrate quality and quantity. Epidemiologic data suggest that a high dietary GL from refined carbohydrates increases the risk of CVD with no previous diagnosis of diabetes mellitus [130]. Furthermore, the adverse metabolic effects of hyperglycemia in response to high carbohydrate intake or dietary GL are greatly exaggerated in the presence of insulin resistance [131, 132]. A study assessing the impact of diets differing by GI on endothelial function showed that FMD was significantly lowered after the high glycemic diet $(-0.9 \pm 3.6\%, P < 0.005)$ compared to low glycemic diet $(2.3 \pm 2.6\%, p > 0.05)$ [133].

When the endothelium is exposed to high levels of glucose (hyperglycemia), an array of disruptive intracellular events occur that can lead to impaired endothelial function and atherosclerosis development and progression. Ex vivo experiments with rabbit aorta treated for 6 hours in hyperglycemic media showed reduced acetylcholine-mediated vasodilation compared to normoglycemic controls [114]. Antioxidant administration (eg., superoxide dismutase, catalase, deferoxamine, or allopurinol) reversed the hyperglycemia-associated impairment in vasodilation [114], suggesting that glucose associated suppression of endothelium-dependent vasodilation is mediated through increased production of oxygen-derived free radicals. Hyperglycemia can increase cellular ROS production by several pathways including direct glucose autooxidation [134, 135], mitochondrial superoxide production [136], eNOS uncoupling [137], and Advanced Glycation Endproducts (AGE)-dependent NAD(P)H oxidase activation [138]. Glucose autooxidation and mitochondrial superoxide are considered to be the primary contributors to ROS-mediated endothelial dysfunction induced by hyperglycemia [139]. A study by Kränkel et al. [140] demonstrated that high glucose impairs proliferation, survival, and function of cultured endothelial progenitor cells (EPC) with concomitant decreased NO production and MMP-9 activity [140]. Correction of hyperglycemia by insulin therapy restored the normal EPC pool. In endothelial cells, eNOS uncoupling in response to ROS production

further augments oxidative stress under hyperglycemic conditions resulting in endothelial dysfunction [137]. Hyperglycemia leading to AGE production also contributes to ROS production through receptor-mediated NAD(P)H oxidase activation [141]. AGEs bind to multiple cell surface receptors including the "receptor for AGE" (RAGE) [142] that are known to regulate AGE-associated endothelial cell dysfunction. Use of the NAD(P)H oxidase inhibitor diphenyliodonium (DPI) has been shown to significantly attenuate the production of ROS, verifying the involvement of NAD(P)H oxidase system, AGE and ROS production [143].

AGEs also decrease eNOS expression and L-citrulline production in endothelial cells [144]. Indeed, Gao et al. [145] showed that the impaired vasodilation in blood vessels of diabetic mice is endothelium dependent and RAGE sensitive [145]. Involvement of AGE in endothelial dysfunction is confirmed by significantly reducing atherosclerotic plaque formation in diabetic ApoE null mice with pharmacological inhibitors of AGE formation, alagebriumchloride (ALT-711), pyridoxamine dihydrochloride [146]. A human clinical trial using ALT-711 has also demonstrated enhanced arterial compliance and improved FMD in older patients with hypertension [147]. These data indicate that controlling hyperglycemia is critical in maintaining or improving endothelial function.

Chronic hyperglycemia may induce changes in the endothelial cell that accelerates endothelial cell aging [148, 149]. Hyperglycemia is often caused by impaired insulin action or insulin resistance. A significant contribution to the accelerated aging process in insulin-resistant individuals is attributable to endothelial senescence, dysfunction and impaired repair. Impaired insulin signaling leads to insulin resistance and affects several aspects involved in premature aging, such as body composition, mitochondrial activity, and endocrine function. The life expectancy of people with diabetes is estimated to be lower than that of the general population by 9.1 years in males and 6.7 years in females [150]. The identification of longevity-associated genes such as sirtuin and p66Shc in the vascular endothelium and metabolically active cells suggests that the aging process, endothelial dysfunction and insulin resistance are closely linked [151]. The mammalian sirtuin (Sirt)–1 is highly expressed in endothelial cells and controls functions that are critical to suppressing the development of atherosclerosis [152]. A series of experimental studies have shown that Sirt1 plays a role in improving the function of

endothelial cells. It has been demonstrated that the positive effects of calorie restriction exerts on diabetes and CVD are mediated by sirtuins [151, 152].

7. Dietary approaches to improve endothelial function and slow aging of the endothelium

Several lines of investigations suggest an important role of dietary factors in modulating endothelial function [153–157]. Diets rich in energy, saturated fat, refined carbohydrates and higher intake of sodium are known to promote endothelial dysfunction [153–156]. Types of fat and carbohydrates play a significant role in modulating endothelial function and have been discussed in previous sections (See dietary fat, lipemia and endothelial function and glycemia and endothelial function). Prospective analysis of the CARDIA study that recruited a population based sample of 5115 black and white men and women aged 18–30 years indicated that dietary patterns are associated with cellular adhesion molecules, known markers of endothelial function [155]. The CARDIA study particularly suggested that cellular adhesion molecules are related longitudinally to dietary patterns. The *A Priori Diet Quality Score* was inversely related to cellular adhesion molecules, E-selectin, P-selectin, sICAM-1, and VCAM-1. Similarly, in a cross-sectional analysis of 732 women from the Nurse's Health Study data, two major dietary patterns: a Prudent dietary pattern and a Western dietary pattern were compared relative to endothelial function. The data indicated that E-selectin, sICAM-1, and VCAM-1 were positively associated with a Western diet derived through principal components analysis. A Prudent diet that is high in fruits and vegetables and low in red and processed meats, and refined grains was only inversely associated with E-selectin [158].

Conversely, adherence to diets such as the Mediterranean diet rich in fruits, vegetables, fish, olive oil, and nuts is known to associate with reduced risk of CVD [159]. From a nutritional perspective, these types of diets are abundant in essential micronutrients, fiber, polyphenols and emphasize quality macronutrient food sources. Polyphenols have recently been at the forefront of interest for their effects on endothelial function. Data from the PREDIMED study showed an inverse association between habitual polyphenol intake and the risk of major cardiovascular events and higher polyphenol intake was inversely associated with hypertension, a sign of endothelial dysfunction [160]. These findings are supported by acute and chronic clinical studies showing improved FMD responses after dietary polyphenols intake [161–164]. Although polyphenolic compounds have been studied for their role on endothelial function due to their direct antioxidant effects, recent research indicates that these compounds offer indirect protection by activating cell signaling molecules related to endothelial function such as activation of eNOS and modulating inflammatory genes such as NF-κB [165]. Certain polyphenols and/or their phenolic metabolites are suggested to increase NO bioavailability leading to EDR [166]. This may be achieved through various intracellular signaling paths such as altered redox sensitive kinase activity leading to increased NO synthesis (eg., PI3/Akt, eNOS) [167–169], and or modulating genes involved with the endogenous antioxidant defense system, reducing $ONOO^-$ formation [168, 170]. Investigations have also found that higher intakes of dietary polyphenols are associated with decreased levels of oxidized LDL and various inflammatory markers [7, 8, 171]. Oxidized LDLs promote vascular inflammation and an array of events leading to endothelial dysfunction [172, 173]. Folic acid and antioxidant vitamins such as Vitamins A, C and E also play an important role in modulating endothelial function; although the data are not consistent [159, 174]. The Dietary Guidelines for Americans (DGA, 2010) recommend that people consume at least 2.5 cups of vegetables and 2 cups of fruits daily (2000 Kcal/day diet) in order to achieve the varied essential nutrients people need for health and disease risk reduction [175]. However, specific recommendations are not given in relation to daily polyphenolic intake. This is an area ripe for review and consideration in updates of the DGA.

The association between high dietary sodium intake and blood pressure is a well-known as a risk factor for the endothelial dysfunction, while potassium intake attenuates these effects. It has been demonstrated that a 5% increase of plasma sodium concentration (sodium excess) changes the hardness of endothelium by 25%, leading to endothelial dysfunction [176]. Diets, such as the Dietary Approaches to Stop Hypertension (DASH) diet, that include sodium reduction while increasing potassium intake have been shown to prevent or control hypertension and decrease cardiovascular morbidity and mortality [177, 178]. Furthermore, it has been shown that blood pressure-independent effects of sodium reduce vascular NO bioavailability limiting endothelium-dependent dilation via a mechanism associated with increased levels of ROS generated by

NAD(P)H oxidase, XO or uncoupled endothelial NO synthase within the vascular wall [179].

Poor dietary patterns promote endothelial dysfunction. In aged endothelium, the effects are accentuated [2, 180, 181]. Based on the derived literature, a reasonable hypothesis is that poor dietary habits practiced routinely likely age the endothelium prematurely. Therefore, dietary strategies based on regular consumption of diets emulating Mediterranean dietary patterns, the DASH diet or other diets emphasizing fruits, vegetables, whole grains and intake of low-fat animal products can be used to improve and maintain endothelial function, and to slow down the aging process of the endothelium.

8. Conclusion

Advancing age is an important risk factor for the development of many cardiovascular diseases such as atherosclerosis and hypertension. Endothelial dysfunction occurs during the human aging process and is accompanied by declines and/or deactivation of vasodilator substances produced by the endothelium. Persistent oxidative stress and inflammation play a significant role in endothelial function; and diet can influence both. In particular, diets rich in energy, saturated fat and refined carbohydrates promote endothelial dysfunction: augmenting dysfunction in aged endothelium and possibly aging the endothelium prematurely. Several overlapping mechanisms are apparent and related to modern lifestyle and dietary habits. Understanding and elucidating the mechanisms of endothelial (dys)function relative to dietary patterns and various constituents with potential health benefits, such as polyphenols will require continued investment in the field, particularly focused on long-term interventional studies. With diet at the cornerstone of health and disease risk reduction, science supporting development of dietary strategies that people can easily adopt in modern times to support a healthy endothelium and slowing of age-associated declines can have a critical impact on cardiovascular disease development, progression and complication.

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