Chapter 12
Oxidative Stress in Diabetes Mellitus and Possible Interventions

Triantafyllos Didangelos, John Doupis, and Aristidis Veves

12.1 Introduction

Diabetes mellitus is considered one of the most important diseases of our time as its prevalence is globally increased every year. A large amount of evidence has proved that there is a strong association between diabetes, oxidative stress, and endothelial dysfunction. It is also well recognized that endothelial dysfunction, which is present even in people at risk of developing diabetes, is strongly connected with oxidative stress and considered as a preliminary risk factor for the development of atherosclerosis and cardiovascular disease. Thus, a lot of research effort has been focused during the last years toward the direction of reducing diabetes-related oxidative stress, either with the use of different pharmaceutical agents or with life style interventions.

In this chapter we are going first to analyze briefly the basis of oxidative stress in diabetes and then to focus on the different studied interventions for the diabetes-related oxidative stress reduction.

T. Didangelos
Diabetes Center, First Propeudetic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, American Hellenic Educational Progressive Association Hospital, S. Kyriakidi 1, 54636 Thessaloniki, Greece

J. Doupis
Internal Medicine and Diabetes Department, Salamis Naval Hospital, Salamis, Greece

A. Veves (✉)
Microcirculation Laboratory and Joslin-Beth Israel Deaconess Foot Center, Beth Israel Deaconess Medical Center and Harvard Medical School, West Campus, One Deaconess Road, Boston, MA 02215, USA
e-mail: aveves@caregroup.harvard.edu

I. Obrosova et al. (eds.), *Studies in Diabetes*, Oxidative Stress in Applied Basic Research and Clinical Practice, DOI 10.1007/978-1-4899-8035-9_12,

12.2 Oxidative Stress in Diabetes Mellitus

Helmut Sies was the first to define oxidative stress in the following way: "Oxidative stress is a change in the pro-oxidant/antioxidant balance in the favor of the former, potentially leading to biological damage" [1]. Diabetes is currently recognized as an oxidative stress disorder [2]. Oxidative stress per se is characterized by high accumulation of reactive oxygen species (highly reactive molecules generated during oxidative metabolism and energy production) that cannot be coerced by the endogenous circulating neutralizing agents and antioxidants [3]. The causative mechanisms of oxidative stress due to hyperglycemia are shown in Fig. 12.1.

12.3 Increased Superoxide Production

Diabetes mellitus is associated with increased production of superoxide (O_2^-), mainly due to hyperglycemia [3]. Hyperglycemia causes an increase in intracellular glucose concentration in insulin-independent cell types, such as endothelium. More particular, increased intracellular glucose concentration results in an increased rate of glycolysis, which in turn increases the flux of pyruvate (the product of glycolysis) through the tricarboxylic acid (TCA) cycle. This increased flux of pyruvate through the TCA cycle appears to be responsible for overproduction of superoxide [3].

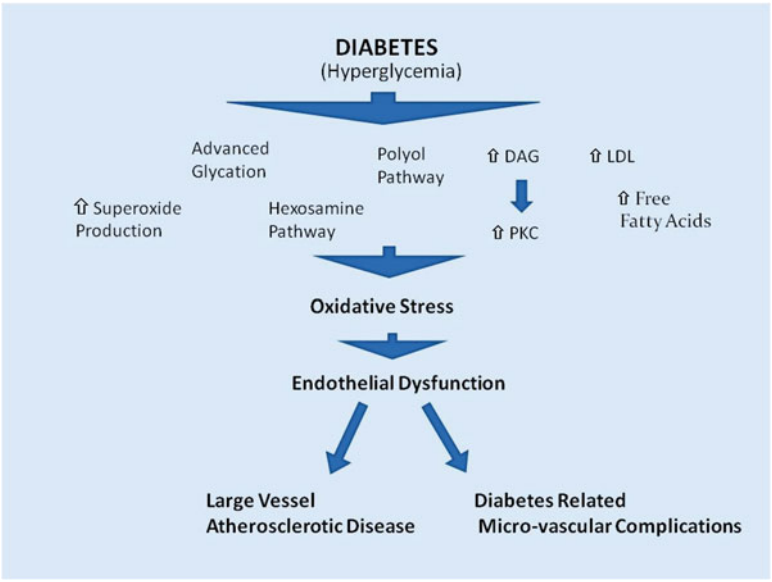

Fig. 12.1 Mechanisms of oxidative stress in hyperglycemia

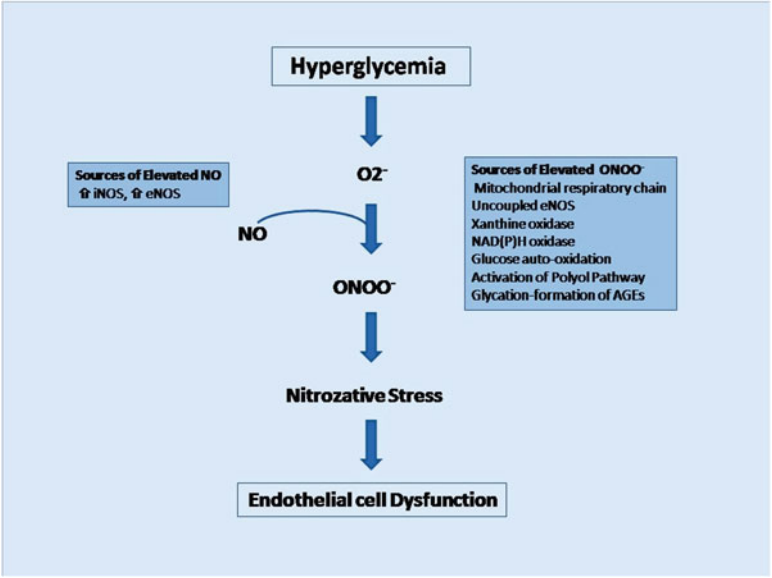

Fig. 12.2 Hyperglycemia-induced endothelial dysfunction. Superoxide produced secondary to hyperglycemia combines with NO to form peroxynitrite. This reduces the bioavailability of NO and induces nitrosative stress by multiple mechanisms including modifications of macromolecules and PARP induction

Hyperglycemia, however, is not the only mechanism by which diabetes causes increased superoxide production. Diabetes is also associated with increased levels of free fatty acids, which contribute to increased superoxide production [4]. Other circulating factors that are elevated in diabetes, such as leptin, also contribute to increased ROS generation [5].

12.3.1 Oxidative Stress and NO

Nitric oxide (NO) plays a key role in vascular health, regulating the endothelial vasodilatation and protecting the vascular wall by inhibiting inflammation, cellular proliferation, and thrombosis [3]. Increased superoxide and reactive oxygen species negatively affect vascular health by downregulating endothelial-derived NO. Decreased NO bioavailability increases the vascular tone, promoting also structural and biological changes that lead to atherosclerosis [3, 4]. NO quenching by peroxynitrite ($ONOO^{-}$) and decreased NO production are the main causes of decreased NO bioavailability [3, 4, 6]. In addition, under certain conditions, the superoxide anion reacts with NO to form peroxynitrite, further reducing the bioavailability of NO in the vasculature leading to impaired protein and lipid function (see Fig. 12.2) [7]. Peroxynitrite, in turn, inactivates the factor (6R)-5,6,7,8-tetrahydro-L-biopterin

(BH4), which plays a significant role in NO production by the endothelial NO synthase (eNOS), leading to further reduction of NO bioavailability. BH4 deficiency uncouples the eNOS complex and promotes production of superoxide by eNOS, thus producing more oxidative stress promoting vascular dysfunction and atherosclerosis [7].

12.3.2 Other Effects of Oxidative and Nitrosative Stress

The degradation of tyrosine nitrated proteins produces free nitrotyrosine. This marker of nitrosative stress has been found in tissues, atherosclerotic lesions, and blood [8–10]. In addition to the modification of biomolecules, peroxynitrite affects important signaling pathways triggering mitochondrial dysfunction and cell death in endothelial cells and cardiomyocytes [11].

12.3.3 PARP Activation

Oxidative and nitrosative stress has been proved to activate poly(ADP-ribose) polymerase (PARP), which is an important mediator of vascular dysfunction in diabetes [7, 11–13] even prior to the onset of microvascular disease [14]. PARP activation initiates a series of cell cycle events (see Fig. 12.2) that deplete intracellular nicotinamide adenine dinucleotide (NAD) and adenosine 5′-triphosphate (ATP) pools, thus limiting glycolysis and mitochondrial respiration, leading to vascular cell dysfunction and death [6].

12.3.4 Protein Kinase C Activation

Hyperglycemia and increased production of free fatty acids increase the activity of protein kinase C (PKC) promoting oxidative stress through activation of mitochondrial NADPH oxidase. Increased PKC activity has also a number of other effects including decreased NO production, increased vascular permeability, increased microvascular protein accumulation, increased plasminogen activator inhibitor-1 (PAI-1) expression and activation of nuclear factor-kappa B (NF-κB) in endothelial cells and vascular smooth muscle, and increased endothelin-1 (ET-1) production. All these actions promote vascular occlusion, stimulate inflammation, and ultimately lead to endothelial dysfunction [2, 15]. PKC may also be activated by increased diacylglycerol (DAG) levels either from de novo synthesis of DAG (from glycolytic intermediates) or from increased activity of the polyol pathway and via ligation of RAGE [16]. Inhibition of PKC with ruboxistaurin (or LY333531) greatly improves microvascular flow to the retina, kidney, endoneural blood supply, and

mesenteric bed in animal models [17–19]. Despite these promising findings, ruboxistaurin has had less robust results in humans [20].

12.3.5 Advanced Glycation End Products

Hyperglycemia may also promote oxidative stress by contributing to the production of advanced glycation end products (AGEs) which are nonenzymatically glycated proteins or lipids susceptible to oxidation after exposure to aldose sugars [21]. AGEs can produce ROS and trigger mechanisms that generate the production of intracellular oxidants. In addition, AGEs have been found to alter extracellular matrix protein function, cause vascular leak, decrease the bioavailability of endothelium-derived nitric oxide (NO), and promote inflammation and endothelial dysfunction [22].

Additionally, AGEs may also induce oxidative stress and endothelial dysfunction by binding and activating RAGE which results in a sustained activation of NF-κB and its target genes increasing also the endothelial cell permeability to macromolecules [23]. Elevated levels of AGEs have been noted in the serum of diabetic patients and correlate with progression of diabetic complications such as nephropathy [24, 25]. Treatment of animals with inhibitors of AGE formation, such as aminoguanide, can prevent diabetic microvascular complications [26].

12.3.6 Polyol Pathway

Hyperglycemia may also promote oxidative stress by increasing polyol pathway flux [27]. The enzyme aldose reductase usually presents low affinity to glucose. However, in a high glucose concentration environment, the increased intracellular glucose results an increased activity of aldose reductase and a consequent increase of the glucose reduction to sorbitol which is further oxidized to fructose. This procedure, which consumes NADPH, decreases the reduced glutathione and increases the PKC activation, subsequently increasing the oxidative stress [3]. Inhibition of aldose reductase has been shown to prevent diabetic nephropathy, retinopathy, and neuropathy in animal models [27]. Larger clinical trials in humans, however, have had mixed results, thus raising questions regarding the importance of this mechanism [28, 29].

12.3.7 Hexosamine Pathway

Hyperglycemia, finally, may also shunt excess glucose through the hexosamine pathway [30]. Excessive intracellular glucose results in conversion of fructose-6-phosphate

to glucosamine-6-phosphate and ultimately to *N*-acetylglucosamine, promoting a series of reactions that increase oxidative stress by NADPH depletion, TGF-beta and plasminogen activator inhibitor-1 (PAI-1) gene expression increase, and endothelium nitric oxide synthase (eNOS) activity inhibition [31].

12.3.8 Diabetes and Cellular Adhesion Molecules (CAMs)

Endothelium can be activated by the effect of various factors including oxidative stress, producing inflammation molecules like iCAM and vCAM MCP and inducing the adhesion and accumulation of monocytes at the arterial wall. This is the first step for the development of endothelial dysfunction and atherosclerosis. This process has been proved to be present not only in diabetes but also in the prediabetic state many years before the diagnosis of diabetes [32].

Diabetes has been found to be closely associated with endothelial dysfunction in both resistance and conduit vessels of the peripheral circulation [33–37] as well as in the coronary circulation [38, 39]. The soluble adhesion molecules, E-selectin, vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1, the presence of which is highly associated with vascular inflammation and oxidative stress, are found to be elevated in subjects with T2DM [40–43]. Similarly, increased levels of von Willebrand factor (vWF), a measure of endothelial cell damage and activation, are found in diabetes [40, 42, 43]. Furthermore, microalbuminuria, which has been proved to be an independent predictor of endothelial dysfunction, may possibly indicate a widespread vascular dysfunction in diabetes [40, 44].

The pathogenetic mechanisms underlying the development of endothelial dysfunction in diabetes have not been fully identified. Oxidative stress and the subsequent reduction on NO bioavailability seem to play the most significant role according to the data so far.

12.4 Methods of Assessing Endothelial Function

Prior to the development of macrovascular and microvascular clinical disease, early changes in endothelial function can be measured. These changes reflect alterations in the regulation of vascular tone or reactivity which is influenced by endothelial NO production (endothelium-dependent vasoreactivity) as well as vascular smooth muscle relaxation in response to NO (endothelium-independent vasoreactivity). In endothelium-dependent vasodilation, acetylcholine, shear stress, or hypoxia can activate endothelial cells to release NO. The stimuli of shear stress and hypoxia are utilized in the flow-mediated dilation (FMD) technique to produce endothelium-dependent vasodilation. In contrast, endothelium-independent vasodilation occurs as a result of smooth muscle cell relaxation in direct response to exogenous NO (from NO donors such as nitroglycerin or nitroprusside). Vascular reactivity refers to both endothelium-dependent and endothelium-independent vasodilation.

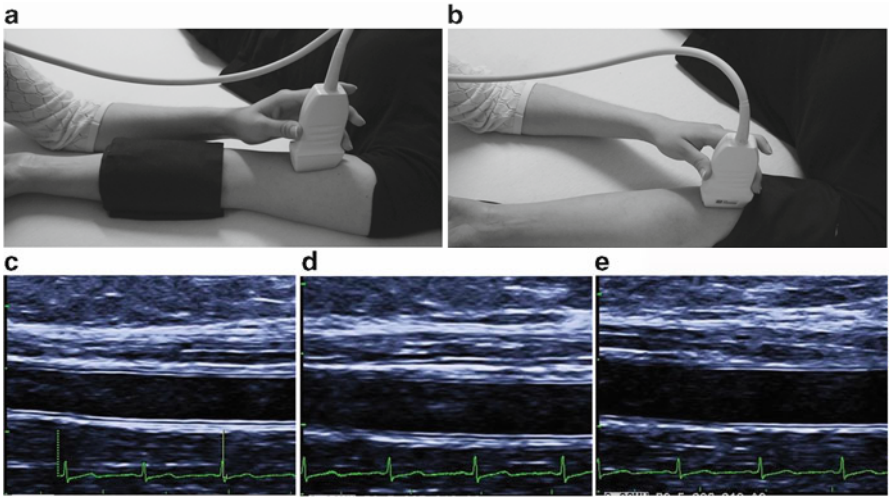

Fig. 12.3 **a** The assessment of flow-mediated vasodilation in the brachial artery. A 7.0 MHz or greater linear array transducer is used to image the brachial artery above the antecubital fossa in the longitudinal plane. A regular sphygmomanometer is employed to occlude the artery blood flow. The sphygmomanometer can be placed either at the forearm (**a**) or at the upper arm level (**b**). Two-dimensional grayscale scans are taken, one at rest, before the cuff inflation (**c**), and 1 min after the cuff deflation that leads to artery dilation (**d**). The percentage of the post-occlusive artery diameter increase over the baseline represents the FMD

12.4.1 Vascular Reactivity Measurements in the Macrocirculation

Macrovascular disease is most commonly assessed by ultrasound measurements of brachial artery diameter and the common carotid intima–media thickness (IMT). Changes in brachial artery diameter after stimuli measure early functional changes associated with atherosclerosis. Endothelium-dependent vasodilation of the brachial artery can be assessed by intra-arterial infusion of substances that act on the endothelium to release NO, such as acetylcholine, or by FMD. FMD is induced by occluding the brachial artery with a pneumatic tourniquet to the upper limb for a total of 5 min [45]. Tissue hypoxia and pH changes in the area distal to the occlusion, causes reactive vasodilation in the skin and muscle microcirculation immediately after release of the occlusion. This process causes a brief period of high blood flow and increased shear stress in the brachial artery that stimulates the endothelial production of NO and vasodilation that can be measured on high-resolution ultrasound (see Fig. 12.3). Endothelium-independent vasodilatory function of the brachial artery can be assessed by intra-arterial or sublingual administration of NO donors such as nitroglycerin or nitroprusside.

In contrast, common carotid IMT identifies anatomic changes consistent with early atherosclerosis. Carotid artery IMT is an ultrasound measure of the distance between the intima to the outer edge of the media. Increased intima–media thickness occurs early in the process of atherosclerotic plaque formation prior to luminal

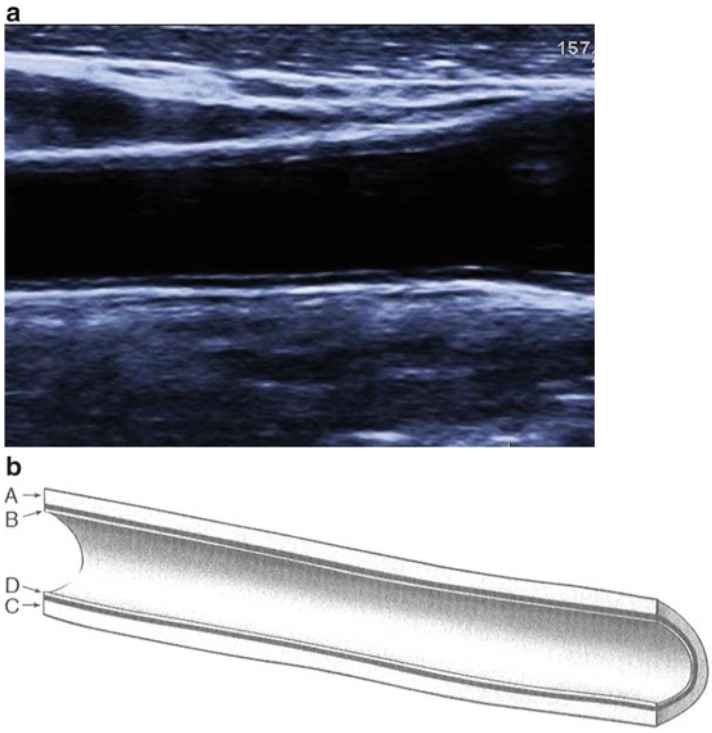

Fig. 12.4 **a** Image of the common carotid artery. A 7.5 MHz linear array transducer and high-resolution ultrasound were used. The carotid bifurcation can be seen on the right of the picture. **b** Simplified diagram of the arterial wall boundaries indicating the adventitia–media (*A*) of the near wall, intima–blood boundaries (*B*) for the near wall, and adventitia–media (*C*) and intima–blood boundaries (*D*) for the far wall

narrowing. IMT is associated with the presence of conventional atherosclerotic risk factors and can predict the development of cardiovascular events [46, 47] (see Fig. 12.4).

12.4.2 Microcirculatory Measurements

Microcirculatory vascular reactivity is most commonly assessed by laser Doppler flowmetry to measure blood flow in the skin. Blood flow is estimated from the combination of number and velocity of moving red cells within arterioles, capillaries, and postcapillary venules. A laser beam is delivered to the skin via a fiber optic light guide, and reflected light is gathered by a second set of photodetectors. Light reflected by moving objects, such as red blood cells, is reflected at a different frequency. The Doppler shifted fraction of the light signal and the mean Doppler frequency shift is calculated to generate a value in mV, which is proportional to the

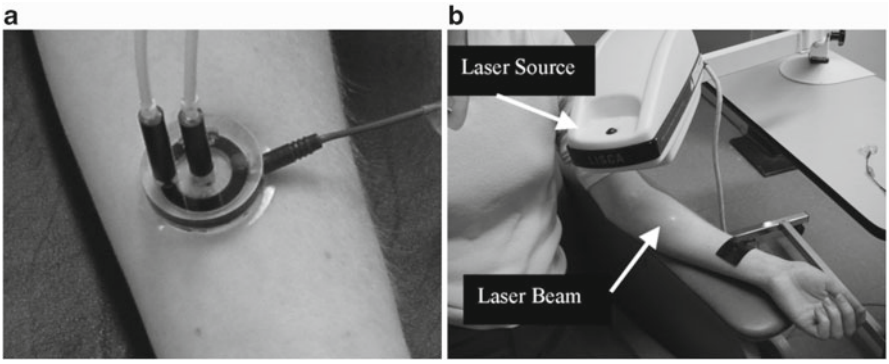

Fig. 12.5 **a** Measurements of direct and indirect effect of vasoactive substance using single-point laser probes: one probe is used in direct contact with the iontophoresis solution chamber (*colored ring*) and measures the direct response. The center probe measures the indirect response (nerve axon-related effect). A small quantity (<1 mL) of 1 % acetylcholine chloride solution or 1 % sodium nitroprusside solution is placed in the iontophoresis. A constant current of 200 mA is applied for 60 s achieving a dose of 6 mC/cm^{-2} between the iontophoresis chamber and a second non-active electrode placed 10–15 cm proximal to the chamber (black strap around the wrist). This current causes a movement of solution to be iontophoresed toward the skin. **b** Laser Doppler flowmetry: A helium-neon laser beam is emitted from the laser source to sequentially scan the circular hyperemic area (seen surrounding the laser beam) produced by the iontophoresed vasoactive substance to a small area on the volar surface of the forearm

quantity and velocity of red blood cells with the measured superficial skin microcirculation [48].

The microcirculation can be studied without systemic side effects by using iontophoresis and microdialysis techniques that allow for precise, local delivery of vasoactive agents. Iontophoresis uses a small charge to facilitate transcutaneous delivery of charged substances into the skin without trauma or pain (Fig. 12.5). The length of stimulation, strength of current used, and area of delivery determine the number of molecules transported. Endothelium-dependent vasodilation is assessed by delivery of acetylcholine using anodal current given its positive charge, whereas endothelium-independent vasodilation is assessed by the delivery of the anion sodium nitroprusside using cathodal current. Microdialysis can be used to deliver larger, water-soluble vasoactive agents that lack a charge. These techniques allow for noninvasive measurement of abnormal endothelial function prior to the development of overt clinical disease.

12.5 Therapeutic Interventions That Modify Oxidative Stress

Significant amount of evidence has proved that oxidative stress may be very harmful for the vasculature, especially in individuals with diabetes; thus, research has been focused the late years in investigating possible therapeutic ways against

oxidative stress in patients with diabetes including the use of therapeutic agents or lifestyle interventions. Agents, including vitamins E, C, α-lipoic acid, statins, angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers (ARBs), and thiazolidinediones, as well as lifestyle interventions, have been evaluated in large clinical trials and will be discussed in the following section. Many other agents have been noted to have antioxidant properties, but have not been evaluated in human clinical trials and are beyond the scope of this chapter.

12.5.1 Vitamin E

Vitamin E is a fat-soluble vitamin that has been found to present significant antioxidant properties. Initial studies showed that vitamins E and C supplementation may improve markers of oxidative stress and endothelium-dependent vasodilation in both experimental diabetic models and clinical trials [17, 49–51]. Specifically, vitamin E supplementation has been initially proved to ameliorate endothelial dysfunction in both cholesterol-fed rabbits and streptozotocin-diabetic rats [49, 52]. Furthermore, in human studies, acute administration of vitamin E has generally been shown to improve endothelium-dependent vasodilatation in both type 1 and type 2 diabetes [53]. The Cambridge Heart Antioxidant Study (CHAOS) that employed vitamin E (400–800 IU) reported a significant risk reduction from nonfatal myocardial infarction after an 18-month follow-up period, accompanied, though, by a nonsignificant increase of cardiovascular deaths in the same group [54].

However, the initial enthusiasm regarding the possible vaso-protective role of vitamin E dropped after the results of subsequent animal and human studies. More particular, animal studies reported that the supplementation of vitamin E or/and C may lead to endothelial dysfunction in both diabetic and healthy animals [16, 17] possibly due to pro-oxidant effects of vitamin E on vitamin C in the presence of NO and/or the de novo synthesis of vasoconstrictive prostanoids [16]. In addition, the PPP trial that included diabetic patients revealed no reduction in cardiovascular events or death after vitamin E supplementation. The study showed also an increased risk of adverse events with vitamin E supplementation, raising further concerns about its use [55].

A study from our unit, which included patients with both type 1 and type 2 diabetes treated with high dose of vitamin E (1,800 IU daily) for 12 months, found no improvement in endothelium-dependent or endothelium-independent vasodilation, in both skin microcirculation and brachial artery macrocirculation tests [56]. In addition, vitamin E supplementation had no effect in left ventricular function [56]. Interestingly in the same study, endothelin (a potent vasoconstrictor) was increased in the treatment group after 6 months and normalized by 12 months. In addition, endothelium-independent vasodilation and systolic blood pressure slightly worsened by the end of the 12-month treatment period. Of interest, C-reactive protein (CRP), a marker of inflammation, was decreased in the vitamin E-treated group, concluding that, although vitamin E may present a beneficial anti-inflammatory

effect, reducing CRP does not seem to have a positive effect on cardiovascular function.

The GISSI-Prevenzione trial employed vitamin E (300 mg per day) and *n*-3 polyunsaturated fatty acids (PUFA) or placebo for a median of 3.5 years [57]. Patients treated with vitamin E had no benefit in preventing cardiovascular events. On the contrary, patients with left ventricular dysfunction (ejection fraction < 50 %) presented a 50 % increased risk of developing congestive heart failure [57, 58]. In the Heart Outcomes Prevention Evaluation study (HOPE), conducted in more than 9,500 subjects, it was concluded that vitamin E supplementation had no effect on cardiovascular outcomes in all subgroups including the individuals with diabetes [59].

The HOPE trial was extended to the HOPE—The Ongoing Outcomes (HOPE-TOO) trial reported no difference in cardiovascular outcomes (including myocardial infarction, stroke, and death from cardiovascular causes) between the vitamin E treatment and placebo groups. On the contrary, subjects treated with vitamin E had higher rates of heart failure and heart failure-related hospital admissions. These findings were present in all groups of patients including the patients with diabetes and were persistent through both HOPE and HOPE-TOO [60]. The reason for the association between the increased rate of heart failure and vitamin E supplementation was unclear; however, the authors expressed the hypothesis that a pro-oxidative effect of vitamin E, in certain circumstances, could possibly depress the myocardial function. Finally initial meta-analyses did not show any effect of vitamin E on survival [61, 62].

In a recent meta-analysis of 19 clinical trials, the relationship between vitamin E supplementation and total mortality was examined. The results showed that in 9 of 11 trials testing high-dose vitamin E (≥400 IU/day), the all-cause mortality risk increased, prompting the conclusion that high doses of vitamin E (≥400 IU/day) should be avoided [63]. Finally, both cardiovascular outcomes and atherosclerosis progression by carotid intima–media thickness are not improved by vitamin E in a group of high-risk patients with vascular disease or diabetes in both HOPE study and SECURE trial [60, 64, 65].

Vitamin E has been also tested in the prevention of type 2 diabetes. Two interventional studies that used vitamin E or β-carotene supplementation did not show any positive effect on the delay of the development of type 2 diabetes [66, 67]. In another recent study [63], vitamin C supplementation was added to vitamin E, for testing the hypothesis that vitamin C is necessary for the regeneration of the oxidized vitamin E. However, the analysis of the study revealed neither benefit nor harm, by the supplementation of vitamin C, vitamin E, and β-carotene on the primary prevention of type 2 diabetes.

In conclusion, as the data, so far, indicate, there is currently no compelling evidence to support the use of vitamin E for preventing cardiovascular disease in diabetes. On the contrary, high doses of vitamin E may be associated with serious side effects. Thus, it is reasonable to suggest that such high dose should be avoided.

12.5.2 Vitamin C

Vitamin C (or ascorbic acid) is a water-soluble vitamin that, except its numerous biological effects, demonstrates a significant antioxidant role. It prevents oxidation of LDL and, as already mentioned, regenerates oxidized vitamin E. In addition, it stabilizes BH4, an eNOS cofactor, subsequently increasing NO production. Initial studies involving acute increases of the vitamin C plasma levels reported a significant improvement of endothelial function in multiple disease models of oxidative stress. Indeed, in a study by Beckman et al., it was reported that hyperglycemia-induced endothelial dysfunction in healthy volunteers was reversed by vitamin C infusion [68]. In addition, intra-arterial infusion of vitamin C has been reported to improve endothelial function in both type 1 and type 2 diabetic patients [69, 70]. Furthermore, other studies presented an immediate improvement of the endothelial function in subjects with essential hypertension, after vitamin C infusion, whereas other antioxidants such as *N*-acetylcysteine did not have similar effect [71].

In a cohort study of 11,348 adults for 10 years (the first National Health and Nutrition Examination Survey (NHANES I) [72], increased vitamin C intake (approx 300 mg per day) was associated with a 45–25 % risk reduction in all-cause mortality including mortality from cardiovascular events in men and women, respectively. Additionally, in an observational study in 85,118 female nurses followed for 16 years, vitamin C supplementation was associated with a significantly lower risk (28 %) of coronary disease (relative risk of 0.72) after statistical correction for other cardiovascular risk factors [7, 73]. This benefit was noted again by researchers in the EPIC-Norfolk prospective population study [74].

Although initial acute studies have shown significant improvement in endothelial function with vitamin C administration, long-term therapy did not present similar results. In a recent study, the combined therapy with vitamins C and E in types 1 and 2 diabetic patients showed an improvement in endothelial function only in patients with type 1 diabetes [53]. In another study, high oral doses of vitamin C did not improve endothelial function in type 2 diabetic subjects [75].

In summary, according to the current data, there is no compelling evidence to support the use of vitamin C for preventing cardiovascular disease in diabetes. New randomized, placebo-controlled studies addressing the cardiovascular benefits of vitamin C supplementation, independent of other vitamin supplements, need to be conducted to support evidence regarding the possible cardiovascular benefit of vitamin C supplementation in patients with diabetes.

12.5.3 α-Lipoic Acid

α-Lipoic acid is a hydrophilic antioxidant allowing it to exert beneficial effects in both aqueous and lipid cellular environments. α-Lipoic acid is reduced to its

conjugate base, dihydrolipoate, which is able to regenerate other antioxidants such as vitamins C and E, as well as reduced glutathione.

A long-term treatment with α-lipoic acid in diabetic animal models demonstrated improvements in metabolic profile including blood glucose, plasma insulin, cholesterol, triglycerides, and lipid peroxidation as well as the microvasculature [76]. In contrast, short-term treatment with α-lipoic acid in rat models of insulin resistance and insulin deficiency did not improve hyperglycemia or fasting triglycerides [77].

In the microcirculation of diabetic rats, α-lipoic acid reduces nitrotyrosine levels and prevents pathologic retinal vessel changes [78]. Additionally, α-lipoic acid has been proved to prevent AGE-dependent depletion of reduced glutathione and ascorbic acid and the subsequent activation of NF-kappa B in endothelial cell culture [79]. Thus, it appears that α-lipoic acid supplementation may reduce oxidative stress improving the metabolic derangements and microvascular function in animal and in vitro models.

Human studies with α-lipoic acid have been mainly focused in the treatment of diabetic polyneuropathy. In initial studies, a 19-day supplementation with α-lipoic acid improved the symptoms of diabetic polyneuropathy [80], while a longer-term therapy (initial IV infusions, then oral treatments for 2 years) objectively improved peripheral nerve function [81].

On the contrary, another trial followed the patients for 7 months, demonstrated no improvements in symptoms in the group with α-lipoic acid [82], while 4 years treatment in the NATHAN 1 trial reported improvements in only some neuropathic deficits and symptoms, but not objective nerve conduction, in patients with mild to moderate distal symmetric neuropathy [83]. In addition, there was a nonsignificant trend of developing serious adverse events in the treatment group indicating that although there may be a possible improvement in neuropathy, the long-term oral therapy may increase the risk of serious adverse events [83].

The effects of α-lipoic acid have been studied also in autonomic diabetic neuropathy and surrogate markers of macrovascular disease in a small number of subjects. A 4-month treatment with α-lipoic acid showed a slight improvement in heart rate variability measurements, without, though, changing the symptoms of autonomic dysfunction [84]. Finally, in a study of 4 weeks of oral α-lipoic acid supplementation, it was reported that there was a significant improvement of the endothelium-dependent vasorelaxation of the brachial artery compared to the placebo group, accompanied by a significant reduction in markers of endothelial activation (interleukin-6 and plasminogen activator-1) [85].

Concluding, the impact of lipoic acid on clinical cardiovascular end points is still unknown. Given also the increased risk of serious adverse events in long-term administration, the use of α-lipoic acid supplements cannot be recommended for patients with diabetes.

12.5.4 Statins

Statins improve the lipid profile by inhibiting the enzyme hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase) reducing the risk of cardiovascular morbidity and mortality [86]. Several studies have proposed that statins may decrease oxidative stress consequently improving the endothelial function.

Indeed, statins decrease NADPH activity, reducing the formation of reactive oxygen species and downregulating the renin–angiotensin system. They also reduce the oxidation of ROS and LDL cholesterol by reducing the activity of the NADPH oxidase in endothelial cells [87–94]. In addition, statins reduce the foam cells formation (responsible for atherosclerotic lesions formation) by decreasing the oxidized LDL uptake by the monocytes [95, 96]. Furthermore, statins downregulate AT1 receptor at the transcriptional level, improving measures of oxidative stress and vascular function [90]. Interestingly, atrovastatin has been proved to demonstrate free radical scavenging abilities through its hydroxymetabolites [97].

By reducing the oxidation of LDL, statins upregulate eNOS expression, consequently improving the vascular function in animal models of type 2 diabetes and hypercholesterolemia [98–100]. Statin-mediated increment in eNOS function was reported to be critical in vascular regeneration and restored myocardial vasorelaxation after experimentally induced myocardial infarction in the mouse model. This benefit was not observed in eNOS−/− mice [101].

It is a common knowledge that treatment with statins reduces the risk of major vascular events [102, 103]. However, its benefit in improving endothelial dysfunction has not been clearly identified so far. Indeed, treatment with statins did not improve vasoreactivity in patients with poorly controlled diabetes [104]. On the other hand, endothelium-dependent vasodilation significantly improved, independently of lipid lowering, in patients with better glycemic and lipid control in both type 1 and type 2 diabetes [105–110].

Statins were also reported to ameliorate postprandial hypertriglyceridemia- and hyperglycemia-induced endothelial dysfunction, reducing also the serum nitrotyrosine levels in type 2 diabetes suggesting that its short-term, lipid-independent vascular benefits are secondary to decreased oxidative and nitrosative stress [111].

In conclusion it seems that statins improve endothelial function prior to reductions in LDL unless there is overwhelming oxidative stress related to type 2 diabetes. The reduced response to statins may also be related to the increased levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of eNOS. Indeed, a recent study has been shown that a 3-week treatment with statin failed to improve vasoreactivity in patients with increased levels of ADMA [112].

12.5.5 ACE Inhibitors and ARBs

ACE inhibitors and ARBs exert their clinical effects by decreasing the binding of angiotensin II to the AT1 receptor, by decreasing levels of angiotensin II and by inhibiting the interaction of angiotensin II to the AT1 receptor, respectively. ACE inhibitors and ARBs have been proposed to improve endothelium-dependent vasorelaxation by decreasing superoxide production and increasing NO bioavailability [113–116]. These actions are mainly derived by the inhibition of angiotensin II which opposes many of the actions of NO. In particular angiotensin II causes vasoconstriction, altered vascular smooth muscle function, increased inflammation via NF-κB, and hypercoagulability by increased formation of PAI-1. In addition angiotensin II induces vascular superoxide production by uncoupling eNOS upon loss of dihydrofolate reductase (DHFR), which is a BH4 salvage enzyme [113].

Recent studies have shown that ACE inhibitors and ARBs improve vascular function and cardiovascular outcomes in type 2 diabetes. Both agents unequivocally improve endothelial function in patients with type 2 diabetes [117–120]. Valsartan therapy improved resting forearm skin blood flow and resting brachial artery diameter after a 12-week treatment in patients with type 2 diabetes. However, their impact on endothelial function in patients with type 1 diabetes is less clear [121–124].

HOPE and LIFE studies have shown that ACE inhibitors and ARBs improve cardiovascular as well as all-cause mortality outcomes in patients with diabetes. The benefit seemed to be higher in patients with diabetes than in nondiabetics [125, 126]. The presence of native LDL increases AT1 receptor expression at least twofold in a sustained manner for 24 h by stabilization of posttranscriptional mRNA [127]. Furthermore, angiotensin II is binding with the AT1 receptor, upregulating the endothelial oxidized LDL receptor (LOX-1) in endothelial cells. This upregulation of LOX-1 receptor is prevented by ARBs and ACE inhibitors, limiting the potential diffusion of oxidized LDL from the blood into the vessel wall, thus reducing the possibility of plaque formation [128]. Given that statins decrease the levels of native LDL which is responsible for the at least twofold increase of theAT1 receptor expression [127], a coadministration of ACE inhibitors/ARBs with a statin may produce a synergic decrease in oxidative stress and vasoconstriction, as well as a decreased uptake of oxidized LDL and improved endothelial function [128].

12.5.6 Thiazolidinediones

Thiazolidinediones is an antidiabetic agent category also known as PPAR-gamma agonists that bind nuclear PPAR-gamma receptors in adipocytes which function as transcription factors for genes important in adipocyte differentiation, lipid metabolism, and insulin sensitivity. PPAR-gamma receptors are also expressed in cells

involved in the process of atherosclerosis including endothelial cells, vascular smooth muscle cells, monocytes/macrophages, and T cells.

Increased amount of evidence supports that apart from enhancing glycemic control, thiazolidinediones improve surrogate measures of vascular disease. Indeed, thiazolidinediones have been proved to improve endothelium-dependent vasodilation as well as measurements of carotid IMT in patients with diabetes [129–133]. In addition, both rosiglitazone and pioglitazone have been reported to increase the regenerative capacity of endothelial progenitor cells in individuals with diabetes [134, 135]. This improvement in vascular function has been found to be associated with reduced NADPH oxidase activity, decreased LDL oxidation, and reduction in vascular inflammation [133, 136].

However, although thiazolidinediones proved to have a significant improvement in oxidative stress and vascular function, there are serious concerns that one of them, rosiglitazone, worsens clinical cardiovascular outcomes. Thus, rosiglitazone has been reported to be associated with increased risk of congestive heart failure, as well as myocardial infarction [137, 138]. Thus, the current consensus is that rosiglitazone may have detrimental effects in patients with previous heart disease and diabetes, and its use cannot be recommended in these patients. Unlike rosiglitazone, larger clinical trials of pioglitazone in high-risk patients with type 2 diabetes and prior MI have demonstrated an improvement in rates of myocardial infarction, but increased edema formation and heart failure remain concerns [139, 140].

12.5.7 Antioxidants and Mediterranean Diet

A study in 34.486 postmenopausal women reported that increased intake of vitamin E through diet was associated with decreased risk of death from coronary artery disease, while vitamin E supplementation did not affect the risk of death from cardiovascular disease [141]. This study exemplifies the paradox noted in several large-scale clinical and epidemiologic studies that diet but not vitamin supplementation seems to improve cardiovascular outcomes.

A great amount of evidence the last few decades has shown that this type of diet has impressive effects in reducing cardiovascular risk [142]. In addition, low adherence to Mediterranean diet has been proven to increase the risk for metabolic syndrome [143]. Olive oil, a main component of the diet, has significant antioxidant properties and is considered one of the primary factors that contribute to these beneficial effects [144].

In a recent study involving subjects with metabolic syndrome, the Mediterranean diet presented anti-inflammatory and antithrombotic properties improving the endothelial function and insulin sensitivity [145]. Therefore, the current consensus is that a diet that encompasses the main components of the Mediterranean diet can greatly reduce cardiovascular risk in diabetic patients.

12.5.8 Green Tea and Coffee

Coffee, a common beverage in western countries, has been reported to possibly have antioxidant effects through minerals (such as magnesium), phytochemicals (in caffeine), and antioxidants. Several studies have shown that coffee decreases the risk of type 2 diabetes although there have been reports that caffeine itself may impair glucose metabolism in type 2 diabetics [146, 147]. However, it is not clear how coffee decreases the risk of type 2 diabetes especially since caffeine (and its phytochemicals) does not seem to play a significant role.

Green tea, another widely consumed beverage, also seems to have protective effects as its polyphenols have antioxidant properties. A study that followed Japanese subjects for 11 years reported that the consumption of green tea was associated with a decrease in all-cause mortality as well as mortality from cardiovascular disease [148]. In another Japanese study, consumption of green tea, coffee, and total caffeine was associated with a decreased risk for type 2 diabetes in a 5-year follow-up period [149].

12.5.9 Exercise

Exercise or physical activity is recommended for the prevention or the initial therapy of type 2 DM and ischemic heart disease [150]. Many studies have also shown that exercise can reduce blood glucose, apolipoprotein B-rich lipoproteins, oxidative stress, or inflammatory cytokines and elevate HDL cholesterol, insulin sensitivity, antioxidant capacity, or mitochondrial function [151–154].

Other studies indicated also that exercise may inhibit the expression of NOX in human arteries [155], possibly providing a novel mechanism for the beneficial effect of exercise and may help diabetic patients to prevent cardiovascular disease. NOX is a transmembrane enzyme located in intracellular organelles and functions in the generation of superoxide.

12.6 Conclusions

Although there was initially much enthusiasm for the antioxidant therapy in diabetes, especially in the form of supplemental vitamins, clinical trials have not shown evidence of decreased risk of cardiovascular outcomes. Vitamins E and C supplementation, therefore, cannot be currently recommended. On the other hand, diet rich in antioxidants, especially Mediterranean diet, can provide considerable reduction in cardiovascular risk and may be of particular benefit to subjects with diabetes. Finally, statins, ACE inhibitors, and ARBs, alone or in combination, seem to present antioxidative properties. However, its use cannot be recommended, as their

indications so far are limited to hypercholesterolemia and hypertension, respectively. Further research is needed in order to be determined whether they could be possibly used for their antioxidant vascular protective properties.

References

1. ed Sies H (1991) Oxidative stress: oxidants and antioxidants. Academic, London
2. King GL, Loeken MR (2004) Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol 122:333–338
3. Nishikawa T, Edelstein D, Du XL et al (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404:787–790
4. Schrauwen P, Hesselink MK (2004) Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. Diabetes 53:1412–1417
5. Yamagishi SI, Edelstein D, Du XL, Kaneda Y et al (2001) Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 276(27): 25096–25100
6. Schwartz D, Malhotra A, Fink M (1999) Cytopathic hypoxia in sepsis: an overview. Sepsis 2:279–289
7. Pacher P, Szabo C (2006) Role of peroxynitrite in the pathogenesis of cardiovascular complications of diabetes. Curr Opin Pharmacol 6:136–141
8. Greenacre A, Ischiropoulos H (2001) Tyrosine nitration: localisation, quantification, consequences for protein function and signal transduction. Free Radic Res 34:541–581
9. Beckmann JS, Ye YZ, Anderson PJ et al (1994) Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. Biol Chem Hoppe Seyler 375:81–88
10. Mihm MJ, Jing L, Bauer JA (2000) Nitrotyrosine causes selective vascular endothelial dysfunction and DNA damage. J Cardiovasc Pharmacol 36:182–187
11. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424
12. Mabley JG, Soriano FG (2005) Role of nitrosative stress and poly(ADP-ribose) polymerase activation in diabetic vascular dysfunction. Curr Vasc Pharmacol 3:247–252
13. Yim S, Malhotra A, Veves A (2007) Antioxidants and CVD in diabetes: where do we stand now. Curr Diab Rep 7:8–13
14. Adaikalakoteswari A, Rema M, Mohan V et al (2007) Oxidative DNA damage and augmentation of poly(ADP-ribose) polymerase/nuclear factor-kappa B signaling in patients with type 2 diabetes and microangiopathy. Int J Biochem Cell Biol 39:1673–1684
15. Craven PA, Studer RK, DeRubertis FR (1994) Impaired nitric oxide-dependent cyclic guanosine monophosphate generation in glomeruli from diabetic rats. Evidence for protein kinase C-mediated suppression of the cholinergic response. J Clin Invest 93:311–320
16. Gorbunov NV, Osipov AN, Sweetland MA et al (1996) NO-redox paradox: direct oxidation of alpha-tocopherol and alpha-tocopherol-mediated oxidation of ascorbate. Biochem Biophys Res Commun 219(3):835–841
17. Alper G, Olukman M, Irer S et al (2006) Effect of vitamin E and C supplementation combined with oral antidiabetic therapy on the endothelial dysfunction in the neonatally streptozotocin injected diabetic rat. Diabetes Metab Res Rev 22(3):190–197
18. d'Uscio LV, Katusic ZS (2006) Increased vascular biosynthesis of tetrahydrobiopterin in apolipoprotein E-deficient mice. Am J Physiol Heart Circ Physiol 290:H2466–H2471
19. Hammes HP, Du X, Edelstein D et al (2003) Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med 9:294–299

20. Marchetti V, Menghini R, Rizza S et al (2006) Benfotiamine counteracts glucose toxicity effects on endothelial progenitor cell differentiation via Akt/FoxO signaling. Diabetes 55:2231–2237
21. Goldin A, Beckman JA, Schmidt AM et al (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 114:597–605
22. Hofmann MA, Drury S, Fu C et al (1999) RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell 97:889–901
23. Lander HM, Tauras JM, Ogiste JS et al (1997) Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem 272:17810–17814
24. Berg TJ, Dahl-Jorgensen K, Torjesen PA et al (1997) Increased serum levels of advanced glycation end products (AGEs) in children and adolescents with IDDM. Diabetes Care 20:1006–1008
25. Berg TJ, Bangstad HJ, Torjesen PA et al (1997) Advanced glycation end products in serum predict changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. Metabolism 46:661–665
26. Hammes HP, Martin S, Federlin K et al (1991) Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proc Natl Acad Sci USA 88:11555–11558
27. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820
28. Sorbinil Retinopathy Trial Research Group (1990) A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. Arch Ophthalmol 108:1234–1244
29. Greene DA, Arezzo JC, Brown MB (1999) Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Zenarestat Study Group. Neurology 53:580–591
30. Kolm-Litty V, Sauer U, Nerlich A et al (1998) High glucose induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. J Clin Invest 101:160–169
31. Du XL, Edelstein D, Dimmeler S et al (2001) Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest 108: 1341–1348
32. Su Y, Liu XM, Sun YM et al (2008) Endothelial dysfunction in impaired fasting glycemia, impaired glucose tolerance, and type 2 diabetes mellitus. Am J Cardiol 102(4):497–498
33. McVeigh GE, Brennan GM, Johnston GD et al (1992) Impaired endothelium- dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35(8):771–776
34. Watts GF, O'Brien SF, Silvester W et al (1996) Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. Clin Sci (Lond) 91:567–573
35. Williams SB, Cusco JA, Roddy MA et al (1996) Impaired nitric oxide-mediated vasodilation in patients with noninsulin-dependent diabetes mellitus. J Am Coll Cardiol 27:567–574
36. Doupis J, Rahangdale S, Gnardellis C et al (2011) Effects of diabetes and obesity on vascular reactivity, inflammatory cytokines, and growth factors. Obesity (Silver Spring) 19(4): 729–735
37. Henry RMA, Ferreira I, Kostense PJ et al (2004) Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not: The Hoorn Study. Atherosclerosis 174:49–56
38. Nitenberg A, Valensi P, Sachs R et al (1993) Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. Diabetes 42:1017–1025
39. Prior JO, Quinones MJ, Hernandez-Pampaloni M et al (2005) Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Circulation 111:2291–2298

40. Woodman RJ, Chew GT, Watts GF (2005) Mechanisms, significance and treatment of vascular dysfunction in type 2 diabetes mellitus : focus on lipid-regulating therapy. Drugs 65:31–74
41. Thorand B, Baumert J, Chambless L et al, for the MONICA/KORA Study Group (2006) Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. Arterioscler Thromb Vasc Biol 26:398–405
42. Lim SC, Caballero AE, Smakowski P et al (1999) Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. Diabetes Care 22:1865–1870
43. Guerci B, Kearney-Schwartz A, Bohme P et al (2001) Endothelial dysfunction and type 2 diabetes. Part 1: physiology and methods for exploring the endothelial function. Diabetes Metab 27:425–434
44. Papaioannou GI, Seip RL, Grey NJ et al (2004) Brachial artery reactivity in asymptomatic patients with type 2 diabetes mellitus and microalbuminuria (from the detection of ischemia in asymptomatic diabetics-brachial artery reactivity study). Am J Cardiol 94:294–299
45. Saouaf R, Arora S, Smakowski P et al (1998) Reactive hyperemic response of the brachial artery: comparison of proximal and distal occlusion. Acad Radiol 5:556–560
46. Heiss G, Sharrett AR, Barnes R et al (1991) Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. Am J Epidemiol 134:250–256
47. O'Leary DH, Polak JF, Kronmal RA et al (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 340:14–22
48. Tooke JE, Ostergren J, Fagrell B (1983) Synchronous assessment of human skin microcirculation by laser Doppler flowmetry and dynamic capillaroscopy. Int J Microcirc Clin Exp 2:277–284
49. Cinar MG, Ulker S, Alper G et al (2001) Effect of dietary vitamin E supplementation on vascular reactivity of thoracic aorta in streptozotocin-diabetic rats. Pharmacology 62:56–64
50. Keegan A, Walbank H, Cotter MA et al (1995) Chronic vitamin E treatment prevents defective endothelium-dependent relaxation in diabetic rat aorta. Diabetologia 38:1475–1478
51. Doupis J, Veves A (2007) Antioxidants, diabetes and endothelial dysfunction. US Endocrine Diseases 2:61–65
52. Stewart-Lee AL, Forster LA, Nourooz-Zadeh J et al (1994) Vitamin E protects against impairment of endothelium-mediated relaxations in cholesterol-fed rabbits. Arterioscler Thromb 14:494–499
53. Beckman JA, Goldfine AB, Gordon MB et al (2003) Oral antioxidant therapy improves endothelial function in type 1 but not type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 285:H2392–H2398
54. Stephens NG, Parsons A, Schofield PM et al (1996) Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 347(9004):781–786
55. Sacco M, Pellegrini F, Roncaglioni MC et al (2003) Primary prevention of cardiovascular events with low-dose aspirin and vitamin E in type 2 diabetic patients: results of the Primary Prevention Project (PPP) trial. Diabetes Care 26:3264–3272
56. Economides PA, Khaodhiar L, Caselli A et al (2005) The effect of vitamin E on endothelial function of micro- and macrocirculation and left ventricular function in type 1 and type 2 diabetic patients. Diabetes 54(1):204–211
57. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 354(9177):447–455
58. Marchioli R, Levantesi G, Macchia A et al (2006) Vitamin E increases the risk of developing heart failure after myocardial infarction: results from the GISSI-Prevenzione trial. J Cardiovasc Med (Hagerstown) 7:347–350

59. Yusuf S, Dagenais G, Pogue J et al (2000) Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 342(3):154–160
60. Lonn E, Bosch J, Yusuf S et al (2005) Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 293(11):1338–1347
61. Eidelman RS, Hollar D, Hebert PR et al (2004) Randomized trials of vitamin E in the treatment and prevention of cardiovascular disease. Arch Intern Med 164(14):1552–1556
62. Shekelle PG, Morton SC, Jungvig LK et al (2004) Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease. J Gen Intern Med 19(4):380–389
63. Miller ER, Pastor-Barriuso R, Dalal D et al (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 142(1):37–46
64. Lonn E, Yusuf S, Hoogwerf B et al (2002) Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes: results of the HOPE study and MICRO-HOPE substudy. Diabetes Care 25:1919–1927
65. Lonn E, Yusuf S, Dzavik V et al (2001) Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). Circulation 103:919–925
66. Kataja-Tuomola M, Sundell JR, Mannisto S et al (2008) Effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes. Diabetologia 51:47–53
67. Song Y, Cook NR, Albert CM et al (2009) Effects of vitamins C and E and β-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr 90:429–437
68. Beckman JA, Goldfine AB, Gordon MB et al (2001) Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 103: 1618–1623
69. Timimi FK, Ting HH, Haley EA, Roddy MA et al (1998) Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. J Am Coll Cardiol 31:552–557
70. Ting HH, Timimi FK, Boles KS et al (1996) Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 97: 22–28
71. Schneider MP, Delles C, Schmidt BM et al (2005) Superoxide scavenging effects of N-acetylcysteine and vitamin C in subjects with essential hypertension. Am J Hypertens 18:1111–1117
72. Enstrom JE, Kanim LE, Klein MA et al (1992) Vitamin C intake and mortality among a sample of the United States population. Epidemiology 3:194–202
73. Tousoulis D, Antoniades C, Tountas C et al (2003) Vitamin C affects thrombosis/fibrinolysis system and reactive hyperemia in patients with type 2 diabetes and coronary artery disease. Diabetes Care 26:2749–2753
74. Boekholdt SM, Meuwese MC, Day NE et al (2006) Plasma concentrations of ascorbic acid and C-reactive protein, and risk of future coronary artery disease, in apparently healthy men and women: the EPIC-Norfolk prospective population study. Br J Nutr 96:516–522
75. Chen H, Karne RJ, Hall G et al (2006) High-dose oral vitamin C partially replenishes vitamin C levels in patients with type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 290(1): H137–H145
76. Kocak G et al (2000) Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes. Diabetes Nutr Metab 13:308–318
77. Black K, Qu X, Seale JP et al (1998) Metabolic effects of thioctic acid in rodent models of insulin resistance and diabetes. Clin Exp Pharmacol Physiol 25:712–714
78. Kowluru RA, Odenbach S (2004) Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. Diabetes 53:3233–3238

79. Bierhaus A, Chevion S, Chevion M et al (1997) Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. Diabetes 46:1481–1490
80. Ziegler D et al (1995) Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). Diabetologia 38:1425–1433
81. Reljanovic M et al (1999) Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Alpha Lipoic Acid in Diabetic Neuropathy. Free Radic Res 31:171–179
82. Ziegler D et al (1999) Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study). ALADIN III Study Group. Alpha-Lipoic Acid in Diabetic Neuropathy. Diabetes Care 22:1296–1301
83. Ziegler D, Ametov A, Barinov A et al (2006) Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial. Diabetes Care 29:2365–2370
84. Ziegler D, Schatz H, Conrad F et al (1997) Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). Deutsche Kardiale Autonome Neuropathie. Diabetes Care 20:369–373
85. Sola S, Mir MQ, Cheema FA et al (2005) Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: results of the Irbesartan and Lipoic Acid in Endothelial Dysfunction (ISLAND) study. Circulation 111:343–348
86. The Scandinavian Simvastatin Survival Study (4S) (1994) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease. Lancet 344:1383–1389
87. Tsubouchi H, Inoguchi T, Sonta T et al (2005) Statin attenuates high glucose-induced and diabetes-induced oxidative stress in vitro and in vivo evaluated by electron spin resonance measurement. Free Radic Biol Med 39:444–452
88. Ceylan A, Karasu C, Aktan F et al (2003) Effects of simvastatin treatment on oxidant/antioxidant state and ultrastructure of diabetic rat myocardium. Gen Physiol Biophys 22:535–547
89. Wagner AH, Kohler T, Ruckschloss U et al (2000) Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. Arterioscler Thromb Vasc Biol 20:61–69
90. Wassmann S, Laufs U, Baumer AT et al (2001) HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species. Hypertension 37:1450–1457
91. Ceylan A, Karasu C, Aktan F et al (2004) Simvastatin treatment restores vasoconstriction and the inhibitory effect of LPC on endothelial relaxation via affecting oxidizing metabolism in diabetic rats. Diabetes Nutr Metab 17:203–210
92. Rosenson RS, Tangney CC, Levine DM et al (2005) Association between reduced low density lipoprotein oxidation and inhibition of monocyte chemoattractant protein-1 production in statin-treated subjects. J Lab Clin Med 145:83–87
93. Maron DJ, Fazio S, Linton MF (2000) Current perspectives on statins. Circulation 101:207–213
94. Morawietz H, Erbs S, Holtz J et al (2006) Endothelial protection, AT1 blockade and cholesterol-dependent oxidative stress: the EPAS trial. Circulation 114:I296–I301
95. Pietsch A, Erl W, Lorenz RL (1996) Lovastatin reduces expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells. Biochem Pharmacol 52:433–439
96. Umetani N, Kanayama Y, Okamura M et al (1996) Lovastatin inhibits gene expression of type-I scavenger receptor in THP-1 human macrophages. Biochim Biophys Acta 1303: 199–206
97. Aviram M, Rosenblat M, Bisgaier CL et al (1998) Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. Atherosclerosis 138:271–280

98. Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J et al (1998) Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. J Clin Invest 101:2711–2719
99. Yu Y, Ohmori K, Chen Y et al (2004) Effects of pravastatin on progression of glucose intolerance and cardiovascular remodeling in a type II diabetes model. J Am Coll Cardiol 44:904–913
100. Jimenez A, Arriero MM, Lopez-Blaya A et al (2001) Regulation of endothelial nitric oxide synthase expression in the vascular wall and in mononuclear cells from hypercholesterolemic rabbits. Circulation 104:1822–1830
101. Landmesser U, Engberding N, Bahlmann FH et al (2004) Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. Circulation 110:1933–1939
102. Colhoun HM, Betteridge DJ, Durrington PN et al (2004) Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet 364:685–696
103. Collins R, Armitage J, Parish S et al (2003) MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 361:2005–2016
104. Mansourati J, Newman LG, Roman SH et al (2001) Lipid lowering does not improve endothelial function in subjects with poorly controlled diabetes. Diabetes Care 24:2152–2153
105. Dogra GK, Watts GF, Chan DC et al (2005) Statin therapy improves brachial artery vasodilator function in patients with type 1 diabetes and microalbuminuria. Diabet Med 22:239–242
106. Mullen MJ, Wright D, Donald AE et al (2000) Atorvastatin but not L-arginine improves endothelial function in type I diabetes mellitus: a double-blind study. J Am Coll Cardiol 36:410–416
107. Tsunekawa T, Hayashi T, Kano H et al (2001) Cerivastatin, a hydroxymethylglutaryl coenzyme a reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. Circulation 104:376–379
108. Economides PA, Caselli A, Tiani E et al (2004) The effects of atorvastatin on endothelial function in diabetic patients and subjects at risk for type 2 diabetes. J Clin Endocrinol Metab 89:740–747
109. Tan KC, Chow WC, Tam SC et al (2002) Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. J Clin Endocrinol Metab 87:563–568
110. Tousoulis D, Antoniades C, Vasiliadou C et al (2007) Effects of atorvastatin and vitamin C on forearm hyperaemic blood flow, asymmetrical dimethylarginine levels and the inflammatory process in patients with type 2 diabetes mellitus. Heart 93:244–246
111. Ceriello A, Taboga C, Tonutti L et al (2002) Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation 106:1211–1218
112. Boger RH (2004) Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. J Nutr 134:2842S–2847S (discussion 2853S)
113. Oak JH, Cai H (2007) Attenuation of angiotensin II signaling recouples eNOS and inhibits nonendothelial NOX activity in diabetic mice. Diabetes 56:118–126
114. Rajagopalan S, Kurz S, Munzel T et al (1996) Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 97:1916–1923
115. Laursen JB, Rajagopalan S, Galis Z et al (1997) Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. Circulation 95:588–593

116. Bendall JK, Rinze R, Adlam R et al (2007) Endothelial Nox2 overexpression potentiates vascular oxidative stress and hemodynamic response to angiotensin II: studies in endothelial-targeted Nox2 transgenic mice. Circ Res 100:1016–1025
117. Cheetham C, O'Driscoll G, Stanton K et al (2001) Losartan, an angiotensin type I receptor antagonist, improves conduit vessel endothelial function in Type II diabetes. Clin Sci (Lond) 100:13–17
118. Cheetham C, Collis J, O'Driscoll G et al (2000) Losartan, an angiotensin type 1 receptor antagonist, improves endothelial function in non-insulin-dependent diabetes. J Am Coll Cardiol 36:1461–1466
119. O'Driscoll G, Green D, Maiorana A et al (1999) Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 33:1506–1511
120. Giugliano D, Marfella R, Acampora R et al (1998) Effects of perindopril and carvedilol on endothelium-dependent vascular functions in patients with diabetes and hypertension. Diabetes Care 21:631–636
121. Komers R, Simkova R, Kazdova L et al (2004) Effects of ACE inhibition and AT1-receptor blockade on haemodynamic responses to L-arginine in type 1 diabetes. J Renin Angiotensin Aldosterone Syst 5:33–38
122. Schalkwijk CG, Smulders LJ et al (2000) ACE-inhibition modulates some endothelial functions in healthy subjects and in normotensive type 1 diabetic patients. Eur J Clin Invest 30:853–860
123. McFarlane R, McCredie RJ, Bonney MA et al (1999) Angiotensin converting enzyme inhibition and arterial endothelial function in adults with type 1 diabetes mellitus. Diabet Med 16:62–66
124. Mullen MJ, Clarkson P, Donald AE et al (1998) Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. J Am Coll Cardiol 31:1330–1335
125. Yusuf S, Sleight P, Pogue J et al (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 342:145–153
126. Dahlof B, Devereux RB, Kjeldsen SE et al (2002) Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 359:995–1003
127. Nickenig G, Sachinidis A, Michaelsen F et al (1997) Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. Circulation 95:473–478
128. Morawietz H, Rueckschloss U, Niemann B et al (1999) Angiotensin II induces LOX-1, the human endothelial receptor for oxidized low-density lipoprotein. Circulation 100:899–902
129. Caballero AE, Saouaf R, Lim SC et al (2003) The effects of troglitazone, an insulin-sensitizing agent, on the endothelial function in early and late type 2 diabetes: a placebo-controlled randomized clinical trial. Metabolism 52:173–180
130. Mittermayer F, Schaller G, Pleiner J et al (2007) Rosiglitazone prevents free fatty acid-induced vascular endothelial dysfunction. J Clin Endocrinol Metab 92:2574–2580
131. Libby P, Plutzky J (2007) Inflammation in diabetes mellitus: role of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma agonists. Am J Cardiol 99:27B–40B
132. Mazzone T, Meyer PM, Feinstein SB et al (2006) Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. JAMA 296:2572–2581
133. Stocker DJ, Taylor AJ, Langley RW et al (2007) A randomized trial of the effects of rosiglitazone and metformin on inflammation and subclinical atherosclerosis in patients with type 2 diabetes. Am Heart J 153(445):e1–e6
134. Sorrentino SA, Bahlmann FH, Besler C et al (2007) Oxidant stress impairs in vivo reendothelialization capacity of endothelial progenitor cells from patients with type 2 diabetes mellitus:

restoration by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. Circulation 116:163–173
135. Wang CH, Ting MK, Verma S et al (2006) Pioglitazone increases the numbers and improves the functional capacity of endothelial progenitor cells in patients with diabetes mellitus. Am Heart J 152(1051):e1–e8
136. Tao L, Liu HR, Gao E et al (2003) Antioxidative, antinitrative, and vasculoprotective effects of a peroxisome proliferator-activated receptor-gamma agonist in hypercholesterolemia. Circulation 108:2805–2811
137. Nissen SE, Wolski K (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 356:2457–2471
138. Couzin J (2007) Drug safety. Heart attack risk overshadows a popular diabetes therapy. Science 316:1550–1551
139. Erdmann E, Dormandy JA, Charbonnel B et al (2007) The effect of pioglitazone on recurrent myocardial infarction in 2,445 patients with type 2 diabetes and previous myocardial infarction: results from the PROactive (PROactive 05) Study. J Am Coll Cardiol 49:1772–1780
140. Negro R, Dazzi D, Hassan H et al (2004) Pioglitazone reduces blood pressure in non-dipping diabetic patients. Minerva Endocrinol 29:11–17
141. Kushi LH, Folsom AR, Prineas RJ et al (1996) Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N Engl J Med 334(18):1156–1162
142. Kris-Etherton P, Eckel RH, Howard BV et al (2001) Lyon Diet Heart Study: benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association step I dietary pattern on cardiovascular disease. Circulation 103:1823–1825
143. Doupis J, Dimosthenopoulos C, Diamanti K et al (2009) Metabolic syndrome and Mediterranean dietary pattern in a sample of young, male, Greek navy recruits. Nutr Metab Cardiovasc Dis 19(6):e7–e8
144. Perez-Jimenez F, Alvarez de Cienfuegos G, Badimon L et al (2005) International conference on the healthy effect of virgin olive oil. Eur J Clin Invest 35(7):421–424
145. Esposito K, Marfella R, Ciotola M et al (2004) Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 292(12):1440–1446
146. Van Dam RM, Hu FB (2005) Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 294(1):97–104
147. Pereira MA, Parker ED, Folsom AR (2006) Coffee consumption and risk of type 2 diabetes mellitus: an 11-year prospective study of 28 812 postmenopausal women. Arch Intern Med 166(12):1311–1316
148. Kuriyama S, Shimazu T, Ohmori K et al (2006) Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 296(10): 1255–1265
149. Iso H, Date C, Wakai K et al (2006) The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 144(8):554–562
150. Doupis J, Schramm JC, Veves A (2009) Endothelial dysfunction, inflammation, and exercise. In: Regensteiner J (ed) Diabetes and exercise, 1st edn. Humana, Clifton
151. Colberg SR, Grieco CR (2009) Exercise in the treatment and prevention of diabetes. Curr Sports Med Rep 8(4):169–175
152. Lumini JA, Magalhaes J, Oliveira PJ et al (2008) Beneficial effects of exercise on muscle mitochondrial function in diabetes mellitus. Sports Med 38(9):735–750
153. Tucker PS, Fisher-Wellman K, Bloomer RJ (2008) Can exercise minimize postprandial oxidative stress in patients with type 2 diabetes? Curr Diabetes Rev 4(4):309–319
154. Nojima H, Watanabe H, Yamane K, Hiroshima University Health Promotion Study Group et al (2008) Effect of aerobic exercise training on oxidative stress in patients with type 2 diabetes mellitus. Metabolism 57(2):170–176
155. Rush JW, Aultman CD (2008) Vascular biology of angiotensin and the impact of physical activity. Appl Physiol Nutr Metab 33(1):162–172