Introduction

Type 2 diabetes mellitus (T2DM) as a global health problem is not only a metabolic but also a vascular disease associated with an increased risk of cardiovascular complications [1]. Long-term hyperglycemia and specific metabolic milieu (i.e. visceral obesity, insulin resistance, hyperinsulinemia, atherogenic dyslipidemia), induce low-grade inflammation, oxidative stress, endothelial dysfunction and platelet hyperactivity, contributing to genesis of hypercoagulable state and consecutive various atherothrombotic complications [2].

Endothelium is a dynamic, defensive regulator of vascular homeostasis. Physiological and Pathophysiology of Vascular Endothelium. Endothelial phenotypic modulation involves five basic characteristics: the expression of leukocyte adhesion molecules, the production of cytokines, change in the shape and the permeability of the endothelium, prothrombotic changes and upregulation of autoantigens.

Obesity, Metabolic Inflammation and Vascular Endothelium

One of the most important pathophysiological manifestations of adiposopathy may be the phenotypic conversion of vascular endothelium. Insulin Resistance and Vascular Endothelium. Under the conditions of insulin resistance and consequent hyperinsulinemia, there is imbalance between the production of endothelial vasoconstrictors and vasodilators, increased expression of adhesion molecules, and platelet hyperreactivity.

Hyperglycemia and Vascular Endothelium.

Hyperglycemia causes endothelial dysfunction by various mechanisms that involve activation of polyol pathway and production of sorbitol, increased formation of advanced glycation end products, activation of various isoforms of protein kinase C and activation of hexosamine pathway. Dyslipidemia and vascular endothelium. Dyslipidemia takes an important role in a cascade of pathophysiological processes that result in endothelial activation and chronic dysfunction. Conclusion. Hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, visceral obesity and low-grade inflammation are the main factors responsible for development of endothelial dysfunction in type 2 diabetes mellitus.

Key words: Diabetes Mellitus, Type 2; Endothelium, Vascular; Insulin Resistance; Obesity; Dyslipidemias; Hyperglycemia

Summary

Introduction. Endothelium is a dynamic, strategically positioned defensive regulator of vascular homeostasis. Physiology and Pathophysiology of Vascular Endothelium. Endothelial phenotypic modulation involves five basic characteristics: the expression of leukocyte adhesion molecules, the production of cytokines, change in the shape and the permeability of the endothelium, prothrombotic changes and upregulation of autoantigens.

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Sažetak


Ključne reči: dijabetes mellitus tip 2; vaskularni endotelijum; insulinska rezistencija; gojaznost; dislipidemije; hiperglykemija
Abbreviations

T2DM – Type 2 diabetes mellitus
NO – nitric oxide
PGI2 – prostacyclin
ET-1 – endothelin - 1
NF-κB – nuclear factor kappa B
ICAM-1 – intracellular adhesion molecule
VCAM-1 – vascular cell adhesion molecule
vWF – von Willebrand factor
PAI-1 – plasminogen activator inhibitor-1
TF – tissue factor
ROS – reactive oxygen species
DAMP – damage-associated molecular patterns
NLRP3 – nucleotide-binding domain, leucine-rich, pyrin domain containing 3
TLR4 – toll-like receptor 4
TNF-α – tumor necrosis factor-alpha
IL – interleukin
PI3K – phosphatidylinositol-3 kinase
MAPK – mitogen-activated protein kinase
AGE – advanced glycation end products
PKC – protein kinase C
TAFI – Thrombin activatable fibrinolysis inhibitor
HDL – high-density lipoprotein
VLDL – very low density lipoproteins
FFA – free fatty acids
LDL – low density lipoprotein
oxLDL – oxidised LDL
AT – adipose tissue
Lp(a) – lipoprotein-a

Endothelial dysfunction is characterized by reduced bioavailability of NO, the most potent vasodilator, is the premise of vascular health [7]. Nitric oxide enables vasodilation, inhibits the proliferation, migration and differentiation of vascular smooth muscle cells to the intima of the blood vessel, stabilizes the inhibitory subunit of nuclear factor kappa B (NF-κB), that maintains this proinflammatory transcription factor in an inactive state, and thereby inhibits the expression of leukocyte adhesion molecules, the production of chemokines and proinflammatory cytokines [6, 8]. Prostacyclin also exhibits vasodilatory, antiplatelet, and cytoprotective action. Under physiological conditions, adhesion molecules responsible for the control of the migration of leukocytes to the vascular endothelium are not expressed on the cell surface [9]. Also, intact vascular endothelium prevents platelet adhesion and balances between the secretion of procoagulant/anti-coagulant and profibrinolitic/antifibrinolytic substances contributing to the equilibrium of coagulation and fibrinolysis [10].

Endothelial dysfunction is characterized by reduced bioavailability of NO that impairs the endothelium-dependent vasodilator capacity of the vessel wall. Considering the protecting abilities of NO, endothelial dysfunction also involves a certain degree of endothelial cell activation that alters the inert endothelial phenotype into proinflammatory, proliferative and procoagulant one [3, 11]. Vascular endothelium in respond to various stimuli (mechanical, biological, chemical, immune, metabolic) expresses phenotypic changes in terms of endothelial activation. Endothelial activation includes de novo gene expression, the synthesis of proinflammatory cytokines and adhesion molecules as well as increased expression of the adhesion molecules like E-selectin, intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and increased permeability of the blood vessel. Endothelial surface becomes prothrombogenic due to reduced production and expression of molecules such as thrombomodulin, heparan sulfate, NO, PGI2, accompanied by increased von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue factor (TF) and platelet activating factor (PAF). Synthesized proinflammatory cytokines and chemokines amplify the acute phase response and monocytes recruitment to the site of injury [12]. The different stimuli of the endothelial cell surface have a common denominator, a transcription factor, NF-κB. Activation of NF-κB stimulates the transcription of genes responsible for the genesis of phenotypic conversion of endothelial cells [13]. Endothelial phenotypic modulation involves five basic characteristics: the expression of leukocyte adhesion molecules, the production of cytokines, change in the shape and the permeability of the endothelium, prothrombotic changes and upregulation of autoantigens [12]. Interactions of leukocyte and endothelial cell such as capture, rolling, and firm adhesion are series of overlapping synergistic interactions among numerous adhesion molecules result-

Physiology and Pathophysiology of Vascular Endothelium

Vascular endothelium plays a critical role in the regulation of blood pressure and the optimum blood flow continuously balancing between vasodilation and vasoconstriction, with the synthesis of a vasodilators, nitric oxide (NO) and prostacyclin (PGI2), and a vasoconstrictors, endothelin - 1 (ET-1) and endothelium - derived hyperpolarizing factor (EDHF) [6]. It is considered that the bioavailability of NO, the most potent vasodilator, is the premise of vascular health [7]. Nitric oxide enables vasodilation, inhibits the proliferation, migration and differentiation of vascular smooth muscle cells to the intima of the blood vessel, stabilizes the inhibitory subunit of nuclear factor kappa B (NF-κB), that maintains this proinflammatory transcription factor in an inactive state, and thereby inhibits the expression of leukocyte adhesion molecules, the production of chemokines and proinflammatory cytokines [6, 8]. Prostacyclin also exhibits vasodilatory, antiplatelet, and cytoprotective action. Under physiological conditions, adhesion molecules responsible for the control of the migration of leukocytes to the vascular endothelium are not expressed on the cell surface [9]. Also, intact vascular endothelium prevents platelet adhesion and balances between the secretion of procoagulant/anti-coagulant and profibrinolitic/antifibrinolytic substances contributing to the equilibrium of coagulation and fibrinolysis [10].

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Vascular endothelium in terms of deranged metabolic milieu of T2DM loses antiflammatory, antiadhesive, antiplatelet abilities, extreme leukocyte and platelet adhesion molecules, promotes smooth muscle cell proliferation and migration. Alteration of endothelium is atherosclerosis-prone site and endothelial dysfunction is not only the initiator of atherosclerotic process but also is an amplifier of cascade of events leading to atherosclerotic plaque development [5]. Therefore, endothelial dysfunction is considered to be a “sentinel” event in early atherosclerosis development [4].

In this review, we discuss numerous metabolic factors and pathophysiologic processes involved in ethiopathogenesis of endothelial dysfunction in T2DM.
Metabolic (meta) inflammation [21]. Potential inflammatory response by classical mechanisms is named initiation and the amplification of the inflammation attempts to restore and maintain homeostasis. Imbalance of pro- and anti-inflammatory (ECM), all could lead to activation of numerous manifestations (adiposity) leads to a number of local (adipocyte and AT morphological and functional abnormalities), as well as systemic pathophysiological disorders termed adiposopathy (“adipose-opathy,” or “sick fat”) [18]. One of the most important pathophysiological manifestations of adiposopathy may be phenotypic conversion of vascular endothelium in both, the microcirculation and the macrocirculation. Thus, the major clinical consequences, arterial hypertension (predominantly-vasoconstrictive vascular phenotype) and accelerated atherosclerosis (predominantly-proinflammatory phenotype), could be present along with accelerated atherosclerosis (predominantly-proinflammatory phenotype) and activation of numerous inflammatory inducers [22].

Molecular mechanisms underlying the metabolic inflammation in dysfunctional AT are very complex. Activation of the inflammasomes is a key function mediated by the innate immune system. These complexes activate inflammatory protease caspase-1 and induce inflammation in response to molecules that result from cells’ damage or death (necrosis/apoptosis), damage-associated molecular patterns (DAMP). Particularly, the inflammasome nucleotide-binding domain, leucine-rich repeat, pyrin domain containing 3 (NLPR3), could recognize certain metabolic stressors. Caspase-1 further regulates the maturation of proinflammatory cytokines interleukin (IL)-1β and IL-18 or pyroptosis (caspase-1-dependent cell death). Activation of NLPR3 inflammasome may also be associated with DAMP stressors, such as extracellular ATP, hyaluron, amyloid-β fibrils and uric acid crystals. Further, potential inducers of this multi-molecular complex could be ROS, potassium and others [23]. In addition, increase in the extracellular concentration of FFA represents an important metabolic stressor, since FFA serve as TLR4 (toll-like receptor 4) ligands. These receptor protein systems, TLR, activate protein kinases, c-jun N-terminal kinases (JNKs). In the human TLR system, TLR 2 and TLR4 have significance in metabolic disorders [24].

The presence (migration) of macrophages in AT present the initial steps of obesity induced metabolic inflammation. Adipose tissue macrophages can be distinguished into M1 and M2 macrophages. In metabolic homeostasis, M2 phenotypic form is predominantly present in visceral AT. In response to perturbation in dysfunctional AT, resident macrophages shift their polarization status. Classically activated or M1 macrophages stimulated by interferon γ (IFN-γ) express pro-inflammatory phenotype and participate in the polarization Th1 adaptive immune response by producing IL 12. The cytokine profile characterizing the M1 phenotype includes tumor necrosis factor-alpha (TNF-α), IL-1β, IL-12, and IL-23. Alternatively activated or M2 macrophages, stimulated by Th2 cytokines, secrete anti-inflammatory cytokines IL-10, IL-1 receptor antagonist (IL-1Ra), transforming growth factor-β (TGF-β) and other factors involved in tissue and fibrous replenishment processes [25].

Signal mechanisms from dysfunctional adipocytes affect the activation and status of local macrophages. Also, chemokines monocyte chemotactic protein-1 (MCP-1) and leukotriene B4 (LTB4) secreted from adipocytes attract monocytes in AT, where they are further differentiated into tissue macrophages. Locally in AT, macrophages form aggregates “crown-like structures” around necrotic adipocytes (necrotic/apoptotic) and residual lipids, and release cytokines, most notably TNF-α. In addition to macrophage, other immune cells have multiple interaction and exhibit their response to hypoxia, necrosis, and/or apoptosis [16]. Moreover, current findings highlight the importance of metabolic stressors (glucose, FFA, palmitylate, cholesterol crystals, ceramides, etc) as possible inflammatory inducers [22].

Obesity, Metabolic Inflammation and Vascular Endothelium

Obesity represents the most important independent risk factor for T2DM. Also, the risk for developing T2DM increases with the degree of obesity. Overlap of association between T2DM and obesity is beyond epidemiological data, one of the main pathogenetic mechanisms of these diseases is a chronic inflammatory process characterized by the activation of innate and acquired immunity [16]. Adipose tissue (AT) is a dynamic and metabolic highly active endocrine organ involved in the regulation of immunological, metabolic and cardiovascular homeostasis [17]. However, fat accumulation (adiposity) leads to a number of local (adipocyte and AT morphological and functional abnormalities), as well as systemic pathophysiological disorders termed adiposopathy (“adipose-opathy,” or “sick fat”) [18]. One of the most important pathophysiological manifestations of adiposopathy may be phenotypic conversion of vascular endothelium in both, the microcirculation and the macrocirculation. Thus, the major clinical consequences, arterial hypertension (predominantly-vasoconstrictive vascular phenotype) and accelerated atherosclerosis (predominantly-proinflammatory vascular phenotype), could be present along with metabolic disturbances found in obesity [19].

According to scientific theory (hypothesis), adiposopathy may initially be present in visceral, pericardial, and perivascular fat depots. Morphofunctional abnormalities in AT, adipocyte hypertrophy and organellar dysfunction (especially mitochondrial dysfunction and endoplasmic reticulum (ER) stress), impaired angiogenesis and hypoxia, insufficient adipogenesis, imbalance between apoptosis and adipogenesis, disturbances in the remodeling or degradation of extracellular matrix (ECM), all could lead to activation of numerous immune mechanisms. Also, adiposopathy is associated with imbalance of pro- and anti-inflammatory adipokines, increased production of reactive oxygen species (ROS) and oxidative stress [20].

Like a classic inflammatory response, the one that develops in dysfunctional AT depots represents an attempt to restore and maintain homeostasis. Initiation and the amplification of the inflammatory response by classical mechanisms is named metabolic (meta) inflammation [21]. Potential inducers of these inflammatory responses could be adipocyte and macrophage related signals in response to hypoxia, necrosis, and/or apoptosis [16]. Moreover, current findings highlight the importance of metabolic stressors (glucose, FFA, palmitate, cholesterol crystals, ceramides, etc) as possible inflammatory inducers [22].
main effector functions in the process of migration control, activation and polarization of macrophages [21]. Regulatory T lymphocytes CD4 + secretes anti-inflammatory cytokines that inhibit macrophage migration and affect their polarization in M2 phenotype. Cytotoxic CD8 + T lymphocytes infiltrate AT and produce proinflammatory cytokines and activate M1 macrophage phenotype. Also, B lymphocytes further promote activation of T lymphocyte and polarization of macrophage to the proinflammatory M1 phenotype. In addition to mastocyte, resident eosinophils can participate in maintaining M2 polarization status by secreting IL-4 and IL-13. Moreover, local macrophages produce chemokines, which then further promote systemic inflammatory response [26]. The development of obesity-associated systemic inflammation may lead to target organ dysfunction and clinical manifestations of adiposopathy, most notably T2DM [27].

Insulin Resistance and Vascular Endothelium

Under the conditions of insulin resistance and consequent hyperinsulinemia, there is decreased activation of the phosphatidylinositol-3 kinase (PI3K) accompanied by an enhanced activation of mitogen-activated protein kinase (MAPK) signaling pathway, that regulates growth, mitogenesis and differentiation. Using MAPK-dependent signaling pathways, insulin stimulates ET-1 production [28]. Consequence of the reduced activation of PI3K is decreased production of NO accompanied by an increased formation of ROS that induces oxidative stress. Activation of this MAPK-dependent signaling pathway also leads to an up-regulation of PAI-1 and increased expression of adhesion molecules, VCAM-1 and E-selectin. This creates a condition for the expression of vasoconstrictive, proliferative, proatherogenic and prothrombotic endothelial phenotype [29].

Defect in insulin action, caused by insulin resistance, is associated with the changes in platelets function. T2DM is associated with persisted abnormal platelet function proven to be present both in vitro and in vivo, and characterized with a systemic rather than localized stimulation of platelet activation, as well as continuous rather than episodic alteration [30]. Increased number of platelet aggregates in circulation, increased aggregation of platelets after platelet agonists’ addition, increased platelet contractility, and presence of elevated plasma levels of their contents [beta-thromboglobulin, platelet factor 4, thromboxane B2], demonstrate platelet hyperreactivity in T2DM. Platelets in diabetic patients adhere to vascular endothelium and aggregate more rapidly than in healthy people. The most important reason for this is loss of sensitivity to the normal restraints exercised by PGI2 and NO, generated by the vascular endothelium [31]. Platelet adhesion occurs at the stage of endothelial dysfunction, before the damage of endothelial structural integrity and is caused by the expression of adhesion molecules on activated endothelial cells, platelets and leukocytes [32].

Hyperglycemia and Vascular Endothelium

Hyperglycemia causes endothelial dysfunction by various mechanisms that involve activation of polyol pathway and production of sorbitol, increased formation of advanced glycation end products (AGE), overexpression of AGE receptors, activation of various isoforms of protein kinase C (PKC) and activation of hexosamine pathway. The common path in which all the previous mechanisms meet and continue to cause vascular damage is the pathway of oxidative stress [33].

In conditions of chronic hyperglycemia due to inability of glucose metabolism in fully aerobic glycolysis, glucose is metabolised by alternative pathways. Activation of the polyol pathway enhances the synthesis of the sorbitol that has toxic, osmotic activity and reduces the concentration of myoinositol. At the same time, due to the oxidation of nicotinamide adenine dinucleotide phosphate (NAD(P)H) and the reduction of nicotinamide adenine dinucleotide (NAD+), redox imbalance is created. It reduces the bioavailability of NO and further enhances the oxidative stress [34].

Nonenzymatic glycosylation of proteins in conditions of chronic hyperglycemia results with increased production of AGE. AGE react with intracellular structures, extracellular matrix and circulating proteins, altering their structure and function [35]. Binding of AGE to AGE receptors on endothelial cells, monocytes, macrophages and smooth muscle cells induces oxidative stress and proinflammatory response [36].

Intracellularly, hyperglycemia increases the synthesis of diacyl-glycerol (DAG) which consequently activates PKC. The consequences of PKC activation are the imbalance between NO/ET-1 ratio, with the consequent predominant vasoconstrictive response and the activation of NF-κB with the increased proinflammatory gene expression [35].

Hyperglycemia stimulates increased production of superoxide anion, O2− [37]. O2− in turn increases the production of hexosamine and AGE, activates PKC, a polyol pathway and NF-κB and also leads to the production of proinflammatory cytokines (IL-1β, TNF-α), expression of adhesion molecules (E-selectin, ICAM-1, VCAM-1) and ET-1 [38]. It is found that concentration of sE-selectin and vWF-Ag was significantly higher in patients with T2DM in regard to non-diabetics [39].

Numerous complex mechanisms contribute to the diabetic prothrombotic state, such as: endothelial dysfunction, platelet hyperreactivity, increased coagulation and decreased fibrinolysis [40]. Production of TF increases in the presence of low-grade inflammation, commonly associated with type 2 diabetes. Both glucose and insulin levels are responsible for increment of circulating levels of TF, and it seems that they have an additive effect [41]. In a large term study over 18 years, hemoglobin A1c positively correlated with PAI-1 levels, and nega-
tively with tissue plasminogen activator (t-PA), implicating glycemia in modulating fibrinolytic potential. Thrombin activatable fibrinolysis inhibitor (TAFI) antigen levels, as well as TAFI activity are significantly increased in T2DM. Inverse correlation of TAFI antigen levels and D-dimer was found in these patients supporting the role of TAFI in diabetes-induced inhibition of fibrinolysis [42].

**Dyslipidemia and Vascular Endothelium**

Dyslipidemia takes an important role in a cascade of pathophysiological processes that result in endothelial activation and chronic dysfunction [43]. In T2DM, dyslipidemia is characterized by an increased level of triglycerides, usually accompanied by high total cholesterol level, low concentration of high-density lipoprotein (HDL) cholesterol and by the presence of small dense low – density lipoprotein (sdLDL) [44]. Hyperglycemia, hyperinsulinemia and insulin resistance are considered to be the main factors responsible for the occurrence of dyslipidemia that is usually expressed phenotypically by the presence of type IV or IIb hyperlipoproteinemia. Long-term hyperglycemia leads to protein glycation, thus resulting with structural and functional disorders of apolipoproteins in different lipoprotein particles, lipoprotein receptors and the enzymes involved in the lipid metabolism [45]. Insulin resistance and subsequent hyperinsulinemia due to reduced insulin sensitivity lead to increased liver synthesis of free fatty acids (FFA), triglycerides and very low density lipoproteins (VLDL), large, triglyceride-rich lipoprotein particles. High affinity of the lipoprotein lipase for chylomicron hydrolysis contributes to slow catabolism and accumulation of VLDL particles as well as chylomicrons and VLDL remnants in blood during postprandial lipemia [46].

In terms of hypertriglyceridemia it increases the activity of the cholesteryl ester transfer protein (CETP), which enables the exchange of triglycerides from the triglyceride-rich lipoproteins to HDL and LDL particles, which in turn give part of their cholesteryl esters to triglyceride-rich lipoproteins. In this way remodeled HDL and LDL particles, now poorer in cholesterol-esters, but richer in triglycerides, become a suitable substrate for hepatic lipase which hydrolyse the triglycerides in these particles, concomitantly creating proatherogenic smaller and denser LDL, sdLDL, also known as sub-populations of LDL-III particles, and the smaller and denser HDL particles [77]. Small dense HDL particles lose their functionality and very rapidly are removed from the circulation, lowering in this way the serum concentration of HDL-cholesterol (especially a protective HDL2 subpopulation) [47].

Due to the structural changes in sdLDL particles, in order to native LDL, sdLDL have a lower binding affinity for LDL receptors, and are predominantly removed from the circulation by binding the scavenger receptors, promoting the formation of foam cells and the development of premature atherosclerosis. The longer retention of sdLDL in subendothelium makes easier their modification by oxidation or the glycation, so their role in the development of endothelial dysfunction is usually attributed to oxidative modification and to the mechanisms of action of oxidised LDL (oxLDL) [47]. Chronic hyperglycemia leads to an increased glycation of LDL particles (glycated LDL, gLDL), with the consequent conformational changes that interfere with their binding to the LDL receptor. Therefore, gLDL stay in circulation for prolonged period of time that may result in oxidation of these particles and the formation of AGE – LDL complex (AGEs-LDL) with pro-inflammatory and pro-atherogenic characteristics. Also, the gLDL have been removed by alternative pathways, independently of LDL receptors. Additionally, gLDL prevents shear stress-mediated L-arginine uptake and NO synthesis and causes increased production of PAI-1 and prostaglandins, while inhibiting the expression of tPA in endothelial cells [46].

In T2DM, there are significant qualitative changes in LDL particles which make them very susceptible to oxidation, especially when they are trapped in intima of blood vessels. In the first stage of oxidation, minimal modifications of LDL particles occur in the form of minimum modified apolipoprotein B, conversion of cholesteryl esters and phospholipids in hydroperoxides, isoprostanes and short length branched-chain aldehydes. These minimally modified LDL particles can stimulate endothelial cells to secrete various chemokines [47].

It is known that oxidatively modified LDL, αLDL, takes the most important role in the initiation of endothelial dysfunction and the damage of endothelial and smooth muscle cells of the vessel wall. OxLDL binds the released NO, reduces endothelial nitric oxide synthase (eNOS) activity and interferes with the endothelial L-arginine/NO metabolic pathway, thus leading to endothelial vasoconstriction [48]. In the interaction of oxLDL with endothelium, lysophosphatidylcholine is created. Lysophosphatidyl-choline activates protein kinase leading to the formation of superoxide, a reactive oxygen radical, which stimulates further oxidation of LDL particles, as well as it binds NO and thereby inhibits endothelium-dependent vasorelaxation. OxLDL promotes expression of various adhesion molecules (ICAM-1, VCAM-1, E-selectin), activation of NF-κB, increases the expression and production of the ET-1 in endothelial cells and exerts chemotactic effect on monocytes and T lymphocytes. Also, its potent cytotoxic activity and role in apoptosis of endothelial cells has been proven. Binding to scavenger receptors, oxLDL is being taken up by macrophages leading to their activation and transformation into the foam cells which represent pathohistological substrate of early atherosclerotic blood vessels changes [49].

Recent investigations showed that lipoprotein(a) (Lp(a)) as a transporter of oxLDL, particularly in terms of the hyper-Lp(a)-lipoproteinemia, could significantly enhance the effect of oxLDL on the devel-
opment of endothelial dysfunction. Additionally, data suggesting that Lp(a) may contribute to a dysfunctional endothelium in vitro are supported by a number of studies that have demonstrated that elevated plasma Lp(a) concentrations contribute to endothelial dysfunction in vivo [50].

Finally, it should be mentioned that a high concentration of FFA caused by excessive influx of FFA from adipose tissue, as well as by impaired uptake by skeletal muscles, participates in the development of endothelial dysfunction in T2DM. Extracellular FFA activate PKC and impair insulin-mediated activation of PI3K, thus reducing the bioavailability of NO, the intracellular vasodilator which keeps the endothelium-dependent vasorelaxation by stimulation of guanyl cyclase and increment of intracellular levels of cyclic guanine monophosphate (cGMP) [36]. Additionally, FFA stimulate ROS production and activation of the redox sensitive transcription factors and nuclear receptor systems. Thereby, FFA continue to increase the level of oxidative stress and vascular damage [37].

Conclusion
Hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, visceral obesity and low-grade inflammation are the main factors responsible for the development of endothelial dysfunction in type 2 diabetes mellitus. Altered vascular homeostasis results in decreased bioavailability of nitric oxide, consecutive vasoconstriction, leukocyte adherence, platelet activation, an imbalance between the secretion of procoagulant/anti-coagulant and profibrinolytic/antifibrinolytic substances, increased oxidative stress and vascular inflammation. Morphologically normal arteries with altered functional endothelial response represent a target site of early atherosclerosis. Processes involving endothelial dysfunction promote atherogenesis and atherothrombotic complications at early stage of type 2 diabetes mellitus. Atherothrombosis is the leading cause of morbidity and mortality in patients with diabetes mellitus.

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