J. BELTOWSKI [2004] MED HYPOTHESES RES 1: 187-206.

MHR

REVIEW

WHY STATINS HAVE NO EFFECT ON PLASMA ASYMMETRIC DIMETHYLARGININE (ADMA) LEVEL? — THE POSSIBLE ROLE OF PARAOXONASE AND PROTEIN HOMOCYSTEINYLATION

JERZY BELTOWSKI*

DEPARTMENT OF PATHOPHYSIOLOGY, MEDICAL UNIVERSITY OF LUBLIN, LUBLIN, POLAND.

ABSTRACT. STATINS (3-hydroxy 3-methylglutarylcoenzyme A reductase inhibitors) are widely used in the treatment of cardiovascular diseases. In addition to reducing plasma cholesterol, they exert pleiotropic effects on inflammatory reaction, coagulation, fibrinolysis, smooth muscle cells, oxidative stress, and others. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is synthesized by methylation of protein arginine residues, released during proteolysis, and degraded by dimethylarginine dimethylaminohydrolase (DDAH). Hyperlipidemia and oxidative stress increase ADMA production and impair its metabolism. Although statins improve lipid profile and attenuate oxidative stress, they do not change plasma ADMA, which suggests that these drugs may also have some opposite undesirable effect on its metabolism. Recent studies suggest that hyperhomocysteinemia increases ADMA level. Homocysteine may be converted to homocysteine thiolactone (HTL) by methionyl-tRNA synthetase. HTL binds to lysine groups of proteins in the process referred to as protein homocysteinylation, leading to decrease in their biological activity. Apart from total homocysteine level, the amount of HTL is regulated by paraoxonase 1 (PON1) which hydrolyzes it to homocysteine. Both in vitro and in vivo studies suggest that statins can decrease PON1 activity. It is possible that statins, by inhibiting PON1, increase the level of HTL leading to homocysteinylation of DDAH, and thus reduce its activity. This undesirable mechanism could oppose positive effect on ADMA metabolism resulting from hypolipidemic and antioxidant properties of statins, resulting in no net changes in plasma ADMA concentration.

*ADDRESS ALL CORRESPONDENCE TO: DR. JERZY BELTOWSKI, DEPARTMENT OF PATHOPHYSIOLOGY, MEDICAL UNIVERSITY, UL. JACZEWSKIEGO 8, 20-090 LUBLIN, POLAND.
PHONE: +48-81-7425837. FAX: +48-81-7425828. E-MAILS: jerzybel@hotmail.com

● MEDICAL HYPOTHESES AND RESEARCH ● THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH ●

1. INTRODUCTION

Statins (3-hydroxy 3-methylglutarylcoenzyme A reductase inhibitors) inhibit in a competitive manner a rate-limiting enzyme in cholesterol biosynthetic pathway converting 3-hydroxy 3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate. Statins decrease plasma low density lipoprotein (LDL)-bound cholesterol by inhibiting hepatic cholesterol synthesis and upregulating LDL receptor expression. In addition, statins decrease plasma triglycerides and moderately elevate atheroprotective high-density lipoproteins (HDL). Treatment with statins efficiently prevents acute cardiovascular events in both primary and secondary prevention of ischemic heart disease. However, clinical benefits of statin treatment are observed not only in patients with hyperlipidemia but also in those with normal lipid profile. “Cholesterol-independent” activity of statins results from their inhibitory effect on vascular smooth muscle cell migration and proliferation, antiplatelet, anti-inflammatory and anticoagulant activity, stabilization of atherosclerotic plaque, and attenuation of oxidative stress. These pleiotropic effects are attributed to the inhibition of formation of nonsteroid isoprenoids such as farnesylpyrophosphate and geranylgeranylpyrophosphate, which are attached to small signaling G proteins of the Ras superfamily in the process referred to as protein isoprenylation. While decrease in LDL-cholesterol results from the effect on hepatic HMG-CoA reductase, pleiotropic activities of statins are associated with inhibition of this enzyme also in other tissues [1-4].

Currently available statins may be divided into two groups. Natural statins are fungal metabolites or their derivatives and include lovastatin, pravastatin and simvastatin. Synthetic statins, i.e., fluvastatin and atorvastatin, have different chemical structure and are more potent HMG-CoA reductase inhibitors. The third synthetic statin, cerivastatin, was withdrawn from the market in 2001 due to many reported cases of fatal rhabdomyolysis. Pravastatin is the only hydrophilic statin which poorly permeates plasma membranes with the exception of hepatocytes, to which it is transported by membrane carrier protein. All other statins are lipophilic and easily enter all cell types. Therefore, although pravastatin may be as effective as other drugs in decreasing plasma cholesterol, it may have less prominent extrahepatic, i.e., pleiotropic activities.

Nitric oxide (NO) continuously generated from L-arginine by endothelial nitric oxide synthase (eNOS) plays an important role in preventing atherogenesis. NO inhibits adhesion of platelets and monocytes to the endothelium, attenuates migration and proliferation of smooth muscle cells, scavenges reactive oxygen species and inhibits their generation in the vascular wall. NO deficiency accelerates atherogenesis whereas drugs increasing NO generation such as L-arginine are antiatherogenic [5-7]. Many studies have demonstrated that statins increase endothelial NO generation both in vitro and in vivo [8-10]. NO availability is determined by multiple factors including expression and activity of NO synthase, local concentration of its substrate, L-arginine, NO scavenging by reactive oxygen species (ROS) and finally, the level of endogenous NOS inhibitors, especially asymmetric dimethylarginine (ADMA). Plasma concentration of ADMA is increased by hyperlipidemia and oxidative stress, therefore, it should be expected that statins decrease ADMA level due to their potent hypolipidemic and antioxidant properties. Surprisingly, most experimental and clinical studies performed so far indicate that statins have no effect on plasma ADMA. These data suggest that statins may have some additional unbeneficial impact on ADMA metabolism which opposes their indirect effect mediated by normalization of lipid profile and improvement of oxidant-antioxidant balance. Herein, I propose a hypothesis which could explain this apparent paradox. According to this hypothesis, statins may influence ADMA metabolism by modulating paraoxonase 1 (PON1) activity and homocysteine thiolactone (HTL) metabolism. In addition, the possible pathways through which fibrates, other commonly used hypolipidemic drugs, could modulate ADMA metabolism will be discussed.

2. METABOLISM AND BIOLOGICAL ACTIVITY OF ADMA

In 1992, Vallance et al. [11] first reported that methylarginines including ADMA, symmetric dimethylarginine (SDMA) and N-monomethyl-L-

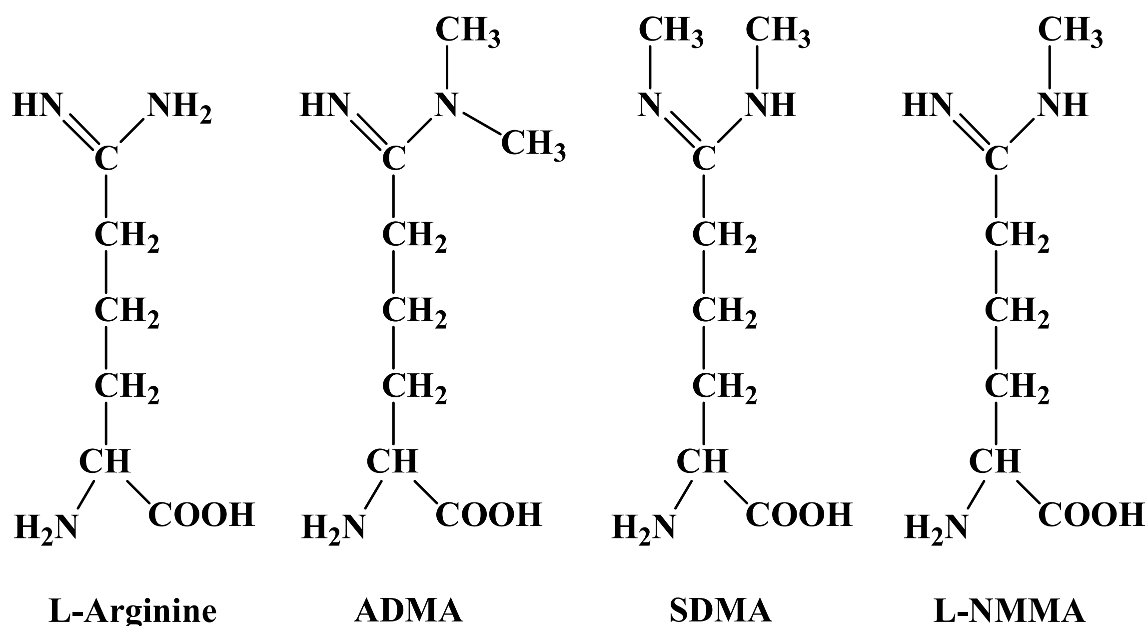

FIGURE 1. STRUCTURE OF ENDOGENOUS METHYLARGININES.

arginine (L-NMMA) are endogenous compounds circulating in human plasma and excreted in urine (FIG. 1). ADMA and L-NMMA are potent inhibitors of NOS, however, the role of the latter is negligible because it circulates at concentration about 10-fold lower than ADMA. Although plasma level of SDMA is comparable to ADMA, it has no effect on NOS activity.

Methylarginines are synthesized during methylation of protein arginine residues by S-adenosylmethionine:protein arginine methyltransferases (protein methylases, PRMT). Two isoforms of this enzyme have been identified. PRMT1 methylates histones and nuclear RNA-binding proteins and yields L-NMMA and ADMA, whereas PRMT2 methylates exclusively myelin basic protein and generates L-NMMA and SDMA [12]. Free methylarginines are released during proteolysis and are not back incorporated into proteins. The generation of these compounds is high during rapid protein turnover, e.g. in catabolic states, and is faster in proliferating than in quiescent cells. Normal plasma level of ADMA is about 1 μM; it increases 2- to 3-fold in patients with different risk factors of atherosclerosis such as hyperlipidemia and diabetes mellitus, and up to 8-fold in patients with end-stage renal disease [13].

All methylarginines are eliminated by renal excretion, however, urinary excretion of SDMA is much higher than of the two remaining compounds because more than 90% of ADMA and L-NMMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH), which degrades them to citruline and dimethylamine or monomethylamine, respectively (FIG. 2) [14]. This enzyme exists in 2 isoforms: DDAH1 is predominantly expressed in tissues containing neuronal NOS and DDAH2 mainly in tissues containing endothelial or inducible NOS [15–17]. Pharmacological inhibition of DDAH increases ADMA elaboration and reduces NO production by isolated vascular tissue [18], whereas transgenic DDAH overexpression has the opposite effect both in vitro [19] and in vivo [20].

ADMA decreases NO production in cell-free preparation and in isolated or cultured cells with IC_{50} of 2-10 μM, which is well within (patho)physiological range [21]. ADMA inhibits not

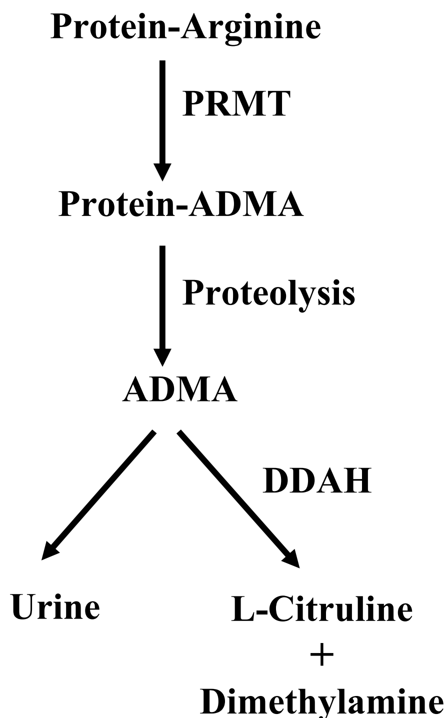

FIGURE 2. SYNTHESIS AND METABOLISM OF ADMA. PRMT, S-adenosylmethionine : protein arginine methyltransferase; DDAH, dimethylarginine dimethylaminohydrolase.

only endothelial or type 3 NOS but also two remaining forms of the enzyme, i.e., neuronal or type 1 (nNOS) and inducible or type 2 (iNOS). Since ADMA is a competitive inhibitor, its effect is counteracted by excess of L-arginine. ADMA may be responsible for so called "arginine paradox" – the observation that L-arginine improves NO generation in many experimental systems although physiological level of this aminoacid in plasma (close to 100 μM) is much above K_M of NOS [22]. In addition, ADMA and L-NMMA may induce "NOS uncoupling", i.e., increase superoxide production by the enzyme [23]. In contrast, synthetic NOS inhibitor, L-NAME, decreases O_2^- generation by isolated NOS. Under physiological conditions L-NAME increases O_2^- production by endothelial cells, because it reduces the availability of NO which scavenges superoxide generated by sources other than NOS. In contrast, in diabetes or hyperlipidemia when ADMA is increased, L-NAME reduces O_2^- production suggesting that under such conditions eNOS is uncoupled and generates superoxide rather than NO [24]. Interestingly, all three methylarginines compete with cellular L-arginine uptake by plasma membrane y^+ basic aminoacid transporter. Thus, also SDMA may limit NO generation by reducing intracellular L-arginine availability [25].

Several biological activities of ADMA have been described, all of them being attributed to shortage of NO. ADMA induces vasoconstriction in vitro and in vivo [11,26], increases blood pressure [27], stimulates leukocyte adherence to endothelium [28], upregulates proinflammatory monocyte chemo-attractant protein-1 (MCP-1) expression in endothelial cells [23], induces the expression of oxidized low-density lipoprotein receptor LOX-1 in endothelial cells and macrophages [29], decreases urinary sodium excretion [30], and augments renal tubuloglomerular feedback, i.e., constriction of glomerular afferent vessel induced by increase in sodium concentration near macula densa [16]. Plasma ADMA level inversely correlates with urinary excretion of nitrites/nitrates, the stable end-products of NO [31]. Several clinical studies have documented that elevated ADMA correlates with increased carotid artery intima-media thickness (a marker of atherosclerosis), and is an independent predictor of acute cardiovascular events both in patients with renal failure and in those with normal renal function [32–35].

3. EFFECT OF HYPERLIPIDEMIA AND OXIDATIVE STRESS ON ADMA METABOLISM

Both animal and human studies indicate that plasma ADMA concentration is increased by hyperlipidemia and oxidative stress. In cultured human coronary artery endothelial cells native or oxidized LDL (oxLDL) increase PRMT mRNA and protein expression as well as release of ADMA to the culture medium. The effect is prevented by S-adenosylhomocysteine, the product of PRMT-catalyzed reaction and inhibitor of SAM-dependent methyltransferases. Oxidized LDL has a more marked effect than native lipoproteins [36]. Oxidized LDL and to a lesser extent native LDL stimulate ADMA release also by human umbilical vein endothelial cells. This is accompanied by reduced DDAH activity [37]. These data suggest

that oxLDL increase ADMA synthesis and impair its catabolism. ROS may inactivate DDAH by oxidizing sulfhydryl group of cysteine contained in active site of the enzyme. This –SH group may also be nitrosylated by nitric oxide when it is produced in excessive amounts by iNOS; the condition referred to as "nitrosative stress" [38,39]. In cultured endothelial cells overexpressing human DDAH-1, this enzyme is not nitrosylated under baseline conditions but becomes nitrosylated when iNOS is upregulated by cytokine treatment [38] Interestingly, DDAH exists in cells in two forms: as a holoenzyme containing Zn^{2+} ion and as an apoenzyme which is not bound to Zn^{2+} [40]. Zinc binding inactivates the enzyme, and it is well known that intracellular concentration of free Zn^{2+} increases under oxidative stress conditions. DDAH-1 isolated from bovine brain is nitrosylated only in its Zn^{2+}-free form; and it is suggested that Zn^{2+} binds to cysteine residues protecting them from S-nitrosylation [39]. Thus, oxidative/nitrosative stress may inactivate DDAH by increasing its Zn^{2+}-bound fraction, as well as by oxidation and/or nitrosylation of cysteine –SH groups of the remaining Zn^{2+}-free fraction.

In vivo studies confirm that plasma ADMA and SDMA increase about 2-fold in cholesterol-fed rabbits, which is accompanied by reduced DDAH activity in cardiovascular system, liver and kidney [37,41,42]. Intravenous administration of isolated LDL increases plasma ADMA in the rat and this is accompanied by increase in lipid peroxidation product, malonyldialdehyde, as well as by the decrease in plasma NO metabolites and impaired endothelium-dependent vasorelaxation [43]. Pretreatment with vitamin E prevents this increase in ADMA suggesting that oxidative stress may be involved. Plasma ADMA is also elevated by about 60% in hyperlipidemic apolipoprotein $E^{-/-}$ mice [44]. In hypercholesterolemic young healthy humans plasma ADMA is increased more than 2-fold whereas SDMA and L-arginine are not changed. ADMA concentration positively correlates with total- and LDL-cholesterol and negatively with urinary excretion of NO metabolites and endothelium-dependent vasorelaxation [45]. Lundman et al. [46] observed about 20% increase in ADMA in men with mild to moderate hypertriglyceridemia in comparisons to the control group. In addition, high-fat meal which transiently elevates plasma triglycerides induced about 2.5-fold increase in plasma ADMA in patients with type 2 diabetes mellitus [47]. Chan et al. [48] reported almost 2-fold increase in plasma ADMA in humans with mixed hyperlipidemia. ADMA concentration was positively correlated with total- and LDL-cholesterol whereas SDMA and L-arginine were unchanged. However, not all studies have demonstrated increase in ADMA in hypercholesterolemic humans. Paiva et al. [49] observed no difference in plasma ADMA between control group and men with heterozygous familial hypercholesterolemia. However, the study group was small (N = 14) and most patients were on long-lasting hypolipidemic therapy which could affect the results.

4. EFFECT OF STATINS ON ADMA LEVEL

Statins effectively reduce LDL-cholesterol and ameliorate oxidative stress due to inhibition of vascular NAD(P)H oxidase and direct radical-scavenging properties [50]. Hypolipidemic and potent lipid-soluble antioxidant drug, probucol, effectively prevents increase in plasma ADMA induced by intravenous administration of LDL in the rat [43]. Thus it should be expected that statin treatment will reduce ADMA level, at least in hypercholesterolemia. Surprisingly, almost all studies performed so far have demonstrated that this is not the case. In a recent randomized double-blind placebo-controlled study neither simvastatin nor atorvastatin administered for 8 weeks at relatively high doses (80 and 40 mg/day, respectively) had any effect on plasma ADMA, SDMA and L-NMMA in middle-aged patients with normal to moderately elevated LDL cholesterol [51,52]. Eid et al. [53] studied the effect of 8-week pravastatin treatment at a dose of 40 mg/day on methylarginines in 32 hypercholesterolemic nondiabetic men without ischemic heart disease. Although initial ADMA was about 60% higher in hyperlipidemic than in control group and pravastatin markedly reduced LDL-cholesterol, average ADMA level decreased after treatment only by 9%, which was not statistically significant. In another study, simvastatin administered for 2 months at 20 mg/day had no effect on ADMA and SDMA in 25 asymptomatic

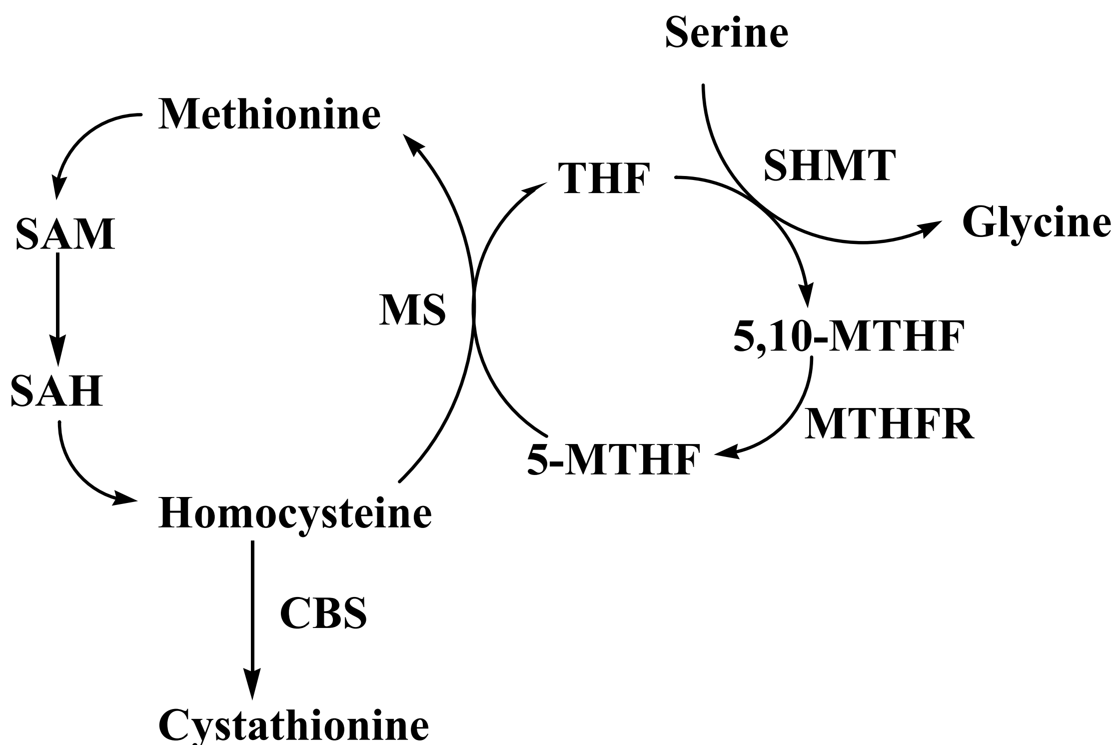

FIGURE 3. HOMOCYSTEINE METABOLISM. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine: MS, methionine synthase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; 5-MTHF, 5-methyl-tetrahydrofolate; SHMT, serine hydroxymethyltransferase; MTHFR, methylenetetrahydrofolate reductase.

hypercholesterolemic patients [54]. Animal studies are consistent with data obtained in humans. Lovastatin (10 mg/day for 12 weeks) failed to reduce ADMA or SDMA in rabbits made hyperlipidemic by high-cholesterol diet. In addition, lovastatin did not correct impaired endothelium-dependent vasorelaxation or deficiency of endogenous NO (measured as reduced renal excretion of NO metabolites and cyclic GMP) [55]. In summary, there is little doubt that statins have no effect on plasma ADMA, although to date no attempts have been made to explain this paradoxical finding. One possibility is that statins exert some beneficial effect on ADMA metabolism resulting from the correction of oxidative stress and lipid profile, but this is counteracted by the other mechanism(s) which acts in the opposite direction. To look for this mechanism, I focused on the relationship between ADMA and homocysteine.

5. HOMOCYSTEINE AND CARDIOVASCULAR DISEASES: IS ADMA A MISSING LINK?

Homocysteine (Hcy) is a non-protein sulfur containing aminoacid which is an intermediate product of methionine metabolism. When S-adenosylmethionine donates a methyl group for methyltransferase-catalyzed reactions (including methylation of protein arginine residues by PRMT), it is converted to S-adenosylhomocysteine (SAH) which is hydrolyzed to adenosine and Hcy by SAH hydrolase (FIG. 3). Homocysteine is metabolized by two alternative pathways. First, it may be remethylated to methionine by methionine synthase which uses 5-methyltetrahydrofolate and vitamin B_{12} as cofactors. Second, in the transsulfuration pathway Hcy condensates with serine to form cystathionine, the reaction catalyzed by cystathionine β-synthase (CBS). Cystathionine is

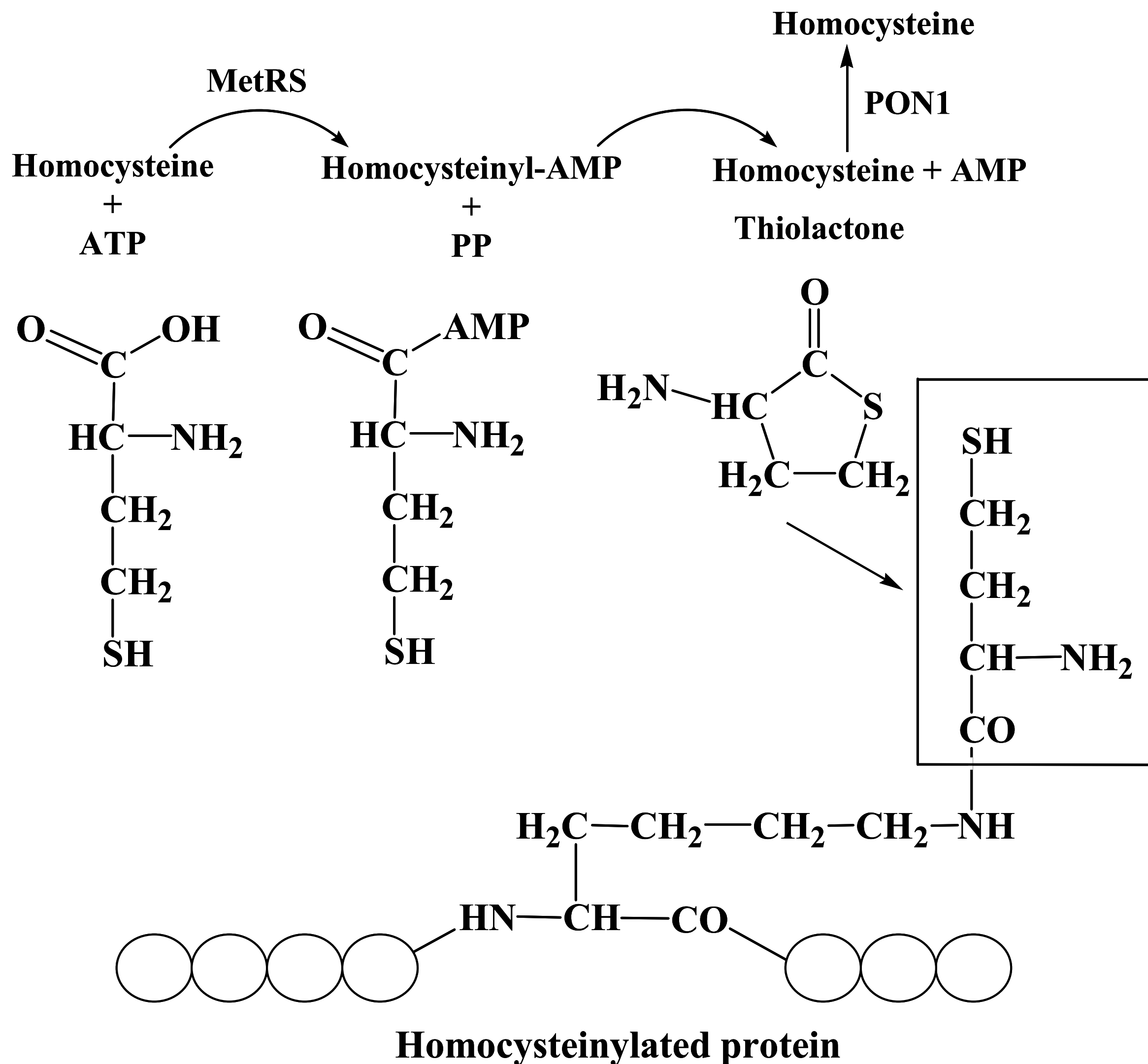

FIGURE 4. METABOLISM OF HOMOCYSTEINE THIOLACTONE AND PROTEIN N-HOMOCYSTEINYLATION. ATP, adenosine triphosphate; AMP, adenosine monophosphate; PP, pyrophosphate; MetRS, methionyl-tRNA synthetase; PON1, paraoxonase 1. On grey and blue backgrounds is indicated protein lysine residue and attached homocysteine, respectively.

then converted to cysteine and α-ketobutyrate by cystathionine γ-lyase (CSE). Both CBS and CSE use pyridoxal 5-phosphate (an active form of vitamin B_6) as a cofactor. Homocysteine is also extruded outside the cell and circulates in the blood at a concentration of ~10 μM. In plasma, about 2/3 of total Hcy is bound to protein cysteine residues by –SS- bridges and most of the remaining 1/3 circulates in oxidized form as low-molecular weight disulfides: homocystine (Hcy-SS-Hcy) or Hcy-SS-cysteine. Only about 1% of total Hcy circulates in free reduced form [56].

It was noted in 1960s that the risk of atherosclerosis is markedly increased in patients with homocystinuria — the inherited disease resulting from homozygous CBS deficiency and characterized by mental retardation, lens dislocation, hepatic steatosis and osteoporosis. In these patients plasma Hcy may be as high as 400 μM. Much more frequent (5-10% of the general

population) is mild (15-30 μM) to moderate (31-100 μM) hyperhomocysteinemia, which also increases the risk of atherosclerosis. The most common causes of hyperhomocysteinemia include heterozygous CBS deficiency, thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) resulting from C677T polymorphism of MTHFR gene, nutritional deficiency of vitamins involved in Hcy metabolism (folate, B_6 and B_{12}), and probably high methionine intake [57]. Although many studies have documented the link between elevated homocysteine and atherosclerosis, the mechanisms involved in proatherogenic effect of hyperhomocysteinemia remain elusive. In general, there are two groups of theories raised to explain it. Some of them attribute proatherogenic effect to Hcy itself and other to excess or deficiency of Hcy-related metabolites [56]. Many potentially atherogenic effects of Hcy have been described including toxicity to endothelial cells, stimulation of oxidative stress, scavenging nitric oxide, stimulation of inflammatory reaction, proliferation of vascular smooth muscle cells, and procoagulant effect. Nevertheless, most of these effects were observed at high supraphysiological Hcy concentrations and are unlikely to be relevant in vivo. In addition, these effects are usually not specific for Hcy and are shared by another sulfhydryl group-containing aminoacid, cysteine. Although cysteine circulates at the level much higher than Hcy, there is no evidence that it is atherogenic. In addition to exerting direct effects, excess Hcy may disturb the related metabolic pathways. For example, Hcy is converted to SAH which inhibits methyltransferases and causes hypomethylation of proteins or nucleic acids [58-60]. Abnormalities of transsulfuration pathway may lead to deficiency of glutathione, a cysteine containing intracellular antioxidant, or to decreased generation of hydrogen sulfide which is synthesized from cysteine by either CBS or CSE and is suggested to be involved in the regulation of vascular tone and inhibiting atherogenesis [61].

Recent studies suggest that homocysteine-induced atherogenesis may be mediated, at least in part, by ADMA. Both methionine and homocysteine increase ADMA elaboration by bovine aortic endothelial cells. The effect is accompanied by reduced DDAH activity despite unchanged protein expression. Hcy (but not methionine) also decreases the activity of recombinant DDAH [62]. Homocysteine reduced DDAH activity and increased ADMA also in cultured neurons [63]. Experimental hyperhomocysteinemia induced in cynomologus monkeys by folate- and vitamin B-deficient diet enriched in methionine (2.7-fold increase in plasma Hcy) was associated with 3-fold elevation of ADMA level [64]. In addition, very strong correlation between Hcy and ADMA (r = 0.8) was noted. In cultured human umbilical vein endothelial cells both methionine and homocysteine increase ADMA production [64]. Holven et al. [65] have observed a 2-fold increase in ADMA in humans with plasma homocysteine exceeding 15 μM in comparison to normohomocysteinemic control. This was accompanied by decreased concentration of NO metabolites. Treatment with folate normalized homocysteine level and reduced ADMA. In 27 patients with peripheral artery occlusive disease (PAOD), elevated homocysteine correlated with high plasma ADMA [66]. Oral methionine loading which transiently increases plasma homocysteine also elevated plasma ADMA in healthy persons as well as in patients with PAOD [67-69].

6. STATINS, HOMOCYSTEINE, PARAOXONASE, AND HOMOCYSTEINE THIOLACTONE

Most studies indicate that statins have no or only minor effect on plasma homocysteine. A recent metaanalysis [70] revealed that in 11 of 15 human studies statins did not change plasma homocysteine. In the remaining four studies including the largest one (2700 patients, 1-year lovastatin treatment) [71] statins have very weak (<10%) reducing effect on Hcy concentration. In any case, statins could not undesirably affect ADMA metabolism by modulating homocysteine level. However, in all these studies only total Hcy was measured, whereas plasma homocysteine is distributed in different fractions. One of these fractions, homocysteine thiolactone (HTL), has attracted much attention in recent years and, as discussed below, statins could modulate its level without affecting total Hcy.

Although Hcy is not translationally incorporated into proteins, it is nonspecifically activated by

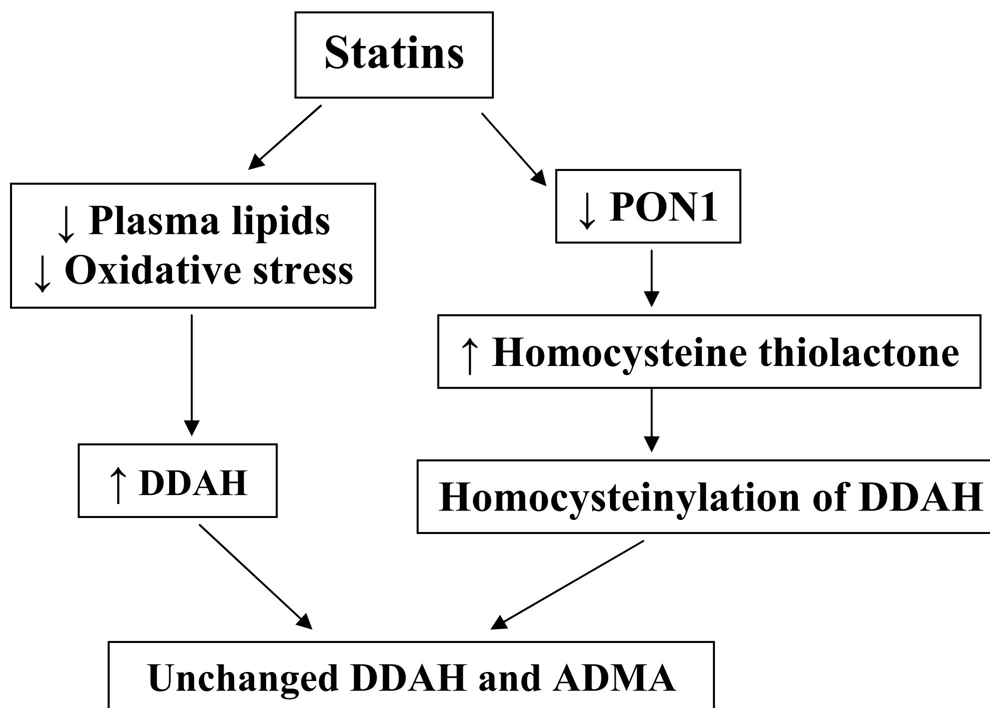

FIGURE 5. HYPOTHETICAL EFFECT OF STATINS ON ADMA METABOLISM IN HYPERLIPIDEMIC SUBJECTS. Statins have no net effect on ADMA because they trigger two opposite mechanisms: (1) normalization of the lipid profile and oxidant-antioxidant balance attenuates hyperlipidemia-induced downregulation of DDAH, (2) inhibition of PON1 increases homocysteine thiolactone level and induces homocysteinylation of DDAH, leading to reduction of its activity.

methionyl-tRNA synthetase which forms homocysteinyl-adenylate (FIG. 4). However, the latter is never transferred to tRNA but is edited to cyclic thioester, homocysteine thiolactone. This is the universal mechanism operating from bacteria to humans to prevent incorporation of Hcy into aminoacid chain [72]. Homocysteine thiolactone is a lipophilic compound which freely permeates plasma membranes and circulates in plasma at a concentration of 0.05-0.2 μM [73]. HTL is highly reactive and acylates free amine groups, especially ε-NH2 groups of protein lysine residues; the process referred to as protein homocysteinylation. In human serum, the highest amount (2.8 μM) of HTL is bound to albumin, which corresponds to homocysteinylation of about 0.36% of albumin molecules. Other proteins such as γ-globulins, transferrin, antithrombin-1, and the apolipoproteins of LDL and HDL are also homocysteinylated [74]. Considering the whole blood, the main reservoir of HTL is hemoglobin followed by albumin, with only <1% of HTL being bound to all other proteins. The susceptibility of a given protein to homocysteinylation depends on the amount of lysine residues and the molecular weight of a protein; large proteins are less prone to homocysteinylation because some lysine residues are not accessible to HTL. It should be noted that routine methods used to determine total plasma homocysteine measure only its free form as well as Hcy-protein, Hcy-Hcy and Hcy-cysteine disulfides; thus HTL contained in homocysteinylated proteins is not detected. Plasma concentration of hemoglobin-linked HTL is about 12 μM, i.e., more than "total" blood homocysteine. Homocysteinylated proteins (FIG. 4) have additional –SH groups which increase their negative charge and enable formation of intramolecular as well as intermolecular–SS- bridges, leading to changes in protein conformation and aggregation of molecules, respectively. Consequently, homocysteinylated

proteins have impaired biological activity. For example methionyl-tRNA synthetase is completely inactivated when 30% of its lysine residues are homocysteinylated whereas trypsin becomes inactive following homocysteinylation of 90% lysines [72]. HTL has been demonstrated to impair insulin signaling in vitro [75], and induces apoptosis of cultured cells at levels much lower than homocysteine [76, 77]. Both L- and D- isomers of homocysteine thiolactone are cytotoxic to chick embryo consistently with their identical chemical activity, whereas only L-homocysteine is cytotoxic, probably because D-isomer can not be converted to HTL by stereospecific enzymatic mechanism [78]. Because homocysteinylation is irreversible, it can especially modulate the properties of long-living proteins such as those of connective tissue. Interestingly, HTL inactivates lysine oxidase – a copper-containing amine oxidase which oxidizes peptidyl lysine allowing it to form covalent cross-linkages between and within molecules of collagen and elastin [79]. It has been suggested that homocysteinylation increases oxidizability of LDL, however, this has not been confirmed in a recent study [80]. Nevertheless, homocysteinylated LDL is taken up by macrophages faster than native lipoproteins leading to accumulation of cholesterol and formation of foam cells — a hallmark of early atherosclerotic lesions. In addition, homocysteinylated LDL induce oxidative stress in cultured endothelial cells [81]. Finally, homocysteinylated proteins may elicit the immune response. Injection of LDL containing homocysteinylated apolipoprotein B induces antibody formation in rabbits [82]. Antibodies recognizing homocysteinylated proteins are detected in human plasma and their level correlates with total homocysteine [83]. Antigen-antibody complexes may induce local inflammatory reaction in the vascular wall leading to plaque formation and/or destabilization, as previously demonstrated for antibodies directed to oxidized LDL. Indeed, plasma concentration of anti-homocysteinyl lysine antibodies is 50% higher in patients with cerebral stroke than in healthy subjects confirming their pathogenetic role in atherosclerosis [83].

Assuming that constant fraction of Hcy undergoes conversion to HTL, hyperhomocysteinemia should augment protein homocysteinylation. Indeed, CBS deficiency, folate or vitamin B_{12} deficiency, and antifolate drug aminopterin increase the amount of HTL formed by cultured endothelial cells [72,84]. The level of both free and protein-bound HTL in plasma correlates with Hcy concentration [73,74]. Uji et al. [85] have observed that in hemodialyzed patients plasma concentration of protein-bound HTL is increased proportionally to increase in total homocysteine. It is suggested that homocysteinylation may be the main mechanism of proatherogenic effect of homocysteine, since it occurs at physiological HTL concentrations and is specific for this aminoacid.

In plasma, homocysteine thiolactone is hydrolyzed to homocysteine by paraoxonase 1 (PON1) – a calcium-dependent esterase contained in high-density lipoproteins [86,87]. PON1 degrades organophosphate insecticides such as paraoxon, however, its physiological function is not entirely clear. It is suggested that PON1 is an antioxidant enzyme and inhibits atherogenesis by protecting plasma lipoproteins and plasma membranes from oxidative modification. PON1 hydrolyzes phospholipid hydroperoxides and cholesterol ester hydroperoxides as well as reduces hydroperoxides to the respective hydroxides [88–90]. Recent prospective study has confirmed that low PON1 activity is an independent predictor of acute coronary events [91]. Two closely related proteins, PON2 and PON3, have been identified. Although they lack paraoxon-hydrolyzing activity, both share with PON1 lactonase and antioxidant activities. Hydrolysis of homocysteine thiolactone may be a principal atheroprotective effect of paraoxonases. In vitro, PON1 protects proteins from homocysteinylation [74]. In addition, rabbits which have very high PON1 activity are resistant to homocysteine-induced atherosclerosis although they are very sensitive to hypercholesterolemia-induced atherosclerosis. Any conditions which decrease PON1 activity will elevate HTL level and augment protein homocysteinylation. Preliminary data suggest that plasma HTL-hydrolyzing activity is lower in patients with ischemic heart disease than in healthy controls [92]. Interestingly, PON1 itself may be homocysteinylated and this is associated with enzyme inactivation [93]. Thus, increased HTL level may initiate a vicious circle leading to

reduction of PON1 activity which causes further thiolactone accumulation.

The effect of statins on PON1 is controversial. Tomas et al. [94] and Deakin et al. [95] have demonstrated that simvastatin therapy results in increase in plasma PON1 activity in patients with hypercholesterolemia. In addition, atorvastatin increased PON2 activity in macrophages isolated from patients with hypercholesterolemia [96] (although it is not clear whether PON2 hydrolyzes HTL, this possibility is very likely because it hydrolyzes many synthetic lactones). However, hypercholesterolemia per se decreases PON1 and PON2 activities due to their inactivation by accompanying oxidative stress [96, 97], therefore, statins could modulate PONs in hypercholesterolemic subjects secondarily to improving their lipid profile. Thus, these studies could not answer whether statins affect PONs independently of correcting plasma lipoprotein metabolism. Interestingly, Tsimihodimos et al. [98] have reported that atorvastatin had no effect on plasma PON1 in patients with type IIA or type IIB hyperlipidemia despite normalizing their lipid profile. These data could suggest that at least some statins have direct negative impact on PON1, which opposes secondary desirable effect resulting from their hypolipidemic and antioxidant properties.

We investigated the effect of statin therapy on plasma and tissue PON1 activity in the rat. Although there are important differences in plasma lipoprotein metabolism between humans and rats, the main of them being low LDL level in the rat, this species is a good model to study "cholesterol-independent" effects of statins because statins have no effect on plasma cholesterol in the rat. We have demonstrated that cerivastatin decreased plasma PON1 activity toward paraoxon and phenyl acetate [99]. Moreover, we have shown that fluvastatin also decreased PON1 activity in plasma [100] as well as in selected tissues such as liver, myocardium, renal cortex and medulla [101]. Pravastatin had no effect on plasma PON1 activity toward paraoxon and phenyl acetate, however, tended to decrease its activity toward γ-decanolactone, as well as significantly reduced renal PON1 activity. These data suggest that, at least in the rat, reduction of PON1 activity is a class effect of statins, although different statins have different ability to affect this enzyme. These results were confirmed by Gouedard et al. [102] who have demonstrated that all tested statins decrease PON1 gene expression in cultured human hepatocytes. Thus, statins could undesirably modulate PON1 activity and thus impair its pleiotropic atheroprotective properties including PON1-dependent HTL hydrolysis.

7. CAN DDAH BE HOMOCYSTEINYLATED?

The mechanism through which homocysteine increases ADMA in vitro and in vivo is not clear. Increase in ADMA synthesis seems unlikely, because SDMA, another product of arginine methylation, is not changed in hyperhomocysteinemia [62,64]. In addition, homocysteine rather inhibits protein methylation by increasing intracellular SAH level [58,103]. Methionine loading could increase ADMA synthesis but it is unlikely to result in elevation of plasma ADMA within hours as observed in human studies [67-69], because more time would be needed for protein methylation, proteolysis, and accumulation of ADMA in extracellular fluid. Thus, it is more likely that homocysteine rather impairs ADMA metabolism. Homocysteine decreases DDAH activity in cultured endothelial cells by inducing oxidative stress, however, the effect is observed at supraphysiological Hcy concentration [62].

It is possible that hyperhomocysteinemia increases ADMA level by causing homocysteinylation of DDAH and thus decreasing its activity. In addition, statins could exert undesirable effect on ADMA by downregulating PON1, increasing HTL concentration, and augmenting DDAH homocysteinylation (FIG. 5). This mechanism could counteract the beneficial effect of statin treatment resulting from their hypolipidemic and antioxidant activity, leading to no net effect on ADMA if drugs are administered to hyperlipidemic humans. Until now it is not known whether intracellular proteins are homocysteinylated in vivo. However, they are readily homocysteinylated in vitro [72]. Intracellular HTL concentration may be higher than in plasma because HTL is formed inside the cells and PON1 activity in tissues is much lower than in plasma [101]. Human DDAH1, human DDAH2, and rat DDAH1 contain 19, 7, and 18 lysine residues,

respectively, so they are good potential substrates for homocysteinylation.

The scheme presented in FIG. 5 is only a working hypothesis and much research is needed to verify it. First, it should be tested whether purified or recombinant DDAH is homocysteinylated in vitro and if so, whether homocysteinylation modulates its activity. Second, it is unclear whether statins affect PON1's HTL-hydrolyzing activity, the level of free HTL in plasma and tissues, and the degree of protein homocysteinylation. Finally, the effect of statin treatment on DDAH activity and ADMA level should be studied under normolipidemic conditions, when the mechanism reducing ADMA level presented on the left side of FIG. 5 is unlikely to operate and the undesirable effect on ADMA metabolism could be unmasked.

Recently, we have obtained some preliminary results which seem to support this hypothesis. We have found that statins reduce HTL-hydrolyzing activity in plasma and tissues of normolipidemic rats. Moreover, DDAH activity is lower in statin-treated animals and this effect correlated with decrease in PON1 activity in a given tissue. We have also observed that preincubation of tissue homogenate obtained from control animals with HTL at concentrations as low as 1 μM results in decrease in DDAH activity. This effect inversely correlates with PON1 activity in a given tissue, is augmented by EDTA which inactivates PON1 by chelating calcium, and is attenuated by adding PON1-containing plasma or isolated HDL but not by plasma with PON1 inactivated by EDTA. Homocysteine at the same concentrations had no effect on DDAH activity. These data suggest that PON1-HTL pathway may at least partially contribute to undesirable effect of statins on ADMA metabolism.

One could suggest that even if this mechanism operates in normolipidemic rats, it is unlikely to affect ADMA metabolism in hyperlipidemic humans in whom statins increase PON1 activity and thus should improve HTL and ADMA metabolism. However, statins are now recommended also to normolipidemic humans, especially in secondary prevention. In addition, although statins increase plasma PON1 activity in subjects with hyperlipidemia, their effect on tissue PON1 is not clear. Tissue PON1 is unlikely to be decreased by hyperlipidemia because it is not subjected to oxidized plasma lipoproteins, thus, the effect of statins on tissue PON1 could be similar in normo- and hyperlipidemic conditions. PON1 activity in tissues seems to be more important than plasma enzyme for protecting intracellular proteins such as DDAH from homocysteinylation. Therefore, the proposed mechanism of DDAH dysregulation by statins could operate also under hyperlipidemic conditions.

Nevertheless, other explanations of "statin-ADMA" paradox should also be considered. For example, statins have been shown to impair insulin signaling both in vitro [104-106] and in vivo [107,108], and impaired insulin sensitivity is associated with increased ADMA level [109]. Second, statins have extremely pleiotropic activities including inhibition of protein isoprenylation. Although DDAH itself is not isoprenylated, its synthesis and/or activity may be regulated by isoprenylated proteins. It has been demonstrated that DDAH interacts with neurofibromin I which increases GTP-hydrolyzing activity of Ras proteins [110]. Ras proteins regulate cell proliferation and differentiation and to become active, they need to be farnesylated. Statins decrease isoprenylation of Ras by inhibiting synthesis of farnesylpyrophosphate, one of the products of mevalonate cascade. Interaction with DDAH increases phosphorylation of neurofibromin by protein kinase A, however, it is not clear whether this interaction has any effect on DDAH activity [110]. If this is a case, statins could modulate DDAH activity by affecting Ras-dependent signaling cascade. Third, C-terminal cysteine residue of isoprenylated proteins is subsequently methylated by SAM-dependent prenylcysteine methyltransferase [111]. It may be speculated that inhibition of isoprenylation pathway by statins increases tissue SAM pool and thus enhances other methylation reactions including methylarginine synthesis. Finally, studies addressing the effect of statins on ADMA performed to date could simply be too short to detect desirable changes resulting from the normalization of patients' lipid profile.

8. FIBRATES AND PON1-HOMOCYSTEINE-ADMA TRIANGLE

Fibrates are commonly used hypolipidemic drugs which reduce plasma triglycerides, increase HDL-cholesterol, and moderately decrease LDL-cholesterol. Their mechanism of action involves activation of peroxisome proliferator-activated receptor α (PPARα) – nuclear transcription factor which regulates the expression of genes encoding enzymes involved in lipoprotein metabolism. Upon activation, PPARα dimerizes with retinoid X receptor (RXR) and this complex binds to peroxisome proliferator response element (PPRE) in the promoter region of target genes.

While the relationship between statins and ADMA is poorly recognized, even less is known about the effect of fibrates. In the only study which addressed this issue [112], 6-week treatment with fenofibrate administered at a dose of 200 mg/day did not change plasma ADMA level in 25 hypertriglyceridemic men, despite reduction of plasma triglycerides. Since hypertriglyceridemia is associated with increased ADMA [46], neutral effect of fibrates is surprising. Moreover, PPRE has been identified in the promoter region of DDAH-2 gene and RXR agonist, all-trans retinoic acid, increases DDAH-2 expression in cultured endothelial cells [113]. Since RXR agonists often exert effects similar to PPAR agonists, fibrates could improve ADMA metabolism also by upregulating DDAH. Indeed, agonists of a related receptor, PPARγ, which also dimerizes with RXR and binds to PPRE, decrease ADMA level in humans [109].

What mechanism(s) could oppose these potentially beneficial effects of fibrates leading to no net changes in ADMA level? First, fibrates have been consistently demonstrated to markedly elevate plasma homocysteine both in animals and in humans [70,112,114,115]. Hcy could downregulate DDAH either directly or through enzyme homocysteinylation. The effect of fibrates on PON1 activity which could modulate HTL/Hcy ratio is not consistent and may be more complex than in the case of statins. In patients with type 2 diabetes and hypertriglyceridemia gemfibrozil increased plasma PON1 activity [116]. The similar was observed in patients with hypercholesterolemia and coronary heart disease following treatment with fenofibrate [117]. In contrast, ciprofibrate tended to reduce, although not significantly, plasma PON1 in patients with familial combined hyperlipidemia [118]. Tsimihodimos et al. [119] found no effect of fenofibrate on PON1 activity in patients with either hypercholesterolemia or hypertriglyceridemia. Also Durrington et al. [120] have reported that neither gemfibrozil nor bezafibrate affected PON1 activity in patients with hyperlipidemia. Since PON1 is reduced in hyperlipidemic patients, the fact that fibrates have little or no effect on its activity despite correcting lipid profile is surprising and suggests that these drugs may negatively affect PON1 in "lipid-independent" manner. The effect of fibrates in normolipidemic humans has not been studied because unlike statins, fibrates are not recommended to normolipidemic subjects. Therefore, studies in experimental animals or on cultured human cells are needed to clarify it. We have demonstrated that fenofibrate markedly decreases plasma PON1 activity in the rat [121]. However, the effect of fibrates on PON1 may be species specific. First, expression of PPARα is much higher in the rat than in humans, at least in the liver where plasma PON1 is synthesized. Second, PPARα has species-specific effect on apolipoprotein A-I expression (stimulation in humans and inhibition in rodents) [122], whereas this apolipoprotein facilitates PON1 transfer from hepatocytes to HDL and stabilizes HDL-bound enzyme [123,124]. Gouedard et al. [102] have recently demonstrated that fenofibric acid, the active derivative of fenofibrate, increases PON1 expression in cultured human hepatocytes but surprisingly, this effect seems to be PPARα-independent because overexpression of this receptor tended to downregulate the enzyme. These data suggest that: (i): PPARα may inhibit not only rodent but also human PON1 which may explain neutral effect of fibrates in some studies despite correction of patients' lipid profile, (ii) fibrates may regulate PON1 in PPARα-independent manner. To make things even more complex, fenofibrate in its ester form in which it is clinically used (but not as fenofibric acid which is the active PPARα agonist generated by hydrolysis of the parent drug in vivo) is a potent antagonist of oxysterol receptor, liver X receptor α and β [125]. Activation of LXR

stimulates PON1 synthesis in hepatocytes [102]. Other fibrates are used in carboxylic acid form, in which they do not bind to LXR. Thus, fenofibrate could have additional LXR-dependent mechanism of action, unique among fibrates. These considerations should be kept in mind when one aims to analyze fibrate-PON1 relationship.

In summary, currently available data suggest that fibrates have neutral effect on ADMA in patients with hyperlipidemia because their desirable mechanisms of action (correction of lipid profile and eventually direct upregulation of DDAH as well as of PON1) are opposed by increase in plasma homocysteine.

9. CONCLUSIONS AND FUTURE PERSPECTIVES

ADMA is increasingly recognized as a key regulator of nitric oxide production; therefore, the effect of drugs used in the treatment of cardiovascular diseases on its metabolism is of special interest. Although currently available data suggest that most widely used hypolipidemic drugs, i.e., statins and fibrates, have no effect on plasma ADMA in hyperlipidemic patients, several issues deserve further consideration. First, only few studies addressing the effect of statins or fibrates on ADMA metabolism have been performed. Second, these studies were relatively short-lasting and it cannot be excluded that prolonged treatment will substantially affect ADMA metabolism. Third, nothing is known about the effect of these drugs on intracellular ADMA concentration which is higher than in plasma and more important for the regulation of NOS activity. Fourth, it is not clear whether statins affect ADMA in normolipidemic subjects. One of the mechanism through which hypolipidemic drugs could regulate ADMA metabolism is their effect on PON1, homocysteine thiolactone, and homocysteinylation of DDAH. This possibility is suggested by three facts: (i) hyperhomocysteinemia is associated with increased ADMA level, (ii) homocysteinylation of proteins is probably the principal mechanism of unbeneficial effects of homocysteine, (iii) statins have an inhibitory effect on PON1, which hydrolyzes homocysteine thiolactone. This hypothesis requires careful experimental verification. In addition, other aspects of homocysteine-ADMA relationship need to be addressed. Is PRMT and the rate of ADMA production regulated by homocysteine thiolactone? Can hypolipidemic drugs affect homocysteine thiolactone through the mechanisms other than regulation of PON1 or total homocysteine level, e.g., by modulating HTL synthesis by methionyl-tRNA synthase? Is reduced PON1 activity causally linked to elevated ADMA through homocysteinylation-dependent mechanism in diseases in which both these effects coincide such as hyperlipidemia, diabetes and preeclampsia? These questions remain to be answered in future studies.

ACKNOWLEDGEMENTS

The author's original work quoted in this paper was supported by grant PW 443 from Medical University of Lublin, Poland.

REFERENCES

[1] ROSENSON RS [2001] Pluripotential mechanisms of cardioprotection with HMG-CoA reductase inhibitor therapy. Am J Cardiovasc Drugs 1: 411-420.

[2] ALCOCER L [2003] Statins for everybody? New evidence on the efficacy and safety of the inhibitors of HMG Co-A reductase. Am J Ther 10: 423-428.

[3] TSIARA S, ELISAF M AND MIKHAILIDIS DP [2003] Early vascular benefits of statin therapy. Curr Med Res Opin 19: 540-556.

[4] NAPOLI C AND SICA V [2004] Statin treatment and the natural history of atherosclerotic-related diseases: pathogenic mechanisms and the risk-benefit profile. Curr Pharm Des 10: 425-432.

[5] BÖGER RH, BODE-BÖGER SM, MUGGE A, KIENKE S, BRANDES R, DWENGER A AND FROLICH JC [1995] Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis 117: 273-284.

[6] BÖGER RH, BODE-BÖGER SM AND FROLICH JC [1996] The L-arginine-nitric oxide pathway: role in atherosclerosis and therapeutic implications. Atherosclerosis 127: 1-11.

[7] BULT H, HERMAN AG AND MATTHYS KE [1999] Antiatherosclerotic activity of drugs in relation to nitric oxide function. Eur J Pharmacol 375: 157-176.

[8] O'DRISCOLL G, GREEN D AND TAYLOR RR [1997] Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 95: 1126-1131.

[9] LAUFS U, LA FATA V, PLUTZKY J AND LIAO JK [1998] Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation 97: 1129-1135.

[10] KANO H, HAYASHI T, SUMI D, ESAKI T, ASAI Y, THAKUR NK, JAYACHANDRAN M AND IGUCHI A [1999] A HMG-CoA reductase inhibitor improved regression of atherosclerosis in the rabbit aorta without affecting serum lipid levels: possible relevance of up-regulation of endothelial NO synthase mRNA. Biochem Biophys Res Commun 259: 414-419.

[11] VALLANCE P, LEONE A, CALVER A, COLLIER J AND MONCADA S [1992] Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 339: 572-575.

[12] RAWAL N, RAJPUROHIT R, LISCHWE MA, WILLIAMS KR, PAIK WK AND KIM S [1995] Structural specificity of substrate for S-adenosylmethionine:protein arginine N-methyltransferases. Biochim Biophys Acta 1248: 11-18.

[13] VALLANCE P AND LEIPER J [2004] Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 24: 1023-1030.

[14] OGAWA T, KIMOTO M AND SASAOKA K [1989] Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. J Biol Chem 264: 10205-10209.

[15] LEIPER JM, SANTA MARIA J, CHUBB A, MACALLISTER RJ, CHARLES IG, WHITLEY GS AND VALLANCE P [1999] Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem J 343: 209-214.

[16] TOJO A, WELCH WJ, BREMER V, KIMOTO M, KIMURA K, OMATA M, OGAWA T, VALLANCE P AND WILCOX CS [1997] Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. Kidney Int 52: 1593-1601.

[17] TRAN CT, FOX MF, VALLANCE P AND LEIPER JM [2000] Chromosomal localization, gene structure, and expression pattern of DDAH1: comparison with DDAH2 and implications for evolutionary origins. Genomics 68: 101-105.

[18] MACALLISTER RJ, PARRY H, KIMOTO M, OGAWA T, RUSSELL RJ, HODSON H, WHITLEY GS AND VALLANCE P [1996] Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. Br J Pharmacol 119: 1533-1540.

[19] SMITH CL, BIRDSEY GM, ANTHONY S, ARRIGONI FI, LEIPER JM AND VALLANCE P [2003] Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. Biochem Biophys Res Commun 308: 984-989.

[20] DAYOUB H, ACHAN V, ADIMOOLAM S, JACOBI J, STUEHLINGER MC, WANG BY, TSAO PS, KIMOTO M, VALLANCE P, PATTERSON AJ AND COOKE JP [2003] Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. Circulation 108: 3042-3047.

[21] BÖGER RH [2003] The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovasc Res 59: 824-833.

[22] TSIKAS D, BÖGER RH, SANDMANN J, BODE-BÖGER SM AND FROLICH JC [2000] Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. FEBS Lett 478: 1-3.

[23] BÖGER RH, BODE-BÖGER SM, TSAO PS, LIN PS, CHAN JR AND COOKE JP [2000] An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. J Am Coll Cardiol 36: 2287-2295.

[24] SYDOW K AND MUNZEL T [2003] ADMA and oxidative stress. Atheroscler Suppl 4: 41-51.

[25] BOGLE RG, MACALLISTER RJ, WHITLEY GS AND VALLANCE P [1995] Induction of NG-monomethyl-L-arginine uptake: A mechanism for differential inhibition of NO synthases? Am J Physiol 269: C750-C756.

[26] SEGARRA G, MEDINA P, VILA JM, CHUAN P, DOMENECH C, TORONDEL B AND LLUCH A [2001] Inhibition of nitric oxide activity by arginine analogs in human renal arteries. Am J Hypertens 14: 1142-1148.

[27] KIELSTEIN JT, IMPRAIM B, SIMMEL S, BODE-BÖGER SM, TSIKAS D, FROLICH JC, HOEPER MM, HALLER H AND FLISER D [2004] Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. Circulation 109: 172-177.

[28] KUROSE I, WOLF R, GRISHAM MB AND GRANGER DN [1995] Effects of an endogenous inhibitor of nitric oxide synthesis on postcapillary venules. Am J Physiol 268: H2224-H2231.

[29] SMIRNOVA I, SAWAMURA T AND GOLIGORSKY M [2004] Upregulation of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) in endothelial cells by nitric oxide deficiency. Am J Physiol Renal Physiol 287: F25-F32.

[30] KIELSTEIN JT, SIMMEL S, BODE-BOGER SM, ROTH HJ, SCHMIDT-GAYK H, HALLER H AND FLISER D [2004] Subpressor Dose Asymmetric Dimethylarginine Modulates Renal Function in Humans through Nitric Oxide Synthase Inhibition. Kidney Blood Press Res 27: 143-147.

[31] BÖGER RH, BODE-BÖGER SM, THIELE W, JUNKER W, ALEXANDER K AND FROLICH JC [1997] Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. Circulation 95: 2068-2074.

[32] MIYAZAKI H, MATSUOKA H, COOKE JP, USUI M, UEDA S, OKUDA S AND IMAIZUMI T [1999] Endogenous nitric oxide synthase inhibitor: A novel marker of atherosclerosis. Circulation 99: 1141-1146.

[33] ZOCCALI C, BODE-BÖGER S, MALLAMACI F, BENEDETTO F, TRIPEPI G, MALATINO L, CATALIOTTI A, BELLANUOVA

I, FERMO I, FROLICH J AND BÖGER R [2001] Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. Lancet 358: 2113-2117.

[34] ZOCCALI C, BENEDETTO FA, MAAS R, MALLAMACI F, TRIPEPI G, MALATINO LS AND BÖGER R; CREED INVESTIGATORS [2002] Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol 13: 490-496.

[35] VALKONEN VP, PAIVA H, SALONEN JT, LAKKA TA, LEHTIMAKI T, LAAKSO J AND LAAKSONEN R [2001] Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 358: 2127-2128.

[36] BÖGER RH, SYDOW K, BORLAK J, THUM T, LENZEN H, SCHUBERT B, TSIKAS D AND BODE-BÖGER SM [2000] LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: Involvement of S-adenosylmethionine-dependent methyltransferases. Circ Res 87: 99-105.

[37] ITO A, TSAO PS, ADIMOOLAM S, KIMOTO M, OGAWA T AND COOKE JP [1999] Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 99: 3092-3095.

[38] LEIPER J, MURRAY-RUST J, MCDONALD N AND VALLANCE P [2002] S-Nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. Proc Natl Acad Sci U S A 99: 13527-13532.

[39] KNIPP M, BRAUN O, GEHRIG PM, SACK R AND VASAK M [2003] Zn(II)-free dimethylargininase-1 (DDAH-1) is inhibited upon specific Cys-S-nitrosylation. J Biol Chem 278: 3410-3416.

[40] KNIPP M, CHARNOCK JM, GARNER CD AND VASAK M [2001] Structural and functional characterization of the Zn(II) site in dimethylargininase-1 (DDAH-1) from bovine brain.

[41] YU XJ, LI YJ AND XIONG Y [1994] Increase of an endogenous inhibitor of nitric oxide synthesis in serum of high cholesterol fed rabbits. Life Sci 54: 753-758.

[42] BODE-BÖGER SM, BÖGER RH, KIENKE S, JUNKER W AND FROLICH JC [1996] Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Commun 219: 598-603.

[43] JIANG JL, LI NS NS, LI YJ AND DENG HW [2002] Probucol preserves endothelial function by reduction of the endogenous nitric oxide synthase inhibitor level. Br J Pharmacol 135: 1175-1182.

[44] JANG JJ, HO HK, KWAN HH, FAJARDO LF AND COOKE JP [2000] Angiogenesis is impaired by hypercholesterolemia: Role of asymmetric dimethylarginine. Circulation 102: 1414-1419.

[45] BÖGER RH, BODE-BÖGER SM, SZUBA A, TSAO PS, CHAN JR, TANGPHAO O, BLASCHKE TF AND COOKE JP [1998] Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 98: 1842-1847.

[46] LUNDMAN P, ERIKSSON MJ, STÜHLINGER M, COOKE JP, HAMSTEN A AND TORNVALL P [2001] Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. J Am Coll Cardiol 38: 111-116.

[47] FARD A, TUCK CH, DONIS JA, SCIACCA R, DI TULLIO MR, WU HD, BRYANT TA, CHEN NT, TORRES-TAMAYO M, RAMASAMY R, BERGLUND L, GINSBERG HN, HOMMA S AND CANNON PJ [2000] Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 20: 2039-2044.

[48] CHAN JR, BÖGER RH, BODE-BÖGER SM, TANGPHAO O, TSAO PS, BLASCHKE TF AND COOKE JP [2000] Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. Arterioscler Thromb Vasc Biol 20: 1040-1046.

[49] PAIVA H, LAAKSO J, LAINE H, LAAKSONEN R, KNUUTI J AND RAITAKARI OT [2002] Plasma asymmetric dimethylarginine and hyperemic myocardial blood flow in young subjects with borderline hypertension or familial hypercholesterolemia. J Am Coll Cardiol 40: 1241-1247.

[50] NORATA GD, PIRILLO A AND CATAPANO AL [2003] Statins and oxidative stress during atherogenesis. J Cardiovasc Risk 10: 181-189.

[51] PAIVA H, LAAKSO J, LEHTIMAKI T, ISOMUSTAJARVI M, RUOKONEN I AND LAAKSONEN R [2003] Effect of high-dose statin treatment on plasma concentrations of endogenous nitric oxide synthase inhibitors. J Cardiovasc Pharmacol 41: 219-222.

[52] VALKONEN VP, LAAKSO J, PAIVA H, LEHTIMAKI T, LAKKA TA, ISOMUSTAJARVI M, RUOKONEN I, SALONEN JT AND LAAKSONEN R [2003] Asymmetrical dimethylarginine (ADMA) and risk of acute coronary events. Does statin treatment influence plasma ADMA levels? Atheroscler Suppl 4: 19-22.

[53] EID HM, ERITSLAND J, LARSEN J, ARNESEN H AND SELJEFLOT I [2003] Increased levels of asymmetric dimethylarginine in populations at risk for atherosclerotic disease. Effects of pravastatin. Atherosclerosis 166: 279-284.

[54] PEREIRA EC, BERTOLAMI MC, FALUDI AA, SALEM M, BERSCH D AND ABDALLA DS [2003] Effects of simvastatin and L-arginine on vasodilation, nitric oxide metabolites and endogenous NOS inhibitors in hypercholesterolemic subjects. Free Radic Res 37: 529-536.

[55] BÖGER RH, BODE-BÖGER SM, BRANDES RP, PHIVTHONG-NGAM L, BOHME M, NAFE R, MUGGE A AND FROLICH JC [1997] Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation 96: 1282-1290.

[56] MEDINA M, URDIALES JL AND AMORES-SANCHEZ MI [2001] Roles of homocysteine in cell metabolism: old and new functions. Eur J Biochem 268: 3871-3882.

[57] LAWRENCE DE KONING AB, WERSTUCK GH, ZHOU J AND AUSTIN RC [2003] Hyperhomocysteinemia and its role in the development of atherosclerosis. Clin Biochem 36: 431-441.

[58] WANG H, YOSHIZUMI M, LAI K, TSAI JC, PERRELLA MA, HABER E AND LEE ME [1997] Inhibition of growth and p21ras methylation in vascular endothelial cells by homocysteine but not cysteine. J Biol Chem 272: 25380-25385.

[59] LEE ME AND WANG H [1999] Homocysteine and hypomethylation. A novel link to vascular disease. Trends Cardiovasc Med 9: 49-54.

[60] JAMES SJ, MELNYK S, POGRIBNA M, POGRIBNY IP AND CAUDILL MA [2002] Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J Nutr 132: 2361S-2366S.

[61] WANG R [2002] Two's company, three's a crowd: Can H2S be the third endogenous gaseous transmitter? FASEB J 16: 1792-1798.

[62] STÜHLINGER MC, TSAO PS, HER JH, KIMOTO M, BALINT RF AND COOKE JP [2001] Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 104: 2569-2575.

[63] SELLEY ML [2004] Homocysteine increases the production of asymmetric dimethylarginine in cultured neurons. J Neurosci Res 77: 90-93.

[64] BÖGER RH, BODE-BÖGER SM, SYDOW K, HEISTAD DD AND LENTZ SR [2000] Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia. Arterioscler Thromb Vasc Biol 20: 1557-1564.

[65] HOLVEN KB, HAUGSTAD TS, HOLM T, AUKRUST P, OSE L AND NENSETER MS [2003] Folic acid treatment reduces elevated plasma levels of asymmetric dimethylarginine in hyperhomocysteinaemic subjects. Br J Nutr 89: 359-363.

[66] SYDOW K, SCHWEDHELM E, ARAKAWA N, BODE-BÖGER SM, TSIKAS D, HORNIG B, FROLICH JC AND BÖGER RH [2003] ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins. Cardiovasc Res 57: 244-252.

[67] BÖGER RH, LENTZ SR, BODE-BÖGER SM, KNAPP HR AND HAYNES WG [2001] Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inaemia in humans. Clin Sci (Lond) 100: 161-167.

[68] STÜHLINGER MC, OKA RK, GRAF EE, SCHMOLZER I, UPSON BM, KAPOOR O, SZUBA A, MALINOW MR, WASCHER TC, PACHINGER O AND COOKE JP [2003] Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. Circulation 108: 933-938.

[69] WANBY P, BRATTSTROM L, BRUDIN L, HULTBERG B AND TEERLINK T [2003] Asymmetric dimethylarginine and total homocysteine in plasma after oral methionine loading. Scand J Clin Lab Invest 63: 347-353.

[70] JANKOWSKI P AND KAWECKA-JASZCZ K [2004] Lipid-lowering drugs and homocysteine. A comparison between statins and other lipid-lowering drugs. Atherosclerosis 172: 191-4.

[71] RIDKER PM, SHIH J, COOK TJ, CLEARFIELD M, DOWNS JR, PRADHAN AD, WEIS SE, GOTTO AM JR AND Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) Investigators [2002] Plasma homocysteine concentration, statin therapy, and the risk of first acute coronary events. Circulation 105: 1776-1779.

[72] JAKUBOWSKI H [1999] Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. FASEB J 13: 2277-2283.

[73] JAKUBOWSKI H [2002] The determination of homocysteine-thiolactone in biological samples. Anal Biochem 308: 112-119.

[74] JAKUBOWSKI H [2002] Homocysteine is a protein amino acid in humans. Implications for homocysteine-linked disease. J Biol Chem 277: 30425-30428.

[75] NAJIB S AND SANCHEZ-MARGALET V [2001] Homocysteine thiolactone inhibits insulin signaling, and glutathione has a protective effect. J Mol Endocrinol 27: 85-91.

[76] MERCIE P, GARNIER O, LASCOSTE L, RENARD M, CLOSSE C, DURRIEU F, MARIT G, BOISSEAU RM AND BELLOC F [2000] Homocysteine-thiolactone induces caspase-independent vascular endothelial cell death with apoptotic features. Apoptosis 5: 403-411.

[77] HUANG RF, HUANG SM, LIN BS, WEI JS AND LIU TZ [2001] Homocysteine thiolactone induces apoptotic DNA damage mediated by increased intracellular hydrogen peroxide and caspase 3 activation in HL-60 cells. Life Sci 68: 2799-2811.

[78] JAKUBOWSKI H [2001] Protein N-homocysteinylation: implications for atherosclerosis. Biomed Pharmacother 55:443-447.

[79] LIU G, NELLAIAPPAN K AND KAGAN HM [1997] Irreversible inhibition of lysyl oxidase by homocysteine thiolactone and its selenium and oxygen analogues. Implications for homocystinuria. J Biol Chem 272: 32370-32377.

[80] FERGUSON E, HOGG N, ANTHOLINE WE, JOSEPH J, SINGH RJ, PARTHASARATHY S AND KALYANARAMAN B [1999] Characterization of the adduct formed from the reaction between homocysteine thiolactone and low-density lipoprotein: antioxidant implications. Free Radic Biol Med 26: 968-977.

[81] FERRETTI G, BACCHETTI T, MORONI C, VIGNINI A, NANETTI L AND CURATOLA G [2004] Effect of homocysteinylation of low density lipoproteins on lipid peroxidation of human endothelial cells. J Cell Biochem 92: 351-360.

[82] FERGUSON E, PARTHASARATHY S, JOSEPH J AND KALYANARAMAN B [1998] Generation and initial characterization of a novel polyclonal antibody directed against homocysteine thiolactone-modified low density lipoprotein. J Lipid Res 39: 925-933.

[83] UNDAS A, PERLA J, LACINSKI M, TRZECIAK W, KAZMIERSKI R AND JAKUBOWSKI H [2004] Autoantibodies against N-homocysteinylated proteins in humans: implications for atherosclerosis. Stroke 35: 1299-1304.

[84] JAKUBOWSKI H, ZHANG L, BARDEGUEZ A AND AVIV A [2000] Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. Circ Res 87: 45-51.

[85] UJI Y, MOTOMIYA Y, HANYU N, UKAJI F AND OKABE H [2002] Protein-bound homocystamide measured in human plasma by HPLC. Clin Chem 48: 941-944.

[86] JAKUBOWSKI H [2000] Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation. J Biol Chem 275: 3957-3962.

[87] JAKUBOWSKI H, AMBROSIUS WT AND PRATT JH [2001] Genetic determinants of homocysteine thiolactonase activity in humans: implications for atherosclerosis. FEBS Lett 491: 35-39.

[88] DURRINGTON PN, MACKNESS B AND MACKNESS MI [2001] Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 21: 473-480.

[89] DRAGANOV DI AND LA DU BN [2004] Genetics of paraoxonases: a brief review. Naunyn Schmiedeberg's Arch Pharmacol 369: 78-88.

[90] MACKNESS MI, MACKNESS B AND DURRINGTON PN [2002] Paraoxonase and coronary heart disease. Atheroscler Suppl 3: 49-55.

[91] MACKNESS B, DURRINGTON P, MCELDUFF P, YARNELL J, AZAM N, WATT M AND MACKNESS M [2003] Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation 107: 2775-2779.

[92] JAKUBOWSKI H [2004] Molecular basis of homocysteine toxicity in humans. Cell Mol Life Sci 61: 470-487.

[93] FERRETTI G, BACCHETTI T, MAROTTI E AND CURATOLA G [2003] Effect of homocysteinylation on human high-density lipoproteins: a correlation with paraoxonase activity. Metabolism 52: 146-151.

[94] TOMAS M, SENTI M, GARCIA-FARIA F, VILA J, TORRENTS A, COVAS M AND MARRUGAT J [2000] Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 20: 2113-2119.

[95] DEAKIN S, LEVIEV I, GUERNIER S AND JAMES RW [2003] Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. Arterioscler Thromb Vasc Biol 23: 2083-2089.

[96] ROSENBLAT M, HAYEK T, HUSSEIN K AND AVIRAM M [2004] Decreased macrophage paraoxonase 2 expression in patients with hypercholesterolemia is the result of their increased cellular cholesterol content: effect of atorvastatin therapy. Arterioscler Thromb Vasc Biol 24: 175-180.

[97] MACKNESS MI, HARTY D, BHATNAGAR D, WINOCOUR PH, ARROL S, ISHOLA M AND DURRINGTON PN [1991] Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis 86: 193-199.

[98] TSIMIHODIMOS V, KARABINA SA, TAMBAKI AP, BAIRAKTARI E, GOUDEVENOS JA, CHAPMAN MJ, ELISAF M AND TSELEPIS AD [2002] Atorvastatin preferentially reduces LDL-associated platelet-activating factor acetylhydrolase activity in dyslipidemias of type IIA and type IIB. Arterioscler Thromb Vasc Biol 22: 306-311.

[99] BEŁTOWSKI J, WÓJCICKA G, MYDLARCZYK M AND JAMROZ A [2002] Cerivastatin modulates plasma paraoxonase/arylesterase activity and oxidant-antioxidant balance in the rat. Pol J Pharmacol 54: 143-150.

[100] BEŁTOWSKI J, WÓJCICKA G AND JAMROZ A [2002] Differential effect of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on plasma paraoxonase 1 activity in the rat. Pol J Pharmacol 54: 661-671.

[101] BEŁTOWSKI J, WÓJCICKA G AND JAMROZ A [2004] Effect of 3-hydroxy-3-methylglutarylcoenzyme A reductase inhibitors (statins) on tissue paraoxonase 1 and plasma platelet activating factor acetylhydrolase activities. J Cardiovasc Pharmacol 43: 121-127.

[102] GOUEDARD C, KOUM-BESSON N, BAROUKI R AND MOREL Y [2003] Opposite regulation of the human paraoxonase-1 gene PON-1 by fenofibrate and statins. Mol Pharmacol 63: 945-956.

[103] LENTZ SR, RODIONOV RN AND DAYAL S [2003] Hyperhomocysteinemia, endothelial dysfunction, and cardiovascular risk: the potential role of ADMA. Atheroscler Suppl 4: 61-65.

[104] MARTINEZ-GONZALEZ J, VINALS M, VIDAL F, LLORENTE-CORTES V AND BADIMON L [1997] Mevalonate deprivation impairs IGF-I/insulin signaling

in human vascular smooth muscle cells. Atherosclerosis 135: 213-223.

[105] TOMIYAMA K, NISHIO E AND WATANABE Y [1999] Both wortmannin and simvastatin inhibit the adipogenesis in 3T3-L1 cells during the late phase of differentiation. Jpn J Pharmacol 80: 375-378.

[106] CHAMBERLAIN LH [2001] Inhibition of isoprenoid biosynthesis causes insulin resistance in 3T3-L1 adipocytes. FEBS Lett 507: 357-361.

[107] JULA A, MARNIEMI J, HUUPPONEN R, VIRTANEN A, RASTAS M AND RONNEMAA T [2002] Effects of diet and simvastatin on serum lipids, insulin, and antioxidants in hypercholesterolemic men: a randomized controlled trial. JAMA 287: 598-605.

[108] KANDA M, SATOH K AND ICHIHARA K [2003] Effects of atorvastatin and pravastatin on glucose tolerance in diabetic rats mildly induced by streptozotocin. Biol Pharm Bull 26: 1681-1684.

[109] STÜHLINGER MC, ABBASI F, CHU JW, LAMENDOLA C, MCLAUGHLIN TL, COOKE JP, REAVEN GM AND TSAO PS [2002] Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. JAMA 287: 1420-1426.

[110] TOKUO H, YUNOUE S, FENG L, KIMOTO M, TSUJI H, ONO T, SAYA H AND ARAKI N [2001] Phosphorylation of neurofibromin by cAMP-dependent protein kinase is regulated via a cellular association of N(G),N(G)-dimethylarginine dimethylaminohydrolase. FEBS Lett 494: 48-53.

[111] ROSKOSKI R JR [2003] Protein prenylation: a pivotal posttranslational process. Biochem Biophys Res Commun 303:1-7.

[112] DIERKES J, WESTPHAL S, MARTENS-LOBENHOFFER J, LULEY C AND BODE-BÖGER SM [2004] Fenofibrate increases the L-arginine:ADMA ratio by increase of L-arginine concentration but has no effect on ADMA concentration. Atherosclerosis 173: 239-244.

[113] ACHAN V, TRAN CT, ARRIGONI F, WHITLEY GS, LEIPER JM AND VALLANCE P [2002] All-trans-Retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylarginine dimethylaminohydrolase. Circ Res 90: 764-769.

[114] Luc G, Jacob N, Bouly M, Fruchart JC, Staels B and Giral P [2004] Fenofibrate increases homocystinemia through a PPARα-mediated mechanism. J Cardiovasc Pharmacol 43: 452-453.

[115] LEGENDRE C, CAUSSE E, CHAPUT E, SALVAYRE R, PINEAU T AND EDGAR AD [2002] Fenofibrate induces a selective increase of protein-bound homocysteine in rodents: a PPARα-mediated effect. Biochem Biophys Res Commun 295: 1052-1056.

[116] BALOGH Z, SERES I, HARANGI M, KOVACS P, KAKUK G AND PARAGH G [2001] Gemfibrozil increases paraoxonase activity in type 2 diabetic patients. A new hypothesis of the beneficial action of fibrates? Diabetes Metab 27: 604-610.

[117] PARAGH G, SERES I, HARANGI M, BALOGH Z, ILLYES L, BODA J, SZILVASSY Z AND KOVACS P [2003] The effect of micronised fenofibrate on paraoxonase activity in patients with coronary heart disease. Diabetes Metab 29: 613-618.

[118] TURAY J, GRNIAKOVA V AND VALKA J [2000] Changes in paraoxonase and apolipoprotein A-I, B, C-III and E in subjects with combined familiar hyperlipoproteinemia treated with ciprofibrate. Drugs Exp Clin Res 26: 83-88.

[119] TSIMIHODIMOS V, KAKAFIKA A, TAMBAKI AP, BAIRAKTARI E, CHAPMAN MJ, ELISAF M AND TSELEPIS AD [2003] Fenofibrate induces HDL-associated PAF-AH but attenuates enzyme activity associated with apoB-containing lipoproteins. J Lipid Res 44: 927-934.

[120] DURRINGTON PN, MACKNESS MI, BHATNAGAR D, JULIER K, PRAIS H, ARROL S, MORGAN J AND WOOD GN [1998] Effects of two different fibric acid derivatives on lipoproteins, cholesteryl ester transfer, fibrinogen, plasminogen activator inhibitor and paraoxonase activity in type IIb hyperlipoproteinaemia. Atherosclerosis 138: 217-225.

[121] BEŁTOWSKI J, WÓJCICKA G, MYDLARCZYK M AND JAMROZ A [2002] The effect of peroxisome proliferator-activated receptors α (PPARα) agonist, fenofibrate, on lipid peroxidation, total antioxidant capacity, and plasma paraoxonase 1 (PON 1) activity. J Physiol Pharmacol 53: 463-475.

[122] VU-DAC N, CHOPIN-DELANNOY S, GERVOIS P, BONNELYE E, MARTIN G, FRUCHART JC, LAUDET V AND STAELS B [1998] The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erb α mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. J Biol Chem 273: 25713-25720.

[123] SÖRENSON RC, BISGAIER CL, AVIRAM M, HSU C, BILLECKE S AND LA DU BN [1999] Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids : apolipoprotein A-I stabilizes activity. Arterioscler Thromb Vasc Biol 19: 2214-2225.

[124] DEAKIN S, LEVIEV I, GOMARASCHI M, CALABRESI L, FRANCESCHINI G AND JAMES RW [2002] Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. J Biol Chem 277: 4301-4308.

[125] THOMAS J, BRAMLETT KS, MONTROSE C, FOXWORTHY P, EACHO PI, MCCANN D, CAO G, KIEFER A, MCCOWAN J, YU KL, GRESE T, CHIN WW, BURRIS TP AND MICHAEL LF [2003] A chemical switch regulates fibrate specificity for peroxisome proliferator-activated receptor α (PPARα) versus liver X receptor. J Biol Chem 278: 2403-2410.

ABBREVIATIONS USED:

ADMA, asymmetric dimethylarginine;
DDAH, dimethylarginine dimethylaminohydrolase;
HTL, homocysteine thiolactone;
PON1, paraoxonase 1;
HMG-CoA, 3-hydroxy 3-methylglutarylcoenzyme A;
LDL, low density lipoprotein
oxLDL, oxidized low density lipoprotein;
HDL, high-density lipoproteins;
NO, nitric oxide;
eNOS, endothelial nitric oxide synthase;
ROS, reactive oxygen species;
SDMA, symmetric dimethylarginine;
L-NMMA, N-monomethyl-L-arginine;
PRMT, protein methylases;
MCP-1, monocyte chemoattractant protein-1;
Hcy, homocysteine;
SAH, S-adenosylhomocysteine;
CBS, cystathionine β-synthase;
CSE, cystathionine γ-lyase;
MTHFR, methylenetetrahydrofolate reductase;
PAOD, peripheral artery occlusive disease;
PPARα, peroxisome proliferator-activated receptor α;
RXR, retinoid X receptor;
PPRE, peroxisome proliferator response element.

RECEIVED 6-18-2004.
ACCEPTED 6-27-2004.