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Reviews

Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome?

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Abstract

Endothelium-derived nitric oxide plays a major role in the regulation of vascular tone and in the maintenance of vascular homeostasis. Endothelial dysfunction with impaired nitric oxide biosynthesis and decreased bioavailability has been implicated in insulin resistance syndrome and Type II (non-insulin-dependent) diabetes mellitus. Nitric oxide is synthesised by nitric oxide synthase. Asymmetric dimethylarginine is a major endogenous nitric oxide synthase inhibitor. Increased circulating asymmetric dimethylarginine was initially found in patients with chronic renal failure and subsequently many other disease states. Increased asymmetric dimethylarginine plasma concentrations could contribute to the development of insulin resistance and coronary heart disease. Understanding of the pathophysiological role of asymmetric dimethylarginine could lead to novel therapies in the prevention of arteriosclerosis and coronary heart disease. [Diabetologia (2002) 45:1609–1616]

Keywords Endothelium, nitric oxide, asymmetric dimethylarginine, dimethylarginine dimethylaminohydrolase, insulin resistance syndrome.

Introduction

The insulin resistance syndrome, or metabolic syndrome X, consists of a cluster of closely linked risk factors which accelerate atherogenesis. These associated risk factors include hyperinsulinaemia, hypertriglyceridaemia, increased low-density lipoprotein (LDL) cholesterol, decreased high-density lipoprotein (HDL) cholesterol, hyperuricaemia, hyperhomocysteinaemia, hypertension and Type II (non-insulin-dependent) diabetes mellitus [1]. There is growing evidence that endothelial dysfunction is also a component of the insulin resistance syndrome and Type II diabetes [2, 3]. Furthermore, defective endothelial function, manifested as impaired endothelium-dependent vasodilatation, has been shown to be independent of obesity [4] and is now widely regarded as an early event in atherogenesis. Recent clinical evidence suggests that dysfunction of the coronary endothelium strongly predicts the progression of long-term atherosclerotic disease [5], even in the absence of obstructive coronary lesions [6]. Hence endothelial dysfunction seems to be linked between insulin resistance and accelerated atherogenesis. The precise pathophysiological basis of defective endothelial function, however, remains to be elucidated.

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Abbreviations: ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; DDAH, dimethylarginine dimethylaminohydrolase; ESRD, end-stage renal disease; IMT, intima-media thickness*L*; -NMMA, N^G-monomethyl-L-arginine; NADPH, nicotinamide-adenine dinucleotide phosphate; NO, nitric oxide; PRMT I, protein arginine methyltransferase type I; PRMT II, protein arginine methyltransferase type II; PKC, protein kinase C; PPAR,; γ , peroxisome proliferator-activated receptor gamma; SDMA, symmetric dimethylarginine.

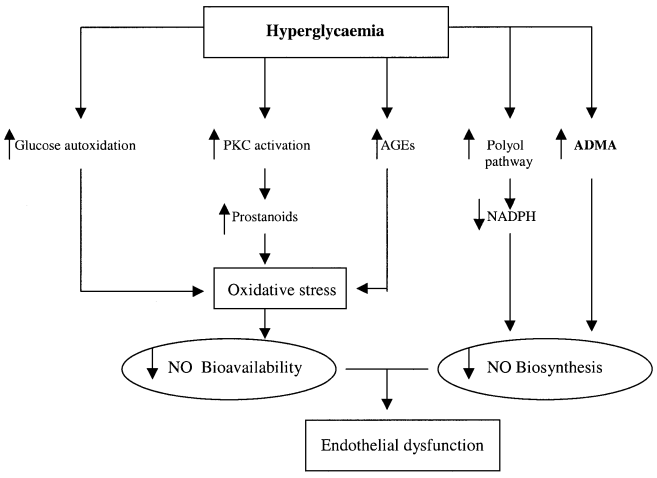

Fig. 1. Complex mechanisms through which hyperglycaemia causes endothelial dysfunction

Nitric oxide pathway, insulin resistance and hyperglycaemia

One of the most critical vasoactive mediators synthesized by the vascular endothelium is nitric oxide (NO), previously known as endothelium-derived relaxing factor [7]. Nitric oxide is synthesized from L-arginine by NO synthase. Endothelium-derived NO is a powerful endogenous vasodilator and also has important roles in the maintenance of vascular homeostasis. For instance, NO inhibits platelet aggregation, leucocyte migration and cellular adhesion to the endothelium, and attenuates vascular smooth muscle cell proliferation and migration. In addition, it inhibits activation and expression of adhesion molecules and the production of superoxide anions [8]. In Type I, Type II diabetes and insulin resistance syndrome, there is evidence that the release and/or bioavailability of NO are diminished [9, 10]. Despite the heterogeneous nature of these conditions, they all share the same feature of increased plasma glucose concentrations which could affect the L-arginine: NO pathway. Nitric oxide bioavailability can be reduced due to increased oxidative stress which can result from increased superoxide anions production from glucose autoxidation. Hyperglycaemia-induced activation of protein kinase C (PKC) followed by that of phospholipase A2, results in increased production of arachidonic acid metabolites which also have potent oxidizing effects. In contrast, reduced NO synthesis can result from activation of the polyol pathway which increases the utilization of nicotinamide-adenine dinucleotide phosphate (NADPH), an important cofactor in the biosynthesis of NO. Furthermore, accumulation of AGE due to non-enzymatic cross-linking of proteins, could quench NO, further reducing its bioavailability (Fig. 1). In addition to these mechanisms, the endogenous NO synthase inhibitor, asymmetric dimethylarginine (ADMA), has recently emerged as a key factor in determining NO biosynthesis. Understanding of the genetic and pathophysiological aspects of this molecule could lead to therapeutic advancement in reversing endothelial dysfunction and more importantly, prevention of diabetic vasculopathy given the unique metabolic milieu of diabetic patients.

Endogenous nitric oxide synthase inhibitor: ADMA

As early as the 1970s, it was recognised that methylated arginines are excreted in human urine [11]. Since then, methylated arginines have been detected in immune cells and neurons of animals [12, 13] and human endothelial cells [14, 15]. It is now clear that two types of endogenous NO synthase inhibitors exist in the human circulation, N^G-monomethyl-L-arginine (L-NMMA) and ADMA [16]. The concentration of ADMA is approximately tenfold higher than that of L-NMMA and is the major inhibitor of the NO biosynthesis in humans [17].

ADMA is derived from the catabolism of proteins containing methylated arginine residues and is released as the proteins are hydrolysed. These proteins are predominately found in the nucleus and are involved in RNA processing and transcriptional control [18]. The synthesis of ADMA (and L-NMMA) requires the enzyme protein arginine methyltransferase type I (PRMT I) which methylates arginine residues [19]. Protein arginine methyltransferase type II (PRMT II) forms symmetric dimethylarginine (SDMA). SDMA is a stereoisomer of ADMA and has no direct inhibitory effect on NO synthase. All three methylarginines (ADMA, SDMA and L-NMMA) enter endothelial cells through the cationic amino acid transporters known collectively as the y^+ transporter. The activity of this transporter was found to co-locate with caveolin-bound NO synthase which suggests that the y^+ transporter activity could be an important determinant of the local concentrations of methylarginines [20]. The three methylarginines compete with each other and with arginine for transport into the cell [21]. Hence high concentrations of ADMA could potentially interfere with intracellular transport of L-arginine resulting in a decrease in NO synthesis. The transport system concentrates methylarginines within the endothelial cells such that intracellular concentrations are greater than circulating concentrations. In this regard, a defective y^+ transporter system could result in a higher concentration of circulating ADMA leading to decreased NO biosynthesis. Hence the y^+ transporter system could be a potential site of defect in disease states.

Degradation of ADMA: role of DDAH

Although a proportion of ADMA is excreted in the urine and therefore accumulates in patients with renal failure [22, 23], its major catabolism is via the enzyme dimethylarginine dimethylaminohydrolase (DDAH) in

vivo [24]. The crystal structure of DDAH has been characterised [25]. Two isoforms of DDAH have been identified in every cell type examined: DDAH I is typically found in tissues expressing neuronal NOS, whereas DDAH II predominates in tissues containing the endothelial isoform of NOS [26]. There is a constant production of ADMA in the course of protein turnover. This is supported by the observation that addition of the DDAH inhibitor 4124 W to a vascular segment in vitro induces vasoconstriction which is reversed by adding L-arginine to the medium [24]. ADMA undergoes extensive metabolism by DDAH to citrulline and dimethylamine, such that only 5% of parenterally administered ADMA is recovered in the urine [23]. DDAH is specific for ADMA and has no effect on SDMA. This is supported by data showing that the use of 4124 W causes accumulation of ADMA in vitro whereas SDMA remains unaffected [24]. Given the central role of DDAH in the regulation of ADMA degradation, any disease that decreases DDAH activity would be expected to increase circulating ADMA concentrations. Increased ADMA in turn leads to diminished NO biosynthesis and endothelial dysfunction. Apart from renal impairment which will reduce ADMA clearance, hypercholesterolaemia could directly reduce DDAH activity resulting in ADMA accumulation. When cultured endothelial cells were exposed to oxidized LDL-cholesterol, ADMA accumulated in the medium at a faster rate than when cells were treated with vehicle or native LDL-cholesterol. This effect was accompanied by a temporally related decline in DDAH activity [27]. Similar observations were also made in the context of hyperglycaemia [17]. The vitamin A derivative, all-trans-retinoic acid has been shown to increase the expression of DDAH II, a likely mechanism through which all-trans-retinoic acid increases NO synthesis by endothelial cells (via reduction of ADMA) [28].

ADMA and endothelial dysfunction in disease states

The role of ADMA in endothelial dysfunction has been studied in several conditions with particular attention given to renal failure. Accumulation of ADMA in humans was first shown in chronic renal failure in the early 1990's [22] and was subsequently confirmed by others [29, 30, 31, 32]. Conversely, short-term reduction of circulating ADMA (amongst other toxins in renal failure) by haemodialysis was shown to improve flow-mediated dilatation (FMD) [31]. In addition, ADMA is a potential mediator of impaired FMD in experimental hyperhomocysteinaemia in humans [33]. It has been shown that by increasing plasma homocysteine concentrations using methionine infusion, FMD is impaired and this is associated with increased plasma ADMA [33]. Increased plasma concentrations of ADMA have also been described in a number of other conditions (Tables 1, 2) including diabetes mellitus, hypercholesterolaemia, hypertriglyceridaemia, hypertension, pre-eclampsia, stroke, peripheral vascular disease, congestive heart failure and acute coronary events [34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54]. Of interest, increased ADMA concentrations have also been implicated in the pathogenesis of conditions not affecting the cardiovascular system such as schizophrenia although the exact mechanism has yet to be clarified [55].

Table 1. Conditions in which increased ADMA concentrations have been found in animals

Conditions	Authors
Hypertension	Matsuoka, et al. 1997 [34] Goonasekera, et al. 2000 [74]
Alloxan-induced hyperglycaemia	Masuda, et al. 1999 [41]
Hyperhomocysteinaemia	Boger, et al. 2000 [47] Stuhlinger, et al. 2001 [42]
Atherosclerosis	Boger, et al. 1997 [49] Boger, et al. 2000 [47]
Hypercholesterolaemia	Boger, et al. 1996 [50] Boger, et al. 2000 [47]
Ageing	Xiong, et al. 2001 [73]

Table 2. Conditions in which increased ADMA concentrations have been found in humans

Conditions	Authors [References]
Chronic renal failure	Vallance et al. 1992 [22] Marescau et al. 1997 [30] Zoccali et al. 2001 [32] Kielstein et al. 2002 [29]
Essential hypertension	Surdacki et al. 1999 [53]
Childhood hypertension	Goonasekera et al. 1997 [36] Goonasekera et al. 2000 [74]
Hypercholesterolaemia	Boger et al. 1998 [38]
Hypertriglyceridaemia	Lundman et al. 2001 [46]
Post-prandial dyslipidaemia	Fard et al. 2000 [40]
Hyperhomocysteinaemia	Boger et al. 2001 [43]
Atherosclerosis	Miyazaki et al. 1999 [35]
Pre-eclampsia	Pattersson et al. 1998 [39]
Type II diabetes mellitus	Ito et al. 1999 [44] Abbasi et al. 2001 [52]
Stroke	Yoo and Lee 2001 [37]
Peripheral vascular disease	Boger et al. 1997 [51]
Congestive heart failure	Usui et al. 1998 [45]
Congenital heart disease	Gorenflo et al. 2001 [75]
Pulmonary hypertension	Gorenflo et al. 2001 [75]
Thrombotic microangiopathy	Herlitz et al. 1997 [48]
Schizophrenia	Das et al. 1996 [55]

ADMA, insulin resistance and cardiovascular disease

Increased ADMA concentrations in plasma are associated with conditions closely related to the insulin re-

sistance syndrome (Table 2). These conditions include hypertriglyceridaemia, hyperglycaemia, hyperhomocysteinaemia and essential hypertension. Furthermore, uremia is strongly linked to insulin resistance. This has been attributed to post-receptor defects in insulin action in muscle, adipose tissue and liver [56]. Given the metabolic abnormalities of diabetic patients and their high risk for renal dysfunction, ADMA could represent an important linking factor for the impaired endothelium-dependent vasodilatation and increased cardiovascular risks in Type II diabetes, especially in the presence of impaired renal function. A cross-sectional study has shown that plasma concentrations of ADMA were positively correlated with insulin resistance independent of other risk factors [57]. In this study, insulin sensitivity was determined by insulin-mediated glucose disposal in non-diabetic, normotensive people. High ADMA concentrations were associated with high fasting triglyceride but not LDL-cholesterol concentrations. It has been shown that treatment with an insulin-sensitizing drug, rosiglitazone, enhanced insulin sensitivity and reduced plasma ADMA concentrations [57]. Although this study proves the direct therapeutic role of rosiglitazone in reducing plasma ADMA concentration, there is a clear association between insulin resistance and high circulating ADMA concentrations. It is now established that activation by thiazolidinediones of the peroxisome proliferator-activated receptor gamma (PPARγ) induces differentiation of preadipocytes into mature fat cells leading to increased non-esterified fatty acid (NEFA) uptake and decreases their plasma concentrations in circulating NEFA. This is often accompanied by a reduction in plasma glucose concentrations and oxidative stress. The ADMA lowering effect of thiazolidinedione [57] could be mediated through a reduction in oxidative stress, increased DDAH activity and/or hence increased ADMA degradation (Fig. 2).

Similarly, metformin has been shown to decrease circulating ADMA both as monotherapy and as add-on therapy to sulphonylurea in poorly controlled Type II diabetic patients [58]. Of note, treatment with both rosiglitazone and metformin results in favourable changes in other metabolic risk factors in addition to improving insulin resistance. The observed reduction in circulating ADMA could relate to the metabolic changes induced and be a direct consequence of improved insulin sensitivity.

The adverse effects of ADMA accumulation on endothelial cell function is well established and is associated with worsening of clinical endpoints. In the MONICA (monitoring of trends and determinants in cardiovascular disease) cohort, high concentrations of ADMA were associated with an increased risk of acute coronary events among non-smoking middle-aged men, especially those with previous coronary heart disease [54]. This finding provided evidence for

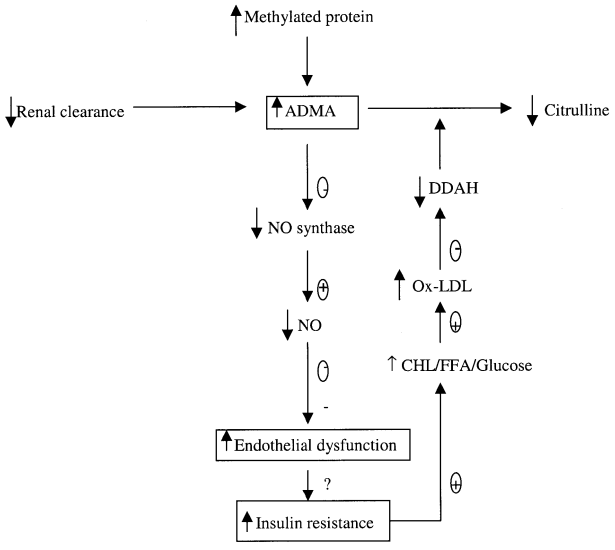

Fig. 2. The complex relations showing the metabolism of ADMA and its putative role in the pathogenesis of insulin resistance. Increased in serum NEFA, glucose and lipid concentrations in insulin resistance syndrome could increase oxidative stress leading to increased oxidized LDL formation which could suppress the activity of DDHA resulting in diminished ADMA degradation. The latter perpetuates the vicious cycle by reducing NO synthase leading to endothelial dysfunction which worsens insulin resistance

the possible aetiological role of ADMA through NO-mediated endothelial dysfunction in the development of coronary heart disease (Fig. 2). Albeit controversies continue with regard to the relation between insulin resistance and endothelial dysfunction, some reports show that insulin resistant subjects, notably those with Type II diabetes and obesity [59, 60], have impaired skeletal blood flow and reduced peripheral glucose uptake even despite high therapeutic insulin treatment. Since the acute vasodilating effects of insulin is primarily NO-mediated, such attenuated vascular response to insulin might be due to ADMA-mediated endothelial dysfunction.

ADMA, inflammation and renal diseases

It is widely accepted that inflammation plays an integral part in atherogenesis [61] and that the increase of inflammatory markers such as C-reactive proteins (CRP) predict future coronary events [62, 63]. At present, there is no proof of any association of ADMA and CRP levels in acute inflammation but since CRP is closely linked to insulin resistance [63], it is tempting to presume such association. This contention is supported by a recent prospective study in patients with end-stage renal disease (ESRD) on haemodialysis in whom changes in carotid intima-media thickness (IMT) were independently related to plasma ADMA concentrations and serum CRP [64]. In parallel, ADMA and CRP were strongly interrelated which

emerged as the sole independent predictor of intimal lesion progression [64]. These findings clearly need to be confirmed in other patient populations as patients with ESRD are not only a heterogeneous group but their underlying renal pathology could well contribute to high plasma CRP concentrations. Nevertheless, these complex inter-relations could exist between ADMA, inflammatory markers, endothelial function, insulin resistance, renal dysfunction and cardiovascular disease.

Therapeutic modulation of ADMA concentrations

Given the pivotal role of ADMA in determining NO bioavailability and the large array of conditions associated with high ADMA concentrations, pharmacological modulation of circulating ADMA could lead to novel therapies for preventing cardiovascular disease in insulin resistant or high risk subjects. Although there is as yet only limited research in this field, a pilot study carried out with incremental doses of rosiglitazone given to seven hyperactive insulin-resistant subjects resulted in a reduction in plasma ADMA concentrations over a 12-week treatment period and improved insulin sensitivity, whereas arterial blood pressure remained unchanged [57]. Although a definitive conclusion cannot be drawn from this study, it supports the hypothesis of a link between ADMA and insulin sensitivity. Similarly, metformin seems to have ADMA-lowering effect in Type II diabetic patients [58], but again it is not clear whether a reduction in ADMA concentration was due to direct metformin action or occurred in parallel with the improvement of other metabolic indices.

Reduction of plasma ADMA concentration has also been seen in response to probucol in rats [65]. In contrast, administration of L-arginine, the key substrate for NO synthase in the biosynthesis of NO, does not alter ADMA concentration but increased the L-arginine to ADMA ratio resulting in improved endothelium-dependent vasodilatation in animals and humans [38, 49, 50]. Of note, patients with unstable angina and reduced endothelium-dependent coronary vasodilatation had markedly improved anginal symptoms after 6 months of therapy with L-arginine (9 g/day) [66]. The same 3 days of oral supplementation with 6 g of L-arginine daily increase treadmill exercise capacity by 30% in patients with stable angina [67]. More recently, the increased risk for CHD in patients with impaired fasting glycaemia in the Cholesterol and Recurrent Events (CARE) trial was substantially reduced by statins [68]. Since statins have various pleiotrophic effects independent of their lipid-lowering effects, it was suggested that statins can also reduce plasma ADMA concentration and hence improve endothelial function [69]. This hypothesis is plausible because statins reduce CRP concentrations [62, 63, 70] and CRP has been shown to be closely related to ADMA concentrations [64]. In this regard, retrospective measurement of ADMA concentrations in various lipid-lowering trials could provide useful information. In the HOPE study, renal insufficiency, a condition in which plasma ADMA is known to be increased, is a predictor of cardiovascular outcomes and ramipril reduces cardiovascular events in both diabetic and non-diabetic subjects with mild renal insufficiency independent of its blood pressure-lowering effect [71]. In a recent small randomised study of hypertensive patients, perindopril and losartan monotherapy, but not bisoprolol have been shown to reduce circulating ADMA [72]. Taken together, these pilot studies suggest that future development of drugs with specific ADMA-lowering effects could prove more effective than conventional drugs in the treatment of specific conditions in which increased ADMA plays a crucial pathophysiological role.

Conclusions and future directions

ADMA is the major endogenous NOS inhibitor, and its accumulation has been associated with atherosclerosis and cardiovascular disease. The association between high ADMA concentrations with various components of the insulin resistance syndrome is particularly intriguing and it is plausible that ADMA may serve as a pivotal link between insulin resistance, intermediary metabolism (lipid and glucose), endothelial dysfunction and cardiovascular diseases. Several potential therapeutic drugs have shown early promise in reducing plasma ADMA concentrations. Whether lowering plasma ADMA concentrations could translate into a reduction in acute cardiovascular events remains to be proven in prospective studies. Additional work would be required to determine the precise mechanisms whereby specific conditions lead to its accumulation. Furthermore, animal studies utilizing DDAH knockout approach or identification of genetic variants of DDAH would provide additional insight into the genetic predisposition of individuals to premature cardiovascular diseases.

Sources. This review is based on the relevant literature published in the English language during the period 1966 to 2002. The sources available to the authors were integrated with sources identified through PubMed searches for "ADMA", "insulin resistance syndrome", and "endothelial dysfunction".

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