

## **ASIMETRIK DIMETHYLARGININE**

**Loo Hariyanto Raharjo**

**DEPARTEMEN BIOKIMIA,**

**DOSEN FAKULTAS KEDOKTERAN UNIVERSITAS WIJAYA KUSUMA**

**SURABAYA**

**E-MAIL : [loo\\_hiandao@yahoo.com](mailto:loo_hiandao@yahoo.com)**

### **ABSTRAK**

dimethylarginine asimetris (ADMA) adalah bahan kimia alami yang ditemukan dalam plasma darah. Ini adalah metabolisme oleh-produk terus-menerus proses modifikasi protein dalam sitoplasma semua sel manusia. Hal ini berkaitan erat dengan L-arginin, asam amino kondisional-penting. ADMA mengganggu dengan L-arginin dalam produksi oksida nitrat, sebuah bahan kimia kunci untuk kesehatan endotel dan karenanya kardiovaskular. konsentrasi ADMA meningkat pada pasien dengan penyakit ginjal stadium akhir (ESRD), sebagian karena diekskresikan melalui ginjal.

**KATA-KATA KUNCI:** asimetrik dimethylarginine (ADMA), oksida nitrat, maka endotel dan penyakit kardiovaskuler, ginjal stadium akhir.

## **ASYMMETRIC DIMETHYLARGININE**

**Loo Hariyanto Raharjo**

**DEPARTEMENT OF BIOCHEMISTRY,**

**LECTURER FAKULTY OF MEDICINE, UNIVERSITY OF WIJAYA**

**KUSUMA, SURABAYA**

**E-MAIL : [loo\\_hiandao@yahoo.com](mailto:loo_hiandao@yahoo.com)**

### **ABSTRACT**

Asymmetric dimethylarginine (ADMA) is a naturally occurring chemical found in blood plasma. It is a metabolic by-product of continual protein modification processes in the cytoplasm of all human cells. It is closely related to L-arginine, a conditionally-essential amino acid. ADMA interferes with L-arginine in the production of nitric oxide, a key chemical to endothelial and hence cardiovascular health. ADMA concentration is elevated in patients with end-stage renal disease (ESRD), in part because it is excreted via the kidneys.

**KEY WORDS :** Asymmetric dimethylarginine (ADMA), nitric oxide, endothelial and cardiovascular hence, end-stage renal disease.

### **INTRODUCTION**

Patrick Vallance and his London co-workers first noted the interference role for asymmetric dimethylarginine. Today biochemical and clinical research continues into the role of ADMA in cardiovascular disease, diabetes mellitus, erectile dysfunction and certain forms of kidney disease.

As the principal endogenous inhibitor of nitric oxide synthase, ADMA (asymmetric

dimethylarginine), regulates rates of nitric oxide (NO) formation. Nitric oxide acts as a signal molecule in the nervous system, as a weapon against infections, as a regulator of blood pressure, and as a gate keeper of blood flow to the organs. Elevated ADMA is a risk factor for hypertension, cardiovascular disease, renal failure, and erectile dysfunction. Factors contributing to elevated ADMA include increased oxidative challenge and folic

acid insufficiency.

Endothelium-derived nitric oxide (NO) plays an important role in the regulation of BP and platelet aggregation. It is synthesized by stereospecific oxidation of the terminal guanidino nitrogen of the amino acid L-arginine. NO is produced by the action of a family of NO synthases, with endothelial, neuronal, and macrophage isoforms.

The synthesis of NO can be selectively inhibited by guanidino-substituted analogs of L-arginine, such as *N*-monomethyl-L-arginine, which act as competitive antagonists at the active site of the enzyme. Asymmetric dimethylarginine (ADMA) has been recently characterized as an endogenous inhibitor of NO synthase. It is synthesized and metabolized by human endothelial cells. ADMA and its biologically inactive stereoisomer symmetric dimethylarginine (SDMA) are at least in part eliminated via urinary excretion. Vallance *et al.* were the first to report elevated plasma levels of ADMA and SDMA in a small group of patients with end-stage renal disease (ESRD). In their study, dimethylarginine (DMA) levels were elevated approximately sixfold, compared with those for healthy control subjects. Those authors suggested that the high incidence of conditions such as hypertension, atherosclerosis, and immune dysfunction among patients with ESRD might be caused at least in part by dysfunction of the L-arginine/NO pathway secondary to accumulation of ADMA.

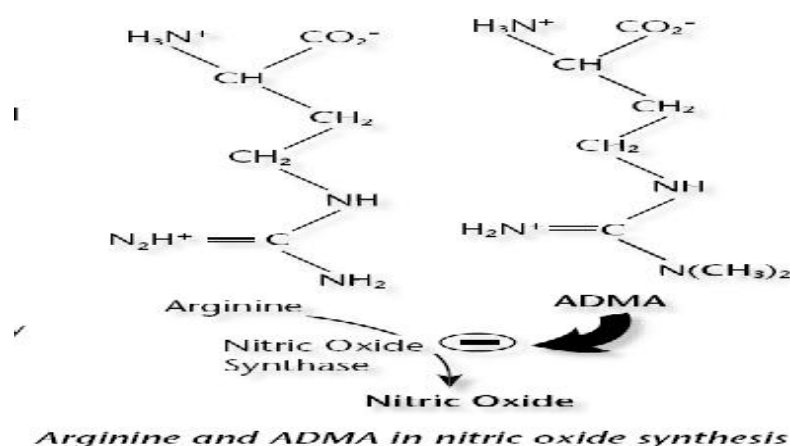
### **Why measure ADMA?**

Because independent studies have shown that:

- ADMA is a better predictor of insulin resistance than any other single marker!

- It is a better predictor of vascular endothelial impairment than cholesterol!
- Homocysteine increases in proportion to ADMA. The inhibition of nitric oxide synthesis may explain why homocysteine has been associated with impaired endothelium mediated, nitric oxide-dependent vasodilatation.
- An elevated concentration of ADMA is a potential contributory factor for pre-eclampsia, and is associated with endothelial dysfunction in some women.
- A glucose-induced impairment causes ADMA accumulation and may contribute to endothelial vasodilator dysfunction in Diabetes Melitus.
- In the cardiovascular system, decreased NO biosynthesis has the potential to increase blood pressure, enhance platelet and white cell adhesiveness, increase vascular smooth muscle growth, alter mitochondrial oxygen consumption and accelerate the development of atherosclerotic-like lesions.
- In pre-clinical and clinical studies, ADMA has been found to be elevated by hypercholesterolemia, hyperglycemia, hypertriglyceridemia, or hyperhomocysteinemia.
- ADMA levels are highly correlated with triglyceride levels.
- ADMA is elevated in peripheral and carotid artery blockage in proportion to the blockage.

## Structure of ADMA



## The role of nitric oxide

Cardiovascular disease remains the number one cause of death in the United States. This fact indicates that there is more to learn regarding risk factors and how these contribute to cardiovascular disease (CVD). Because of its cell regulatory functions, nitric oxide (NO) is involved in the pathogenesis of many chronic degenerative disorders. Atherosclerotic heart disease, type 2 diabetes, metabolic syndrome (X), Crohn's disease, and arthritis are some of the major diseases where pathogenesis involves NO metabolism. Anytime a molecule with such powerful and wide ranging physiological consequences is produced as a normal cell regulatory mechanism, there must be offsetting ways of controlling its synthesis. The focus of

this review is on the control of NO synthesis by the endogenous regulator ADMA and its relationship to cardiovascular disease.

Nitric oxide is a gas with molecular dimensions similar to molecular oxygen ( $\text{O}_2$ ). In fact, the competitive blocking of mitochondrial oxygen uptake is the mechanism used by fireflies for flashing. In humans, one mechanism of NO action is to signal the formation of the intercellular messenger, cyclic guanosine monophosphate (cGMP), thereby eliciting cellular responses like smooth muscle relaxation. NO is formed from the amino acid L-arginine under the action of a family of enzymes, the nitric oxide synthases, found in endothelial, neuronal, and inducible isoforms.

## SYSTEM

### Cardiovascular system

### Brain

### Peripheral nervous system

### Immune system

## FUNCTION OF NITRIC OXIDE

Exerts tonic vasodilator tone. Prevents cellular adhesion to vessel walls. Inhibits platelet activation. Retards development of atherosclerosis. Stimulates angiogenesis. Induces regression of preexisting intimal lesions.

Modulates neurotransmission. Excess causes neurodegeneration

Regulates pain perception. Mediates non-adrenergic, non-cholinergic neurotransmission in the gut, genitourinary system and vasculature.

Mediates cytotoxicity and regulates immune cell function.

## Nitric Oxide and the cardiovascular system

It is now widely understood that heart disease is really a problem of the vascular

system and vascular health is largely a question of endothelial function. In a single adult human, the approximately 100,000 miles of blood vessels are lined

with endothelial cells comprising the surface area of eight tennis courts. It is the response of that tissue that determines cardiovascular health.

Endothelial cells respond to signals for dilation or contraction. The response is mediated first by stimulation of nitric oxide synthase enzymes in the endothelial cells to produce nitric oxide. The gas quickly diffuses into the muscular layer where it triggers the formation of cyclic GMP, thus causing the shifting of ionic calcium and culminating in relaxation of the smooth muscle cells surrounding the arteries. This process must function smoothly and continuously during waking activities and digestion to regulate changes in blood flow.

#### **Beneficial effects of NO on blood vessels:**

- Stimulates dilation and sustained relaxation of arteries
- Reduces platelet and macrophage adhesiveness, thus lowering the inflammatory cascade
- Keeps blood vessels pliant and elastic
- Regulates oxidative enzymes preventing free radical pathology
- Slows plaque growth
- May reverse plaque formation to clear atheroma

#### **Clinical benefits of increased NO production:**

- Less discomfort from angina and other symptoms of heart disease
- Increased pain-free exercise time
- Improvement in erectile dysfunction

#### **Nitric oxide formation from L-arginine**

The normal precursor of NO is the amino acid L-arginine. Thus, the first modifiable factor is to increase plasma arginine by supplementation with 3gm L-arginine or more. Arginine is not considered an essential nutrient because it is formed in human tissues as an intermediate in the urea cycle. Thus, the ubiquitous amino acid glutamate is transformed to citrulline that combines with carbamoyl phosphate (from CO<sub>2</sub> and NH<sub>3</sub>) to form arginine. So, it might seem that one would never run out of arginine because it can easily be made from virtually any amino acid source. The problem is that arginine is also required for synthesis of almost every protein in the body and supply of glutamate is limited. Demands for arginine in the urea cycle and in protein synthesis can cause circulating free arginine concentration in plasma to fall below optimal for NO synthesis.

L-Arginine infusion has been shown to enhance nitric oxide generation and inhibit lesion formation after balloon angioplasty. The communication of vascular dilation signals occurs via NO diffusion from the endothelial layer into the muscular layer. However, the supply of arginine as substrate to the endothelial nitric oxide synthase (NOS) may depend on arginine status in vascular smooth muscle cells, which have unique affinity for uptake of arginine, even in polymeric forms.

Other modifications act on the enzyme activity of NOS. Adding 10 mg of folic acid per day to healthy subjects prevents nitric oxide synthase dysfunction induced by nitroglycerin and nitrate tolerance in the arterial circulation. The results point to oxidative stress as the origin of NOS activity decrease.

| Interventions to increase NO                   |                           |
|------------------------------------------------|---------------------------|
| • Increase circulating arginine concentrations | • Restore folate status   |
| • Increase exercise                            | • Increase phytoestrogens |
| • Lower ADMA (see below)                       |                           |

#### **Nitric oxide regulation by ADMA**

Because it elicits a wide array of powerful cellular responses, NO production must be tightly regulated. The small NO molecule

permeates all surrounding cellular compartments and rapidly travels into adjacent cells. NO is converted to nitrite in 10 seconds or less, so cellular

concentrations are dependent on rates of synthesis by the enzyme nitric oxide synthase (NOS). Therefore, cellular concentrations of NO depend on NOS regulation. The primary factors controlling NOS activity are the concentrations of the substrate, L-arginine, and the NOS inhibitor, ADMA. Enzyme concentrations are also variable; genes controlling the inducible form of NOS (iNOS) are activated by inflammatory cytokines.

When hyperhomocysteinemia is induced in humans by oral methionine loading, the resulting endothelial vasodilator dysfunction is temporally related to, and quantitatively correlated with, elevations in plasma ADMA. These results support and extend the findings that patients with ischemic stroke have significantly higher concentrations of plasma ADMA than controls, and that homocysteine impairs the NO synthesis pathway.

ADMA is produced by methylation of specific arginine residues of certain cellular proteins. Most of the proteins that have been found to undergo significant arginine methylation are found in the nucleus. The methylation produces monomethylarginine and two forms of dimethylarginine, depending on whether the methyl groups are on the same nitrogen atom (ADMA) or two different nitrogen atoms in the side chain. In the latter case, the product is symmetrical dimethylarginine (SDMA), which has insignificant inhibitory effects on NOS. When the proteins are degraded, ADMA and the other isomers are released. Tables 2 and 3 illustrate the range of studies that have demonstrated clinically significant associations with ADMA levels.

### **Mechanisms of ADMA action and effects on cardiovascular function**

An early observation that we now know is related to ADMA was that atherosclerosis is marked by impaired vasodilation in response to normal physiological stimuli, and the impairment can be relieved by intravenous L-arginine. A number of standard heart disease risk factors (smoking, hypertension, hyperlipidemias) are related to vasodilatory impairment, but the strongest correlation may be the ratio of arginine to ADMA in plasma. This ratio

has been used to assess the balance of stimulatory and inhibitory effects on NO synthesis. Elaboration of endothelium-derived nitric oxide affects the behavior of circulating T lymphocytes and monocytes. Mononuclear cell adhesiveness is inversely correlated with the plasma L-arginine/ADMA ratio, and the effect is reversed by restoration of the ratio to control levels with oral administration of 14–21 grams of arginine. These changes lead to lower rates of atherosclerotic plaque formation and lower risk of heart disease.

Exposure of cultured endothelial cells to glucose, homocysteine, low-density lipoprotein as well as oxidized low-density lipoprotein stimulates the activity of the L-arginine-methylating enzyme and also inhibits the activity of demethylating enzymes, such as the dimethylarginine dimethylaminohydrolase, by means of reactive oxygen species. This results in increased intracellular and secondary extracellular ADMA concentrations and, subsequently, to decreased endothelial nitric oxide-mediated nitric oxide production and endothelial dysfunction. Accordingly, treatment of these cells with antioxidants has been demonstrated to restore the activity of dimethylarginine dimethylaminohydrolase, leading to a normalization of cellular ADMA levels and endothelial nitric oxide production. Infusion of ADMA in pathophysiologically relevant levels causes significant hemodynamic changes in humans. Thus, increased synthesis of ADMA and the subsequent impairment of nitric oxide synthesis may provide a common pathway by which many of the proatherogenic factors amount to clinically relevant cardiovascular risk.

There is growing evidence that the well-known connection of elevated homocysteine with cardiovascular disease is mediated by a mechanism involving ADMA. Homocysteine has become recognized as an important risk factor for heart disease. Multiple data sets on homocysteine elevation have been compiled in a meta analysis giving estimates that lowering average homocysteine concentrations by 3 micromol/L would reduce the risk of

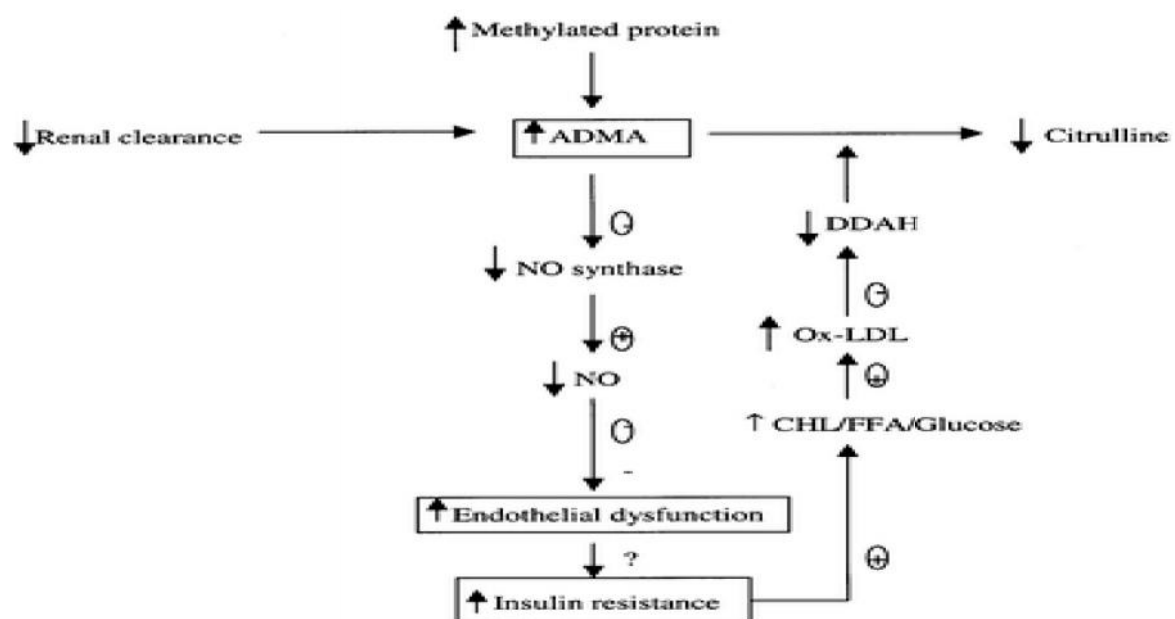
ischaemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24%. Recent research indicates that the effects of homocysteine on cardiovascular health are mediated by ADMA.

In addition, the association of elevated LDL cholesterol with increased risk of heart disease may be as a covariable in the oxidative activation of ADMA synthesis. Oxidized LDL particles stimulate the expression of enzymes that generate ADMA. These enzymes are discussed further below.

In patients with type two diabetes mellitus, plasma ADMA increases from 1.0 to 2.5 mmol/L five hours after ingestion of a high-fat meal. ADMA may contribute to abnormal blood flow responses and to atherogenesis in type 2 diabetics. In hypertensives, the urinary excretion rate of nitrite plus nitrate (NOx), an index of endogenous NO production, was lowered from 77 to 56 micromol/mmol creatinine, while plasma levels of ADMA were increased from 1.1 to 2.4, indicating ADMA inhibition of NO production. ADMA plays an important role in the pathogenesis of hypertension associated with the experimental focal and segmental glomerulosclerosis.

Elevated ADMA is associated with:

- Increase in blood pressure during high salt intake.
- Pathophysiology of coronary vasospasm.
- Carotid intima-media thickness in end-stage renal disease.
- Borderline hypertension in young subjects – independent of hypercholesterolemia.
- Endothelium dysfunction as a mediator of homocysteine degenerative effects.
- Hyperhomocysteinemia in patients with ischemic stroke. ADMA levels above the 90<sup>th</sup> percentile increased risk for stroke by six-fold.
- Increases in endothelin-1 and reductions in insulin-induced increments in plasma NOx and cGMP in patients with cardiac syndrome X (angina pectoris and normal coronary arteriograms). All of the effects are reversed by intravenous L-arginine.
- Acute coronary events. For men who do not smoke, those in the highest quartile for ADMA have 4-fold greater risk.
- Essential hypertension, but not post-transplant hypertension.
- Congestive heart failure, via NO-mediated vasodilatation inhibition by endothelin-1.



### **Generation and clearance of ADMA**

The relationship of methylation to the formation of methylated arginines is important for understanding the rates of formation of ADMA. The conversion can be stimulated by methionine as a direct donor of methyl groups via enzymes called protein arginine N-methyltransferases (PRMTs). Many, if not most proteins that contain ADMA are found in the nucleus and they interact with RNA, exerting transcriptional control. PRMT activity is increased by methionine loading. In fact, acute methionine loading in human subjects induces endothelial dysfunction because of increased methylation of L-arginine residues in endothelial proteins, forming ADMA residues. Upon proteolysis, free ADMA is released for down-regulation of NOS. Once ADMA is released from methylated proteins by proteolysis, the two principal factors controlling levels are renal clearance and hepatic metabolism. Normally, slightly more ADMA is cleared by hepatic metabolism than by renal excretion. The hepatic enzyme dimethylarginine dimethylaminohydrolase (DDAH) is important for clearing ADMA by conversion to citrulline. Over 4 mmol (>1gm)/day of ADMA is extracted by the liver (700 times the amount circulating in blood). Several lines of evidence show that renal clearance is the other primary mechanism for control of ADMA concentrations. ADMA is not excreted in patients with chronic renal failure, and concentrations in plasma are two to six times higher in uremic patients than in healthy control individuals. All-trans-retinoic acid increases the expression of DDAH II, the predominant DDAH isoform in endothelial cells, resulting in increased synthesis of NO. On the other hand, nerve damage causes marked increase in the expression of DDAH as part of the mechanism to protect neuronal cells from the effects of excess NO. Other data suggests that human endothelial cell PRMT activity is up-regulated by LDL cholesterol, due in part to the enhanced gene expression of PRMTs. Thus, we find ADMA intermeshed with both cholesterol and homocysteine cardiovascular risk factors. Hyperglycemia is another factor

related to ADMA. Human endothelial cells exposed to high glucose concentrations show impairment of DDAH and accumulation of ADMA that may contribute to endothelial vasodilator dysfunction in diabetes mellitus. Improved glycemic control in patients with type 2 diabetes results in lowering of plasma ADMA levels. Metformin treatment achieved the lowering of ADMA without simultaneous sulfonylurea treatment. Feedback regulation of NO on DDAH is achieved by nitrosylation of active site residues. This explains why expression of iNOS often leads to inhibition of constitutively expressed NOS isoenzymes. As average nitric oxide concentrations increase, nitrosylation increases and enzyme activities fall. Other lines of evidence indicate that reactive oxygen species are responsible for loss of DDAH activity. For example, the endothelial dysfunction caused by methionine loading can be reversed by administration of ascorbic acid. Since vascular responses are so strongly dependent on NO production, it is important to assess the interplay of other treatments for hypertension and hypercholesterolemia. Plasma ADMA concentrations are unaffected by statin treatments that lower LDL cholesterol. On the other hand, angiotensin converting enzyme (ACE) inhibitors lower plasma ADMA concentrations from 1.69 to 1.41  $\mu\text{mol/L}$  and the effect is independent of blood pressure. Long-term use of ACE inhibitors caused reduced ADMA, increased NOx, and increased ratio of L-Arg/ADMA in syndrome X patients, giving improved coronary microvascular function and reduced myocardial ischemia. Estrogen replacement therapy with oral conjugated estrogen lowers plasma ADMA. Some studies have examined the relative benefits of homocysteine normalization vs NO stimulation. Peripheral arterial occlusive disease patients with hypercholesterolemia and hyperhomocysteinemia showed no improvement in endothelium-dependent vasodilation with combined B vitamin therapy designed to lower homocysteine (folate, 10mg; vitamin B12, 200mcg; vitamin B6, 20 mg/day). Oral L-arginine (24 g/d) significantly improved endothelial

function in these patients. The NO stimulatory effect of arginine supplementation in this study was more beneficial for this group of patients than was homocysteine lowering. Thus, under hyperhomocysteinemic conditions, the controlling factor for endothelial functions is accumulation of ADMA that is relieved by arginine supplementation. There are multiple dietary and nutrient-dependent steps involved in the control of ADMA synthesis and clearance. L-Arginine stimulates NO synthesis to overcome the inhibitory effects of high ADMA. Low fat meals help to reduce the synthesis of ADMA by PRMT. Vitamin A induces expression of DDAH, causing a lowering of ADMA. Antioxidants prevent the inhibition of DDAH, thus enhancing the removal of ADMA. Normalization of elevated homocysteine with vitamins B6, B12, and folic acid reduces ADMA by controlling endothelial oxidative effects. Folic acid supplementation alone has been shown to lower both ADMA and arginine in plasma of hyperhomocysteinemic individuals. In addition, individuals with persistent ADMA elevations may be managed with hormonal interventions such as ACE inhibitors and natural estrogen replacement therapies. Exercise has a strong positive effect on the arginine/ADMA ratio. Pain-free walking distance is linearly correlated with lower ADMA levels in atherosclerotic patients.

Elevated ADMA is associated with:

- Multiple organ failure in intensive care units.
- Regulation of maternal and fetal circulation.
- Risk factor of ICU mortality.
- Hyperthyroidism (ADMA increases with TSH).
- Incipient primary chronic renal disease.
- Insulin resistance and metabolic syndrome.
- Low serum folic acid and high serum homocysteine.
- The pathophysiology of erectile dysfunction.
- Overall mortality and cardiovascular outcome in hemodialysis patients. Thus, ADMA is an important risk factor for

cardiovascular disease in chronic renal failure.

- Shorter survival and higher rate of incident cardiovascular complications in end-stage renal disease.
- A 3.9-fold higher risk of acute coronary events compared with lower quartiles.
- Type I diabetes and hypercholesterolemia. Pravastatin had no effect on the levels of ADMA in hypercholesterolemic men.
- The pathophysiology of insulin resistance syndrome and non-insulin-dependent diabetes mellitus.
- Normoglycemic women with previous gestational diabetes. The ADMA increase is independent of other risk factors or surrogate markers for diabetes or cardiovascular events.
- Renal insufficiency that causes reduction of ADMA clearance to less than 10% of controls.
- Nerve injury, where ADMA in neurons profoundly modulates NOS function and suppress NO-mediated injury.

#### **Laboratory methodology:**

ADMA has been assayed using high pressure liquid chromatography. This method is time consuming and not suitable for clinical laboratory throughput. A simple, sensitive and fast HPLC – tandem mass spectrometric method was developed with limits of detection of 1 ng/ml. Refinements in this method are making measurement of ADMA more routine.

Effect of diet and omega-3 fatty acid intervention on asymmetric dimethylarginine

The clinical benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) of marine origin are well recognized. The exact mechanism by which n-3 PUFAs exert their cardioprotective effect is, however, still not fully understood. In addition to substantial reduction in serum triglyceride level, they have been shown to be antithrombogenic and antiarrhythmic, and also to improve endothelial dysfunction. An important role of dietary

factors in modulating endothelial function by improving endothelium-dependent vasodilatation has also been suggested by mechanisms still unknown.

In the present study we wanted to explore whether n-3 PUFA supplementation and/or diet intervention have beneficial influence on endothelial function assessed as plasma ADMA and L-arginine levels in a male population with long-standing hyperlipidemia. As increased plasma concentrations of ADMA have been reported to be strongly related to components of the metabolic syndrome.

Increased endogenous NO production following supplementation with fish oil has been reported, which may be responsible for the improvement in endothelial function observed with n-3 PUFA. Previous studies have shown that administration of L-arginine has improved impaired endothelial function and inhibited the progression of atherosclerosis in humans, especially in healthy elderly individuals, as well as in experimental animal studies. Bode-Boger et al. demonstrated that dietary L-arginine supplementation elevated the L-arginine/ADMA ratio by increasing plasma L-arginine levels, while the concentrations of ADMA were unaffected, through mechanisms not known. Previous studies using different intervention principles known to improve endothelial dysfunction, have revealed that to influence ADMA levels directly are difficult.

Several studies have reported correlation between ADMA and serum lipid variables. Despite the reduction in intake of dietary fat and triglycerides after long term dietary intervention and in triglycerides and total cholesterol after n-3 PUFA supplementation, this was not accompanied by reduction in ADMA levels or increased nitrate/nitrite concentrations.

The so called Mediterranean diet has previously been reported to be favorable regarding recurrence of myocardial infarction in the LYON Heart Study, and for delaying all cause of death after a heart attack in the GISSI study. The exact mechanism for the reduced risk for cardiovascular disease after dietary

changes towards the Mediterranean diet is not known. Fard et al. have recently demonstrated acute elevation of ADMA levels and impaired endothelial function in response to high-fat meal in patients with type 2 diabetes. They proposed that the increase in ADMA levels resulted from a reduction in the expression or enzyme activity of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme selectively responsible for degradation of ADMA. Down regulation of DDAH has also been associated with oxidative stress. Ambring et al. did not find any beneficial effects either on oxidative stress evaluation or in plasma concentrations of insulin and glucose in their short-term diet intervention study of healthy subjects. The lack of effect of diet in our study might also be due to the fact that there was a tendency towards improved diet also in the group receiving no dietary advice.

Recently, elevated ADMA concentrations have been linked to metabolic variables related to cardiovascular risk factors like glucose, insulin and insulin resistance, and glucose *per se* has been shown important in the regulation of DDAH and ADMA. Lack of effects of both intervention principles on these variables may, therefore, explain why ADMA levels were not affected in the present study.

Direct alteration of ADMA levels with supplements of L-arginine have been suggested. The hope is that such intervention might not only improve endothelial function but also reduce clinical symptoms of overt cardiovascular disease. However studies show inconsistency in results in a clinical context, and the recent results with manipulating homocysteine levels warrant extreme care with what clinical outcomes might arise from this approach.

Statins, as well as affecting circulating cholesterol levels, also increase nitric oxide levels and so have a direct effect on blood supply to the heart. Elevated levels of ADMA seems to modify this effect and so may have consequences for patients' responsiveness to taking statins.

### Plasma Asymmetric Dimethylarginine Concentrations Are Elevated in Obese Insulin-Resistant Women and Fall with Weight Loss

Plasma ADMA levels are higher in obese, Insulin resistance women than in equally obese, Insulin sensitive women and decrease in response to weight loss when associated with enhancement of insulin sensitivity. The fact that plasma ADMA concentrations are also elevated in patients with type 2 diabetes, CVD, and renal failure, clinical syndromes known to be associated with insulin resistance, lends further support for the existence of a relationship between insulin resistance and increases in plasma ADMA concentrations.

ADMA and homocysteine metabolism are related because the methyl groups of ADMA are derived from *S*-adenosylmethionine, thus rendering *S*-adenosylhomocysteine. Another common feature of both atherogenic amino acids is the potential influence of sex hormones on their plasma levels. Plasma levels of homocysteine are higher in postmenopausal than in premenopausal women. Furthermore, hormone replacement therapy (HRT) was effective in lowering plasma homocysteine levels. Fewer studies of ADMA are available and data on the effect of menopause are sparse. Recently, it was demonstrated that ADMA was reduced by HRT with conjugated equine estrogen.

The ADMA concentrations in these individuals were normalized to those of their Insulin sensitivity counterparts, even though their insulin sensitivity did not improve to the same degree. This finding is important for two reasons. First, it suggests a mechanism by which weight loss may lead to enhanced endothelial function and lower CVD risk. Second, the current findings extend those of our prior study, in which we found that enhancement of insulin sensitivity with TZD treatment is capable of decreasing plasma ADMA concentrations.

### Consequences of Reduced NO Elaboration in ESRD

Aside from volume changes during HD, acute alterations in NO formation during

HD may contribute to peridialytic BP instability in HD-treated patients. Changes in ADMA-induced inhibition of endothelial NO production may contribute to this phenomenon. In the long term, chronic reduction of NO synthase activity secondary to accumulation of ADMA may contribute to the pathogenesis of hypertension and atherosclerosis. Chronic inhibition of NO synthesis has been shown to produce hypertension and to accelerate atherosclerosis in animal models. Chronically elevated ADMA concentrations may induce similar proatherogenic effects, like those observed in these experimental models.

Our group previously reported that plasma ADMA concentrations were elevated two- to threefold in patients with generalized atherosclerosis and peripheral arterial occlusive disease. This increase, which was associated with reduced urinary excretion of nitrate and cGMP, was independent of the presence of impaired renal excretory function. Moreover, we found a twofold elevation of ADMA levels in the plasma of asymptomatic young hypercholesterolemic subjects with normal renal function. From these data it remains unresolved whether patients with ESRD with atherosclerosis exhibited higher ADMA levels because of the presence of atherosclerosis or whether they suffered from atherosclerosis as a consequence of higher ADMA levels. However, the elevation of ADMA levels in patients with peripheral arterial occlusive disease with normal renal function, compared with control subjects, suggests that mechanisms other than reduced renal excretion may also contribute to the greater accumulation of ADMA in patients with ESRD with peripheral arterial occlusive disease, compared with patients with ESRD without atherosclerosis. The concept of accelerated atherosclerosis in patients chronically undergoing dialysis has been widely accepted since it was first published. However, in a recent review, London and Drüeke argued that the high mortality rates resulting from cardiovascular disease are not proof of accelerated atherosclerosis in patients undergoing maintenance dialysis. Many patients undergoing dialysis have more or

less marked vascular lesions at the start of dialysis treatment, and the risk factors present in the predialysis phase may be of primary importance for the manifestation of cardiovascular disease. Multiple known cardiovascular risk factors have been shown to be present in patients with ESRD. Elevated ADMA levels may be a newly identified, preexistent risk factor for atherosclerosis.

## CONCLUSION

The origins of cardiovascular disease are clarified by knowledge of the mechanism of formation and action of ADMA. Endothelial function depends on finely tuned regulation of NO production. That regulation revolves around two processes. The first is the rate of formation of NO by isoforms of NOS. The therapeutic use of nitroglycerin and the use of supplemental L-arginine are ways of direct stimulation of increased NO production. Increased intake of folic acid protects NOS activity. Finally, exercise stimulates NOS formation, providing the link to another long-recognized lifestyle modification known to improve cardiovascular health.

The second controlling process is the rate of formation of the NOS inhibitor, ADMA. The enzyme that forms ADMA is stimulated by high dietary fat and by high plasma methionine concentrations. Thus, Plasma ADMA concentrations are elevated in Insulin resistance, hyperinsulinemic individuals and that they decrease significantly in association with enhanced insulin sensitivity.

The increased levels of L-arginine observed after long-term n-3 PUFA supplementation, counteracting the age related decrease seen in the placebo group, especially in lean individuals.

The presence of atherosclerosis is associated with higher ADMA levels in patients with normal renal function, as well as in patients undergoing dialysis, but this phenomenon may be unrelated to renal handling of ADMA.

Reduced NO elaboration secondary to accumulation of ADMA may be an important pathogenic factor for atherosclerosis in chronic renal failure.

PRMT1 activity is modifiable by dietary factors. DDAH, the enzyme that degrades ADMA is susceptible to oxidative damage. Consequently, antioxidant protection conserves DDAH function and lowers ADMA. Oxidant challenge encompasses factors such as cigarette smoking, exercise-induced myocardial infarction, and oxidized cholesterol (the main link associating total cholesterol). Antioxidant intervention studies include those using purified vitamin E, vitamin C, and dietary antioxidant-rich foods. ADMA levels may be reduced by bringing inflammatory processes under control, by increasing antioxidant protection, and by assuring adequate functional status of nutrients that are required for the control of inducing factors such as homocysteine and metabolic syndrome. Macronutrient intake also affects ADMA, especially high fat meals that acutely elevate ADMA levels. Exercise has strong positive effects on ADMA, lowering the plasma arginine to ADMA ratio. Plasma or serum ADMA measurements can be used to monitor the net effect of these multiple health-promoting factors. Significant reduction of morbidity and mortality may be achieved by reducing ADMA in patients with high levels of this inhibitor of cellular nitric oxide production.

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