Asymmetric dimethyl l-arginine, endogenous inhibitor of nitric oxide synthase in congestive heart failure; relation to cardiac function

Thesis submitted for partial fulfillment of master degree in cardiology

By

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ABSTRACT:

Nitric oxide and its endogenous inhibitor Asymmetric dimethyl-l-arginine both are increased in the heart failure milieu and are related to pathophysiology of systolic impairment and endothelial dysfunction.

Objective: To evaluate the levels of nitric oxide metabolites (NOx) (as indirect evidence of nitric oxide level) and the level of ADMA in patients with moderate to severe congestive heart failure; both ischemic and idiopathic etiologies , find any etiology-specific difference and test their relations to EF and endothelial function as measured by FMD. **Methods:** This was a case control study comparing cases of heart failure with normal control subjects. We studied serum levels of ADMA and NOx, echocardiographic parameters for LV dimensions and systolic function and the endothelial function by FMD. **Results:** We found that both ADMA and NOx were significantly elevated in sera of patients with heart failure compared to normal control *[p <0.001 for both]*, there was an inverse correlation between EF and serum ADMA level *[p = 0.008 , r = -0.403]*, EF and NOx *[p = <0.001 , r = -0.943]* A significant direct correlation was found between ADMA and NOx *[p = <0.001 , r = 0.743]*. There was no difference between ischemic and idiopathic groups in ADMA or NOx. Increasing age was directly correlated to ADMA *[p = 0.002 , r = 0.470]*, to nitrites *[p = 0.004 , r = 0.436]* and to nitrates *[p = <0.001 , r = 0.542]*, also endothelial function was worse in heart failure than in control subjects and inverse correlation was found between FMD and ADMA *[p = 0.027 , r = -0.340]* and between FMD and NOx *[p = 0.011, r = -0.389]*. However, neither of ADMA, NOx, EF nor FMD was proved to be correlated to NYHA FC.

Conclusion: Increased NO in heart failure whether due to ischemic or idiopathic cardiomyopathy may be one of the mechanisms for systolic impairment and high ADMA level may explain endothelial dysfunction in heart failure although total NO production is increased.

Key Words: ADMA, Nitric Oxide, Heart Failure, cardiomyopathy, Endothelial Dysfunction.

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CONTENTS:

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ADMA:	Asymmetric Dimethyl –l-Arginine
ADP:	Adenosine di-phosphate
ATP:	Adenosine tri-phosphate
bFGF:	Basic fibroblast growth factor
CAD:	Coronary aytery disease
CBC:	Complete Blood Count
cAMP:	Cyclic Adenosine mono-phosphate
CCS:	Coronary calcium scoring
CESs:	Circulating endothelial cells
cGMP:	Cyclic guanosine mono-phosphate
CHF:	Congestive Heart Failure
CI:	Cardiac index
CIMT:	Carotid intima-media thickness
hsCRP	High sensitiviy C-reactive protein
GC:	Guanosine cyclase
DDAH:	Dimethyl-arginine Dimethylaminohydrolase
E/A ratio:	Early diastolic/atrial contraction phases of diastolic filling
ECG:	Electrocardiogram
ECPT:	Enhanced external counterpulsation therapy
EDRF:	Endothelium-derived relaxing factor
EDHF	Endothelium-derived hyperpolarizing factor
EF:	Ejection Fraction
ELISA:	Enzyme-linked immunosorbant assay
eNOS:	Endothelial Nitric Oxide Synthase
EPCs:	Endothelial progenitor cells
ESC:	Europian Society of Cardiology
ET-1:	Endothelin receptor-1
FMD:	Flow-Mediated Dilatation
g-IFN:	g-Interferon
HDL:	High Density Lipoprotien
H-HYC:	Hyperhomocystinemia
HPLC:	High Performance Liquid Chromatography
IGF:	Insulin-like growth factor
IL:	Interleukin
sICAM-1	Soluble intracellular adhesion molecule-1
iNOS:	inducible Nitric Oxide Synthase
LDL:	Low Density Lipoprotien
MHz:	Mega Hertz

MI:	Myocardial infarction
NMMA:	NG Monomethyl-L-Arginine
nNOS:	neuronal Nitric Oxide Synthase
NO:	Nitric Oxide
NYHA FC:	New York Heart Association Functional Classification
MMP:	Matrix metalloproteinase
PAP:	:ulmonary arterial pressure
PCWP :	Pulmonary capillary wedge pressure
PVR :	Pulmonary vascular resistance
RAAS:	Renin-angiotensin- aldosterone system
RAP :	Right atrial pressure
SD:	Standered Deviation
SDMA:	Symmetric Dimethyl Arginine
SNS:	Sympathetic nervous system
SVI :	Stroke volume index
SVR	Systemic vascular resistance
VSMC:	Vascular smooth muscle cell
TC:	Total Cholestrol
TG:	Triglyceride
TGF-b:	Transforming growth factor-b,
TNF-α:	Tumor necrosis factor-alpha
TVI DF:	Time Velocity Integral of Diastolic Function
TVI:	Time Velocityt Integral
vWF:	Von-Willebrand factor

INTRODUCTION

Arginine N-methyl transferases (PRMTs) catalyses methylation of guanidine nitrogen(s) of arginine to produce NG-monomethyl arginine (L-NMMA),asymmetric NG-NG dimethyl –L-arginine (ADMA) and symmetric NG-NG dimethyl arginine (SDMA)[359].

Free intracellular NMMA and ADMA,but not its stereoisomer SDMA are inhibitors of all three isoforms of nitric oxide synthase (eNOS, nNOS and iNOS). NMMA and ADMA but not SDMA are metabolized by dimethyl-arginine dimethylaminohydrolase (DDAH) to citruline and dimethyl amine[360].

These free methyl arginines are detectable in cell cytosol, plasma and tissues. Elevated plasma level of ADMA has been detected in many diseases including: end stage chronic renal failure[361],congestive heart failure[362],preeclampsia[363] and hypertension[370]

Altered nitric oxide synthesis has been implicated in the pathogenesis of these diseases and it is possible to consider the accumulation of endogenous NMMA and ADMA underlies the impaired nitric oxide generation which implicated in the pathogenesis of these diseases[363]

The vascular endothelium plays a pivotal role in controlling blood vessels' tone and preventing the development of atherosclerosis, principally through production and release of vasoactive compounds such as nitric oxide(NO).

Nitric oxide has several antiatherogenic roles including vasodilatation[364], inhibition of vascular smooth muscle cell proliferation, inhibition of platelet adhesion and aggregation, and inhibition of monocyte adhesion and migration[365].

The production of nitric oxide by vascular smooth muscle is continuous and contributes to the resting tone of the vessels.

Nitric oxide is produced by constitutive endothelial nitric oxide synthase (eNOS). The production is stimulated by: acetyl choline, bradykinin, hypoxia, and stress and inhibited by ADMA[360]

In coronary artery disease, including stable angina, unstable angina, and myocardial infarction there is a primary decrease in coronary blood flow due to vasospasm and thrombosis and the clinical syndromes are almost

exclusively associated with atherosclerosis of epicardial coronary arteries[366].

So efforts have been made to correlate the syndromes of coronary heart diseases to the biology of atherosclerosis. Nitric oxide plays an important role in the regulation of cardiovascular functions.

ADMA as an endogenous inhibitor of nitric oxide synthase has been implicated in the impairment of nitric oxide production in a variety of cardiovascular diseases[367].

The finding that ADMA is elevated in chronic renal failure led to speculation that ADMA may in part be responsible for increased cardiovascular disease risk and hypertension in these patients[368].

Subsequent studies have shown strong association between raised ADMA levels and cardiovascular risk factors, endothelial dysfunction, hypertension, atherosclerosis, and cardiovascular mortality.

ADMA levels are also significantly higher in patients with ischemic heart diseases[369]. A recent analysis of large group of patients with ischemic heart failure demonstrated an inverse relationship between ADMA levels and left ventricular (LV) ejection fraction[346), (370].

ADMA was shown to be independent and strong predictor of LV ejection fraction[346]. These findings raised the possibility that causal relationship between ADMA and LV ejection fraction may exist.

AIM OF THE WORK

The aim of this work is to evaluate the plasma level of ADMA and its relation to NO production, measured as plasma nitrites and nitrates in patients with congestive heart failure with different etiology and to correlate with the severity of the disease as assessed by cardiac function tests and parameters of endothelial function.

REVIEW OF LITERATURE
Chapter I

Endothelial Function
Normal Endothelial Function;

The vascular endothelium lies between the lumen and the vascular smooth muscle. Although only one cell layer thick; it's able to "sense" changes in hemodynamic forces, or blood-born signals by membrane receptor mechanisms and respond to physical and chemical stimuli by synthesis or release of a variety of vasoactive and thromboregulatory molecules and growth factors. The endothelium is a key component of the vessel wall because it serves several major physiological roles, as follows:

- ✓ They regulate the diffusion of substances of cells out of and into the circulating blood, across cell junctions and through their cytoplasm.

- ✓ They participate in the coagulation process by secreting clotting factors and in the process of fibrinolysis.

- ✓ They have selective phagocytic activity and extract substances from the blood besides other metabolic activities.

- ✓ They can synthesize (evidenced by in-vitro cultures) fibronectin, laminin, collagen, elastin and other subendothelial components [(1)].

- ✓ They have renewal capability by proliferating to provide new cells during increasing size of the blood vessel, to replace damaged or exfoliated endothelium, and to provide growing solid cords of cells, which are the forerunners of new blood vessels during neovascularization process.

- ✓ Substances released by the endothelium include Prostacyclin, Nitric Oxide (the endothelium-derived relaxing factor), Endothelins, Endothelial Cell Growth Factor(s), Interleukins, Plasminogen Inhibitors and Von Willebrand factor [(1)]

- ✓ In addition to these universal functions; the endothelium may have organ-specific functions that are differentiated for various parts of the body such as gas exchange in the lungs, control of myocardial function in the heart or phagocytosis in the liver and spleen.

Of the above functions, the functions of vascular tone control and substances secreted from the endothelium are concerned in our current work:

Regulation of vascular tone;

The central role of the endothelium in controlling vascular tone has been appreciated since the discovery of the potent vasodilators prostacyclin and NO in early eighties [2].

The endothelium controls underlying smooth muscle tone in response to certain pharmacologic and physiologic stimuli[3].

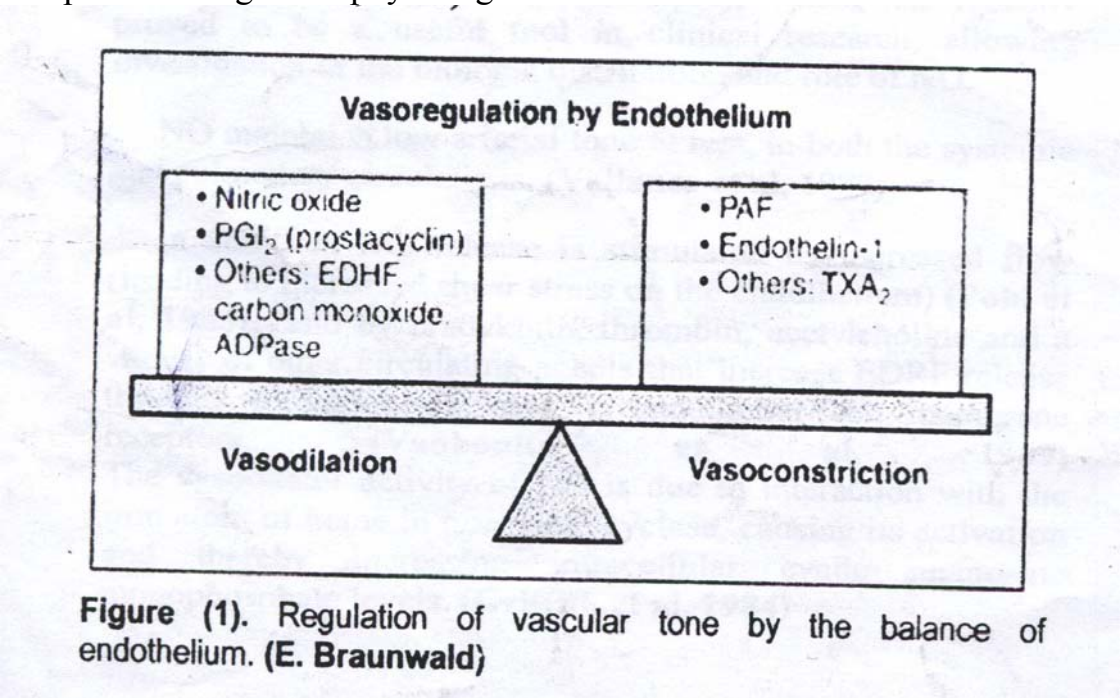

Figure (1). Regulation of vascular tone by the balance of endothelium. **(E. Braunwald)**

This involves a number of lumen membrane receptors and complex intracellular pathways and the synthesis and release of a variety of relaxing and constricting substances (see Fig. 1).

In addition to making their own vasoactive mediators, endothelial cells may transduce signals from, or even inactivate circulating vasodilators and constrictors such as thrombin, bradykinin, ADP and ATP[1] .

1) No , an EDRF:

Furchgott and Zawadzki in 1980 [2] first described the action of a labile substance they termed endothelium-derived relaxing factor (EDRF) that vasodilated rabbit aortic rings preconstricted with norepinephrine after stimulation with acetylcholine

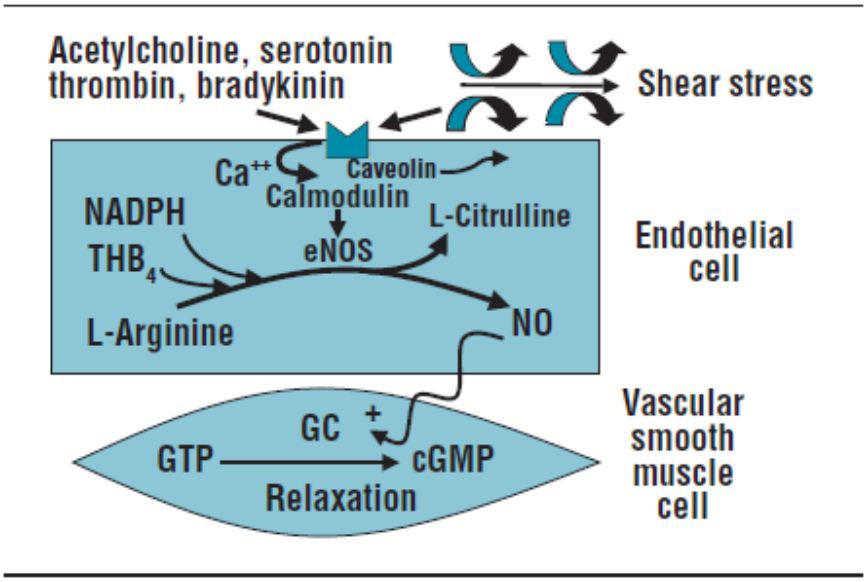

Figure 2: Endothelial cell production of NO by the action of NO synthase (eNOS) on L-arginine. This reaction requires a number of cofactors, including tetrahydrobiopterin [THB4] and NADPH. A rise in intracellular Ca++ in response to vasodilator agonists or shear stress displaces the inhibitor caveolin from calmodulin activating eNOS. NO diffuses to vascular smooth muscle and causes relaxation by activating guanylate cyclase [GC], thereby increasing intracellular cyclic guanosine monophosphate [cGMP].

Denudation of endothelial cells abolished this acetylcholine-induced vasodilation. Subsequently this factor was identified as NO that is produced from L-arginine by constitutive activity of endothelial nitric oxide synthase (eNOS) an enzyme present in endothelial cells [(4)].

The reaction is stereospecific and L-arginine is converted to NO and L-citrulline. Nitric oxide is a diffusible molecule with a very short half-life (3-6seconds) (Fig. 2).

Four recent discoveries have greatly increased interest in nitric oxide:

(1) It has been shown that the presence of NO is involved in several important biological events including vascular smooth muscle relaxation [(29)], platelet deaggregation [(30),(31),(35)], neuronal communication [(32)], and possibly photoreceptor signaling [(33), f(34)].

(2) This involvement of NO has been found to occur through activation of guanylate cyclase, a heme-containing enzyme which catalyzes the reaction GTP to cGMP [(36)].

(3) Nitric oxide released by murine macrophages and other cells after

immunological activation acts as a cytotoxic molecule for invading intracellular microorganisms and tumor cells [37), (38)].

All of the enzymes affected by cytotoxic NO including ribonucleotide reductase (the rate-limiting enzyme in DNA replication) contain catalytically active non-heme iron coordinated to sulfur atoms [(39)]. In all cases the inhibition of enzyme activity was accompanied by the loss of intracellular iron from the target cells [(40)].

(4) An enzyme, nitric oxide synthase, converts L-arginine to NO [(7), (41),(42)]. The endogenously produced NO is known as endothelium-derived relaxation factor (EDRF) because of its role in the relaxation of vascular smooth muscles.

These and related observations have raised the title question, "why NO?" What makes NO so special that it, rather than CO, O_2 or other ligands, is used as a trigger for these important processes.

A possible answer is simply that NO is an extraordinarily different ligand with regard to its reaction with hemes and non-heme iron proteins.

NO maintains low arterial tone at rest, in both the systemic and pulmonary circulations [(43)].

In addition, NO release is stimulated by increased flow (leading to increased shear stress on the endothelium)[(44)] , bradykinin, thrombin, acetylcholine and a variety of other circulating agents that increase EDRF release through activation of specific endothelial cell membrane receptors [(45)].

The vasodilator activity of NO is due to interaction with the iron atom of heme in guanylate cylase, causing its activation and thereby increasing intracellular cyclic guanosine monophosphate levels [(46)].

In smooth muscle cells this results in a reduction of intracellular calcium and thereby relaxation [(47)]. The same pathway is involved in the mechanism of action of exogenous nitrovasodilators, such as sodium nitroprusside and nitroglycerine, further details about NO are coming in chapter II.

2) Endothelium-derived hyperpolarizing factor (EDHF):

Stimulation of the normal endothelium by acetylcholine also produces hyperpolarization of the underlying smooth muscle and thereby vasorelaxation. This is not mediated by NO, but by another endothelium-derived factor, which acts by increasing K+ conductance. The resulting vasodilation is not inhibited by L-NMMA, the specific antagonist of NO[(63)].