

Review Article and Clinical Experience:**DIABETES MELLITUS AND ENDOTHELIAL DYSFUNCTION:
A CLINICAL APPROACH (Molecular Basis for Clinical Application)**

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ABSTRACT

SYNDROME-36 (presented in the year 2002) compiled by the Author since 1992 (SYNDROME-11) represents a cluster of 36 components (comprises Risk & Protective Factors, and Predictors), which determines the quality of Endothelial Function. The most frequent causes of Endothelial Dysfunction (ED) are Hypertension, Diabetes Mellitus, and Lipid (Dyslipidemia) which can be abbreviated as "HDL-Syndrome". In clinical practice, cigarette should be included as a major risk factor in the pathogenesis of atherosclerosis ("HDL-C Syndrome"). Four functional targets for the quality of the Endothelial Cell function are: 1. Lumen (Vasoconstrictors: ET-1, and Vasodilators: NO, Bradykinin, Hyperpolarizing Factor), 2. Growth (Stimulators: PDGF, ET, All, and Inhibitors: NO, PGI₂, TGF), 3. Inflammation (Proinflammatory: Adhesion Molecules, and Anti Inflammatory), and 4. Hemostasis (Prothrombotic: PAI-1, and Anti Thrombotic: PGI₂, tPA). The Endothelial Cell produces mediators that induce vasoconstriction, including ET (Endothelin), Prostaglandins (PGG₂ and PGH₂), and Angiotensin-II (AII). The key enzyme that regulates the local generation of AII is Angiotensin Converting Enzyme (ACE). Angiotensin-II also stimulates the generation of Superoxide. Nitric Oxide (NO) is not only involved in the control of vasomotor tone, but also in vascular homeostasis and immunological functions; eleven (11) roles of NO as atheroprotector of Endothelial Dysfunction have been summarized by the author. The five principles of the treatment of ED esp. in Diabetes Mellitus are: 1. Insulin Sensitizer (in DM: excellent glycemic control is obligatory), 2. ACE-inhibitors (Tissue ACE-I_s: Quinapril etc), 3. Lipid Modulators (Statins: Atorvastatin etc, and or Fibrates), 4. Antioxidants (Raxofelast 600 mg bid, Vit. C 500 mg/day, Vit. E 400-800 mg/day, Beta Carotene), and 5. Arginine Supplementation (Diet-KV, tablet Arginine 450 mg/day). Diet-KV comprises 68% cal. Cbh, 12% cal. Protein, 20% cal. Fat (Cholesterol < 300 mg/day), Fiber ± 35 g/day, Enriched with food supplements containing Arginine, Folate, Vit. B₆, and Vit. B₁₂. Several studies, ie. TREND (1996), BANFF (2000) have established that a potent tissue ACE-I (Quinapril) improves Endothelial Function in humans. Interestingly, the BANFF study showed this anti hypertensive agent from other classes has no effect on Endothelial Function. These results are strengthened by those from QUO VADIS (2000). In this study the treatment with Quinapril significantly reduced clinical ischemic events during the one-year period after coronary bypass graft surgery. The rank order of potency of several different ACE inhibitors have been determined by several investigators as follows (by sequent numbers): 1. Quinapril = Benazepril, 2. Ramipril, 3. Perindopril, 4. Lisinopril, 5. Enalapril, 6. Fosinopril, and 7. Captopril. Conclusion: Endothelial Dysfunction (ED) has been widely documented in patients with Diabetes Mellitus and more frequently in those with "HDL Syndrome". Five principles of treatment of ED are summarized. Tissue-ACE-I_s (Quinapril is the most potent one) play pivotal roles in the improvement of ED and these lines of evidence are strengthened by the results of TREND, BANFF, and QUO VADIS studies.

Keywords: diabetes mellitus, endothelium, hypertension, dyslipidemia

THE L-ARGININE-NITRIC OXIDE PATHWAY

Endothelium-dependent vasodilators such as Acetylcholine activate a Ca²⁺-dependent constitutive enzyme NOS (cNOS) that catalyses conversion of L-Arginine to L-Citrulline and NO (Cockcroft et al 2000). Once synthesized, NO diffuses to the underlying VSMC where it activates soluble Guanylate Cyclase (sGC), leading to a rise in cGMP and relaxation, cNOS can be competitively inhibited using guanidine substituted analogues of L-Arginine such as L-NMMA, L-NAME, and ADMA. Inorganic nitrates such as GTN (Glycerol Tri Nitrate) can activate the same effector pathway by providing a source of NO, and their activity is thus not dependent on the functional integrity of the vascular endothelium. Finally, hypercholesterolemia may activate L-NMMA, L-NAME and ADMA (production

of NO will be decreased), whereas statins and testosterone stimulate NOS (increased production of NO).

Tjokroprawiro (2001) summarized minimally 11 (eleven) atheroprotective effects of NO, such as:

1. Platelet Aggregation
2. Neutrophil Adhesion
3. Adhesion Molecules Expression
4. MCP-1
5. Endothelin
6. Ox-LDL
7. cGMP
8. Activates K⁺ Channel
9. Activates Na⁺/K⁺-ATP-ase Channel
10. Inhibits SMC proliferation
11. Suppresses Collagen Synthesis

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SYNDROME-36: A CLUSTER OF 36 DETERMINANTS COMPONENT OF ENDOTHELIAL FUNCTION

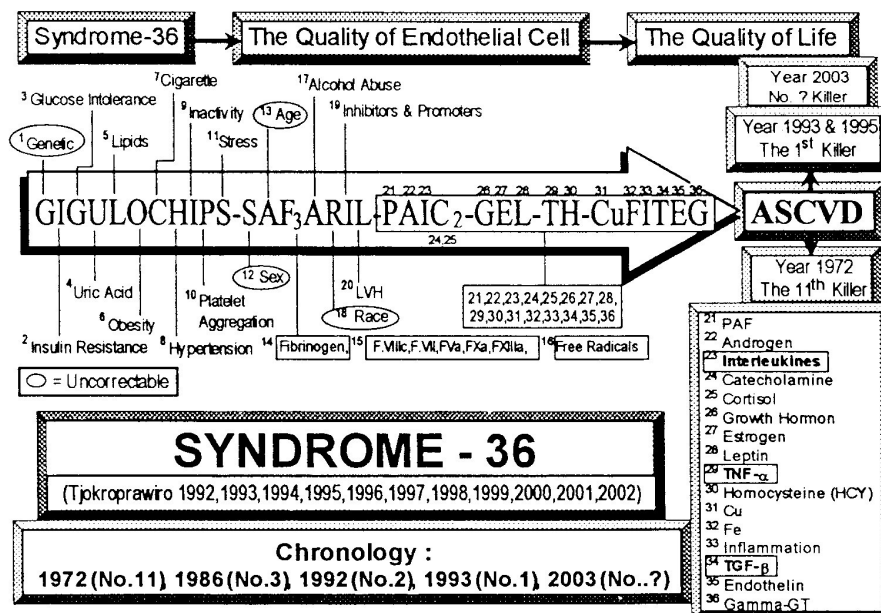
SYNDROME-36 : GIGULOCHIPS-SAF₃ARIL-PAIC₂-GEL-TH-CuFIT
 (Tjokroprawiro, 1992,1993,1994,1995,1996,1997,1998,1999,2001,2002)

Based on House to House Survey in Indonesia, it was reported that Atherosclerotic Cardiovascular Diseases (ASCVD_s) was claimed as the 11th, the 3rd, the 2nd, and the 1st killer in Indonesia by the years 1972, 1986, 1992, and 1993-1995, respectively.

Syndrome-11 (1992 & 1993), Syndrome-20 (1994), Syndrome-23 (1995), Syndrome-26 (1996), Syndrome-27 (1996), Syndrome-29 (1997), Syndrome-30(1998), Syndrome-32 (1999), Syndrome-33 (2000), Syndrome-34 (2001), and finally Syndrome-36 (2002) have been coined consecutively by Tjokroprawiro since 1992, in which the latest described about 36 determinant factors that are responsible for the quality of Endothelial Cell and Blood Vessels, hence, responsible for the quality of Life.

SYNDROME-36 (GIGULOCHIPS-SAF₃ARIL-PAIC₂-GEL-TH-CuFITEG) can be used as ASCVD_s-check list, and shows the image of the quality of blood vessels of patients.

This syndrome stands for : Genetic, Insulin Resistance, Glucose Intolerance, Uric Acid, Lipids, Obesity, Cigarette, Hypertension, Inactivity, Platelet Dysfunction, Stress-Sex, Age, Fibrinogen, F-VIIIc & F-VII, Free Radicals, Alcohol Abuse, Race, Inhibitors (Growth Inhibitors), Left Ventricle Hypertrophy, PAF, Androgen, Interleukines, Cortisol, Cate-cholamine, Growth Hormon, Estrogen, Leptin, TNF- α , Homo-cysteinemia, Cu, Fe, Inflammation, TGF- β , Endothelin, and Gamma-GT.



CONCLUSION : Physically, The Quality of Life completely depends on The Quality of the Endothelial Cells. By controlling factors described in Syndrome-36, lower prevalences of ASCVD_s may pursue. Hence, it is hoped that the prevalence of ASCVDs by the year 2003 can be pushed back towards the figure as reported in the year 1972.

THE QUALITY OF ENDOTHELIAL FUNCTIONS COMPLETELY DEPEND
 ON
 THE QUALITY OF SYNDROME-36
 THE QUALITY OF LIFE "IS IN LINE WITH" THE QUALITY OF ENDOTHELIAL FUNCTIONS
FIGURE-1. SYNDROME-36: A Cluster of 36 Components (Tjokroprawiro 2002)

TISSUE ACE AND TISSUE A-II: CLINICAL ASPECTS

Angiotensin-Converting Enzyme (ACE) is primarily localized (more than 90%) in various tissues and organs, most notably on the endothelium, but also within parenchyma and inflammatory cells (Dzau et al 2001). Tissue ACE is now recognized as a key factor in cardiovascular and renal diseases. Endothelial Dysfunction, in response to a number risk factors, especially "HDL-C Syndrome" (Hypertension, Diabetes, Lipids, - Cigarette), disrupts the balance of vasodilation and vasoconstriction, SMC-growth, the inflammatory and oxidative state of the vessel wall, and is associated with the activation of tissue ACE.

The activation of ACE may increase the generation of tissue A-II, and the latter will stimulate:

1. vasoconstriction
2. inflammation
3. plaque instability

4. thrombus formation
5. vascular remodeling

The degree of functional in vivo inhibition of tissue ACE produced by an ACE inhibitor is directly dependent on the binding affinity of the inhibitor and the concentration of the free inhibitor in the tissue. The rank order of potency of several different ACE inhibitors has been determined by investigators using competition analyses (Fabris et al 1990A, 1990B, Johnston et al 1989, 1993) and by direct binding of Tritium-labeled ACE inhibitors to tissue ACE (Kinoshita et al 1993), and the potency is (Table 1):

1. Quinaprilat = Benazeprilat
2. Ramiprilat
3. Perindoprilat
4. Lisinoprilat
5. Enalaprilat
6. Fosinopril
7. Captopril

Table 1. The Rank Order of Potency of ACE-I (Summarized: Tjokropawiro 2002)

The Rank Order of Potency of ACE-Inhibitors Quinapril is the Most Potent ACE-I		
Fabris et al 1990 1990A, 1990B, Johnson et al 1993, Dzau et al 2001		
① Quinaprilat = Benazeprilat	ACE and ACE-Is	
② Ramiprilat	Tissue	Circulating
③ Perindoprilat	± 90%	± 10%
④ Lisinopril		
⑤ Enalaprilat		
⑥ Fosinopril		
⑦ Captopril		
	Long term "Pleiotropic"	Short term "Vasodilation"

With regard to tissue ACE and its relation to coronary artery disease, the most clinical mechanistic studies include TREND, BANFF, and the Quo Vadis study are summarized (Table 2). The TREND study (Mancini et al 1996) was the first to show improved endothelial function in coronary artery disease patients who were normotensive but did not have severe hyperlipidemia or evidence of heart failure. A total of 105 secondary prevention patients were randomized to Quinapril 40 mg/day or placebo and observed for 6 months. Using quantitative coronary angiography, luminal diameter changes in response to acetylcholine were measured in both cohorts at baseline and at study completion.

After 6 months, patients in the Quinapril group showed significant improvement in endothelial response over the Placebo group ($p=0.002$), suggesting that ACE inhibitor attenuates the vasoconstrictive and superoxide-generating effects of Angiotensin-II while promoting endothelial cell release of NO consequent to accumulation of Bradykinin. The BANFF study (Anderson et al 2000) compared the effect of Quinapril 20 mg, Enalapril 10 mg, Amlodipine 5 mg, and Losartan 50 mg on blood flow and dilation of brachial artery.

The results were assessed by measuring flowmediated vasodilation of brachial artery in response to hyperemia

through highresolution intravascular ultrasound. Patients who all had evidence of CAD confirmed by angiography, were randomized in a crossover design to 3 drugs for 8 weeks each, with a 2-week washout period in between. Although all of the agents improved blood pressure, they differed in their ability to improve endothelial function. Quinapril was the only agent that

produced a significant improvement ($p < 0.02$) in endothelial function versus baseline. These results are strengthened by those from QUO VADIS (not detail described), a 2-phase, parallel-arm, phase 3 study of ACE inhibition in CAD patients scheduled to undergo coronary artery bypass graft surgery (Oostergera et al 2000).

Table 2. ACE Inhibition Improves Endothelial Function in Humans

TREND (Mancini et al 1996)	The Trial on Reversing Endothelial Dysfunction ACE-I with Quinapril Improves Endothelial Vasomotor Dysfunction in pts with CAD
BANFF (Anderson et al 2000)	The Brachial Artery Normalization of Forearm Function Quinapril Improves Endothelial Function (Vasodilation) in CAD, whereas other Hypotensive Agents <u>do not</u>
QUO VADIS (Oostergera et al 2000)	The Quinapril on Vascular ACE & Determinants of Ischemia Treatment with Quinapril Significantly reduced Clinical Events during the 1-year period after Coronary Bypass Graft Surgery

FIVE PRINCIPLES OF THE TREATMENT OF ENDOTHELIAL DYSFUNCTION (Focus on "HDL-C Syndrome")

The most frequent causes of Endothelial Dysfunction are Hypertension, Diabetes, Lipids - Cigarette which

can be shortened as "HDL-C Syndrome" (Tjokropawiro 2001). The summarized five principles of the treatment of Endothelial Dysfunction can be seen in Table 3.

Table 3. Five Principles of Treatment Endothelial Dysfunction in DM

1	Insulin Sensitizer (DM: Excellent Glycemic Control, TZDs)
2	ACE-Inhibitor (Tissue ACE-I): Quinapril etc
3	Lipid Modulators: Statins (Atorvastatin, Etc)
4	Arginine Supplementation: Diet-KV, Tablet: 450 mg, Etc
5	Antioxidants: - Raxofelast 600 mg bid - Vit C 500 mg OD, Vit.E 400-800 mg/day, Betacaroten
The patients should quit smoking!!	
TZDs: Thiazolidinediones	

CONCLUSION

The endothelial function is minimally determined by the quality of SYNDROME-36. Eleven roles of Nitric Oxide are summarized. More than 90% of ACE is primarily localized in various tissues and organs, most notably on the endothelium but also within parenchyma and inflammatory cells. Tissue ACE is now recognized as a key factor in cardiovascular and renal diseases. The rank order of potency of several ACE-Inhibitors is: Quinaprilat = Benazeprilat > Ramiprilat > Perindoprilat > Lisinopril > Enalaprilat > Fosinopril > Captopril. Five principles of the treatment of Endothelial Dysfunction esp. in "HDL-C Syndrome" should be adhered perfectly. TREND and BANFF studies have established that tissue ACE inhibition improves Endothelial Dysfunction in humans. Interestingly, the BANFF study showed that other anti hypertensive agents have no effect on endothelial function. These results are strengthened by those from Quo Vadis, study of ACE inhibition in coronary artery disease patients scheduled to undergo coronary bypass graft surgery. The treatment with Quinapril significantly reduced clinical ischemic events during the 1-year period after coronary bypass graft surgery.

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