Endothelial Dysfunction in the Pathogenesis and Treatment of Myocardial Ischemia

Thomas C. Andrews, MD

Internal Medical Grand Rounds
September 25, 1997

Thomas C. Andrews, MD
Assistant Professor of Internal Medicine, Cardiology Division

Research interests: Endothelial dysfunction in atherosclerosis and hyperlipidemia
Health care delivery

I. Introduction

There is a growing body of evidence suggesting that dysfunction of the vascular endothelium may occur early in the pathogenesis of atherosclerosis and that such dysfunction may play a pivotal role in the clinical manifestations of coronary artery disease.[1-3] With an increasing understanding of atherogenesis has come clues to the mechanism by which a variety of different therapies interact to improve endothelial function and (perhaps) thereby improve clinical outcome. Thus, the vascular endothelium has become the meeting point of basic scientists and clinicians approaching the problem of atherosclerosis from opposite directions. This grand rounds will discuss this collaboration from the clinician's viewpoint, primarily using the treatment of hypercholesterolemia as an example of how endothelial dysfunction may be reversed and clinical outcome improved.

II. The lipid lowering paradox

Data from the Framingham study first demonstrated that the likelihood of sustaining a cardiovascular event varies directly with the level of serum cholesterol. Further data from the MrFIT study showed that there is an approximate 2% increase in the likelihood of a coronary event for each 1% increase in serum cholesterol.[4] In the 1980's, a series of primary and secondary prevention trials demonstrated that lowering serum cholesterol decreases the likelihood of a cardiovascular event, but none of these early trials demonstrated a beneficial effect on all-cause mortality.[5] Subsequently, a series of atherosclerosis "regression trials" were undertaken to test the hypothesis that lowering serum cholesterol can cause regression of fixed atherosclerotic lesions. In general, the results of the regression trials were disappointing as the mean improvements in coronary lumen diameter were very small.[6] However, paradoxically these small changes in angiographic regression were accompanied by marked improvements in clinical events. Finally in the last three years, three landmark trials demonstrated conclusively that cholesterol lowering translates into important clinical benefit, including improvements in all-cause and cardiovascular mortality, myocardial infarction, cerebrovascular events, and need for coronary revascularization with angioplasty or coronary artery surgery. There has been considerable interest in understanding the mechanism involved.[7-9]

The first clue to understanding this "lipid lowering paradox" came from a series of observational studies which demonstrated that myocardial infarction is more commonly associated with plaque rupture at an angiographically non-critical stenosis (<50%) rather than a critical one (>75%).

		Angiographic severity of stenoses		
Study	# of pts	<50%	50-75%	>75%
Ambrose[10]	23	12	6	5
Little[11]	58	36	15	7
Hackett[12]	10	9	1	0
Giroud[13]	92	73	9	10
Totals	183	130 (71%)	31 (17%)	22 (12%)

Table 1: Severity of culprit lesion responsible for myocardial infarction. Subjects underwent coronary angiography before and after myocardial infarction, the stenosis responsible for the infarction (infarct-related artery) was determined by comparing the two studies from each patient.

Some have postulated that certain types of plaques may be more vulnerable to rupture, and that these plaques often are not the most angiographically severe -- rather they are composed of a substantial lipid core covered by a thin fibrous cap. New analyses of the more recent angiographic regression studies, such as the Lipoprotein and Coronary Atherosclerosis Study (LCAS), suggest that while improvements in mean coronary diameter are modest, there is a marked decrease in new lesion formation with lipid lowering therapy.[6] Presumably, these new lesions are the result of plaque rupture, as the progression of these isolated lesions is out of proportion to the surrounding coronary vasculature which is exposed to similar pro-atherogenic factors. Since plaque rupture is the mechanism for myocardial infarction, it is not surprising that the approximate 33% relative risk reduction seen in myocardial infarction in the large secondary prevention studies such as the Scandinavian Simvastatin Survival Study is similar to the relative risk reduction in new lesion formation seen in angiographic regression trials such as the Lipoprotein and Coronary Atherosclerosis Study.[6, 9] The inter-relationship between coronary endothelial function, plaque growth and rupture, dynamic coronary tone, and myocardial ischemia will be the topic of the remainder of these grand rounds.

III. Endothelial function in health and disease

As recently as 20 years ago, the endothelium was felt to be a relatively passive single-cell layer lining of the vasculature. In 1980, Furchgott and Zawadski reported their discovery of endothelium-derived relaxing factor (EDRF), which is released from the endothelium in response to acetyl choline and causes vascular relaxation.[14] Subsequent

work by Moncado and colleagues suggested that EDRF is nitric oxide (or a similar compound), synthesized from L-arginine in a reaction catalyzed by the enzyme nitric oxide synthase.[15, 16] Nitric oxide synthase can be blocked by arginine analogs, such as N^G-monomethyl-L-arginine (L-NMMA), and the production of biologic activity of nitric oxide is enhanced by the administration of excess L-arginine substrate.[17] Among its many biologic properties, nitric oxide acts as an arterial vasodilator, is anti-thrombotic and prevents leukocyte adhesion. In an LDL-receptor knockout mouse model, nitric oxide has important protective effects against the development of atherosclerosis.[18] The vasodilator function of nitric oxide is accomplished by binding to the heme iron of guanylate cyclase with the subsequent generation of cGMP and the relaxation of vascular smooth muscle by reducing the levels of intracellular calcium.[19] Nitric oxide is released from endothelial cells in response to a number of physiologic stimuli including increased blood flow (and thus increased sheer stress), as well as in response to a number of substances such as bradykinin, thrombin, and acetyl choline. Its extremely short half life suggests that nitric oxide acts locally on the arterial wall rather than systemically.

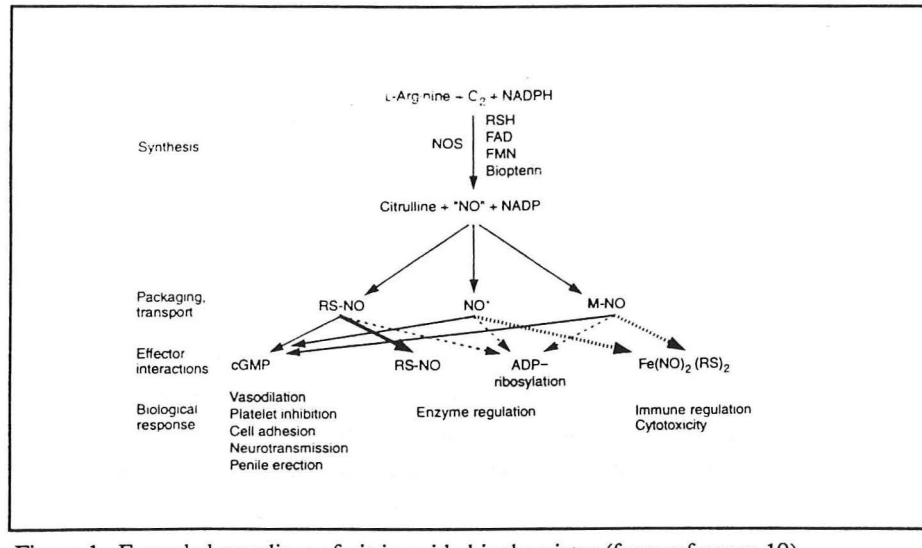


Figure 1. Extended paradigm of nitric oxide biochemistry (from reference 19)

Along with nitric oxide, the endothelial layer produces a number of other vasoactive substances. For example, endothelium-derived hyperpolarizing factor is as yet a poorly characterized substance released by the normal endothelium in response to acetyl choline. Its effects may be blocked by ouabain, a Na^+/K^+-ATPase inhibitor. The endothelins are a

family of potent vasoconstrictor substances, one of which (ET-1) is released from human endothelial cells. These and other vasoactive substance are important determinants of basal vascular tone. In addition to the role of the endothelial cell layer in the maintenance of vascular tone, this cell layer is critical to other processes such as vascular growth, monocyte adhesion, and the maintenance of an appropriate antithrombotic state.

Vasodilator substances	Vasoconstrictive substances
Endothelium dependent relaxing factor (nitric oxide)	endothelins
peptidoleukotrienes	peptidoleukotrienes
Endothelium dependent hyperpolarizing factor	angiotensin
PGE_2	vasopressin
$PGF_{2\alpha}$	
PGI_2	
acetyl choline	
ADP	
bradykinin	
serotonin	
catecholamines	
histamine	

Table 2: Partial listing of vasoactive substances produced by or acting through the vascular endothelium

When the normal functioning of the endothelial layer becomes disturbed in conditions such as hypercholesterolemia, the vessel wall shifts to a pro-atherogenic, prothrombotic state that tends towards inappropriate vasoconstriction, with decreased production of nitric oxide, over-production of monocyte adhesion molecules, perturbation in the normal ratio of plasminogen activator inhibitor (PAI) to tissue plasminogen activator (tPA), increased platelet adhesiveness and activation of smooth muscle cells.[20] All these factors are important in the clinical manifestations of coronary artery disease -- myocardial infarction and ischemia.

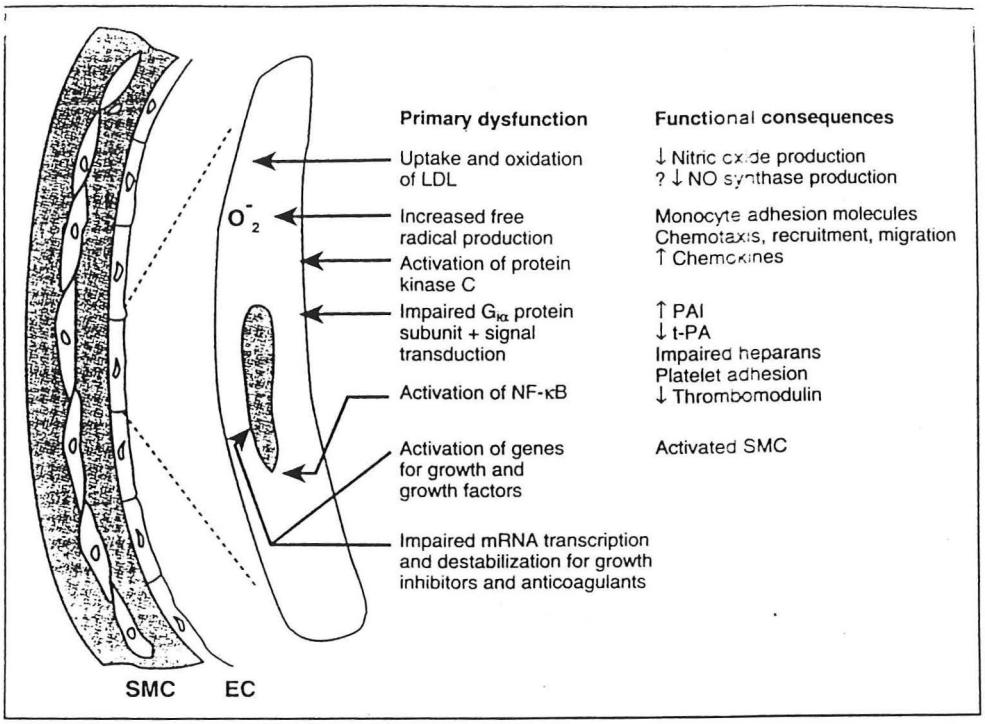


FIGURE 2. Some of the known primary subcellular and molecular disturbances that lead to vascular endothelial cell activation and atherogenesis. These alterations result from adverse interactions with oxidized low-density lipoprotein (LDL) cholesterol and give rise to the functional consequences typical of atherosclerosis. EC = endothelial cells; NF-κB = nuclear factor-kappa B; NO = nitric oxide; PAI = plasminogen activator inhibitor; SMC = smooth muscle cells; t-PA = tissue plasminogen activator.

Figure 2 (from reference 20)

IV. Assessing the L-arginine/NO pathway *in vivo*

The L-arginine/nitric oxide pathway can be studied in human subjects *in vivo*, and may be an appropriate marker for overall endothelial function. Ludmer and colleagues at the Brigham and Women's Hospital were the first to assess this aspect of endothelial function in the large epicardial coronary arteries by performing quantitative angiography before and after the intracoronary administration of acetyl choline. In patients without atherosclerosis, the response of the coronary arteries was vasodilation. However, in patients with angiographic atherosclerosis there was a paradoxical vasoconstriction induced by acetyl choline.[21] In a series of elegant physiological human experiments, Selwyn and Ganz and colleagues showed that this paradoxical vasoconstriction in atherosclerotic arteries can also be induced by mental stress,[22] increased flow (which increases sheer

stress),[23, 24] and the cold-pressor test.[25] Unfortunately, this technique requires cardiac catheterization, and therefore widespread application is limited.

A somewhat less invasive *in vivo* measure of endothelial function utilizes the changes in blood flow induced by infusion of acetyl choline into the brachial artery. A small brachial arterial catheter is inserted and used for drug administration. Forearm blood flow is estimated by volume plethysmography, with blood flow directly related to the change in circumference of the forearm when venous outflow is occluded. (figure) In contrast to Selwyn and Ganz's technique, this technique (referred to as "strain-gauge plethysmography") measures endothelial function in the small resistance vessels.[26]

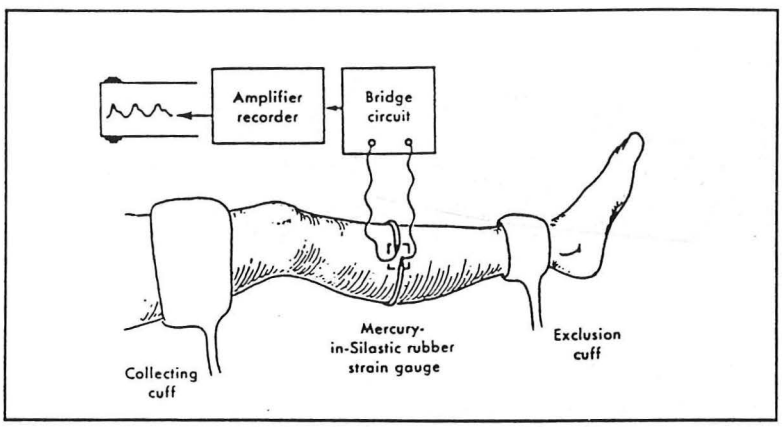


Figure 3: Strain-gauge plethysmography of the lower extremity. The exclusion cuff is inflated to suprasystolic pressures, and the collecting cuff intermittently sufficiently inflated to exclude venous outflow. The strain gauge measures changes in limb circumference, which can be mathematically related to blood flow into the limb segment. Acetyl choline and other vasoactive substances are infused intra-arterially above the isolated limb segment, and their effects on flow determined by serial studies.

Recently, a major breakthrough was made by Celermajer and his colleagues who developed an alternate method to assess endothelial function by measuring flow-mediated vasodilation.[27] They used high-resolution ultrasound to measure the brachial artery diameter at baseline and after five minutes of forearm ischemia. In normal subjects, relief of ischemia is associated with a several-fold increase in forearm blood flow (reactive hyperemia) which leads to increased sheer stress, release of nitric oxide and subsequent

vasodilation of the brachial artery. This flow-mediated vasodilation can be blocked by the administration of L-NMMA, an inhibitor of nitric oxide synthase.[28] A normal response has been defined as a 5-12% increase in brachial artery diameter.[29, 30] Endothelium-independent vasodilation is tested in this model after the administration of sublingual nitroglycerin.

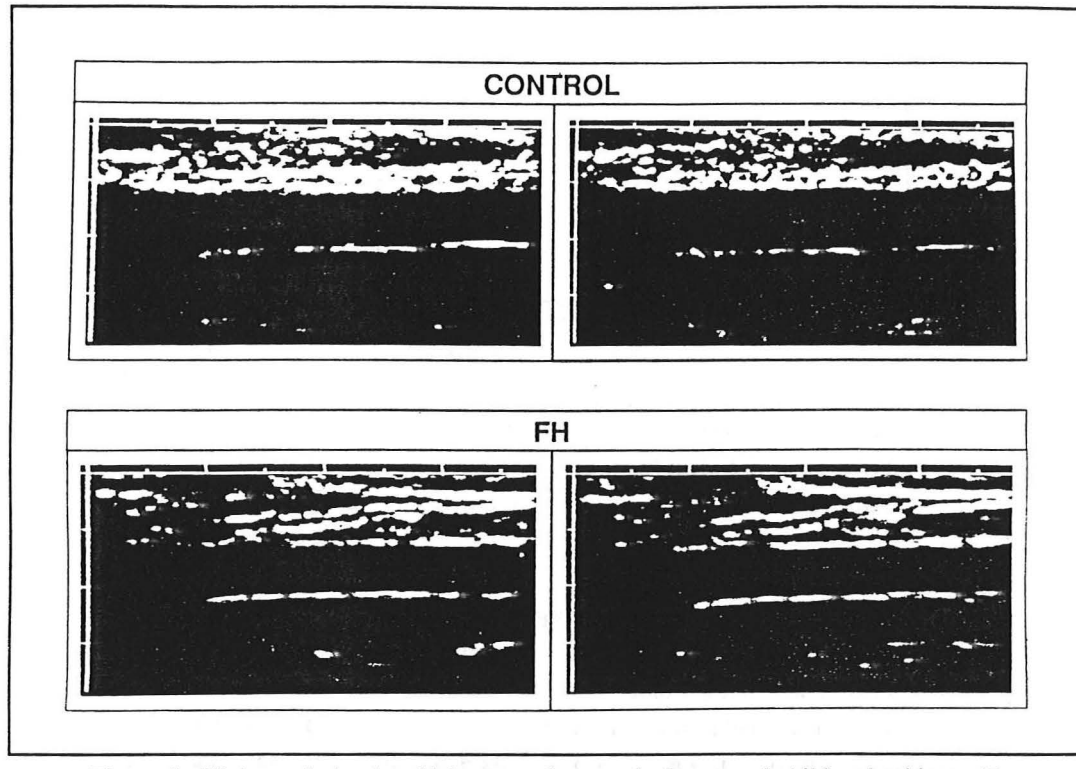


Figure 4: High-resolution brachial artery ultrasound of a control child and subject with familial hypercholesterolemia. Scans on the left are at baseline, and on the right after reactive hyperemia. An increase in arterial diameter can be appreciated in the control subject, but not in the subject with FH.

There is controversy to what extent endothelial function (or dysfunction) of the brachial artery reflects the functional status of the coronary arteries. Todd Anderson working with Selwyn and Ganz and the Brigham and Women's Hospital measured endothelial function in the brachial and coronary arteries of the same patients and found a

statistically significant correlation, although the absolute association was weak (r=0.36, p=0.01).[30] There is also a concern that the brachial artery seldom develops the structural changes of atherosclerosis typical of the coronary circulation, although a recent report suggests that nonobstructive atherosclerosis is more common than previously realized.[31] Therefore, endothelial function measured with the brachial artery model may not reflect the status of the coronary endothelium in all circumstances.

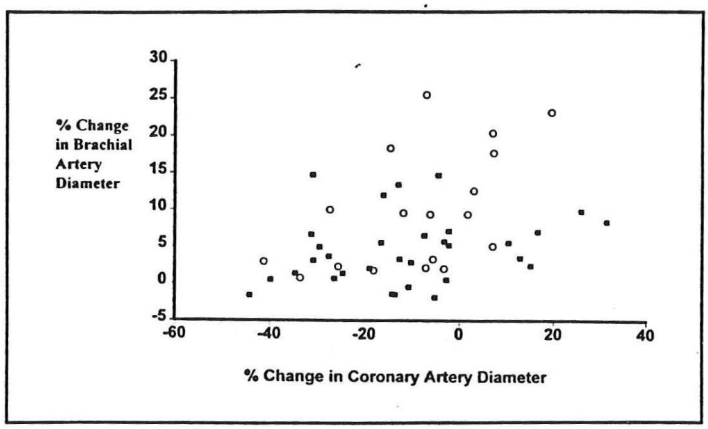


Figure 5: Relationship between brachial artery flow-mediated vasodilation and coronary response to acetyl choline in patients with (squares) and without (circles) coronary artery disease. From reference 30.

V. Endothelial function as a therapeutic target

Some evidence suggests that endothelial dysfunction is associated with adverse clinical events. Okamura and colleagues studied endothelial function using intracoronary acetyl choline testing in patients undergoing catheterization after a myocardial infarction. They found that the "infarct-related artery" demonstrated significantly more vasoconstriction than non-infarct related arteries of the same patient.[32] In addition, Bogaty et al showed a difference between stable and unstable angina patients in the response of the coronary arteries to the cold-pressor test or exercise: abnormal vasoconstriction was found in the arteries of patients with unstable angina but not in the stable angina patients. Our group has performed a pilot study of brachial artery endothelial function in 64 subjects undergoing cardiac catheterization. Subjects were divided into three groups based on their indication for catheterization: Group 1, stable angina, valvular heart disease or atypical chest pain; Group 2, "mild to moderate" unstable angina (Braunwald Class I or II), and

Group 3, "severe" unstable angina (Braunwald Class III) or acute myocardial infarction. Subjects were studied as soon as possible after cardiac catheterization, usually within 18 hours. No difference in brachial artery endothelial function could be demonstrated and no correlation existed between angiographic variables associated with plaque rupture and intracoronary thrombosis and brachial artery endothelial function.[33] Hence, to date there is no evidence for endothelial dysfunction in the brachial artery of patients with unstable angina or acute myocardial infarction, although the hypothesis has not been definitely tested in a trial with adequate statistical power to control for baseline confounding.

	Stable angina, atypical chest pain or valvular disease (n=28)	Braunwald Class I or II unstable angina (n=14)	Braunwald Class III unstable angina or acute MI (n=22)	p value
Clinical variables				
Age	61±2	65±2.9	64±2.3	NS
LDL (mg/dl)	115±9.6	72±12.8	90±12.8	<0.05
HDL (mg/dl)	38±3.3	26±4.4	32±4.4	<0.10
Male gender	61%	79%	64%	NS
Hypertension	63%	36%	73%	NS
Current cigarette use	35%	7%	13%	<0.10
Diabetes mellitus	21%	43%	18%	NS
Angiographic variables				
Ulcerated plaque	12%	30%	28%	NS
Intracoronary thrombus	8%	10%	16%	NS
Eccentric stenosis	23%	40%	61%	<0.05
Flow-mediated vasodilation	7.1±1.4%	5.9±1.1%	5.8±1.0%	NS

Table 3: Flow-mediated vasodilation in acute coronary syndromes (from reference 33)

A number of pharmacologic interventions have been shown to improve endothelial dysfunction. For example, intracoronary L-arginine has been shown to dilate coronary stenoses, probably by effecting the synthesis and release of nitric oxide.[34] Estrogen therapy has a beneficial effect on endothelial function in postmenopausal women, and this findings may at least partially explain the improvements in cardiac morbidity and mortality associated with this therapy.[35] The effect of progesterone in combination with estrogen are less well known. Some experimental data from a dog model suggest that progesterone may antagonize estrogen's effects on the endothelium.[36] Using serial measures of flow-mediated vasodilation in the brachial ultrasound model, our group studied endothelial function in 20 premenopausal women to determine the effects of endogenous estrogen's and progesterone during the menstrual cycle. We found that endothelium-dependent

vasodilation is most pronounced when estrogen is unopposed, and that when both estrogen and progesterone are elevated endothelial function is similar to that when neither hormone is naturally expressed. Serum progesterone level was a weak negative correlate of flow-mediated vasodilation ($R=-0.261$, $p<0.05$).[37] Our data differ from a previous study by Hashimoto and colleagues, in that they failed to detect an anti-estrogenic effect of endogenous progesterone in their study of 17 subjects.[38]

	Follicular	Mid-cycle	Luteal	p value
FMD (%)	8.0	10.9	7.6	0.04
TNG (%)	24.0	28.2	23.6	0.06
Estradiol (pmol/L)	49	174	157	<0.001
Progesterone (nmol/L)	0.7	0.9	9.4	<0.001
LDL-chol (mg/dl)	94.2	96.3	91.3	NS
HDL-chol (mg/dl)	47.2	49.5	49.8	NS
Triglycerides (mg/dl)	67.3	77.8	71.6	NS

Table 4: Flow-mediated vasodilation (FMD) and nitroglycerin-induced vasodilation (TNG) in premenopausal women during three phases of the menstrual cycle. Endothelium-dependent vasodilation was improved approximately 36% by unopposed endogenous estrogen's. From reference 37.

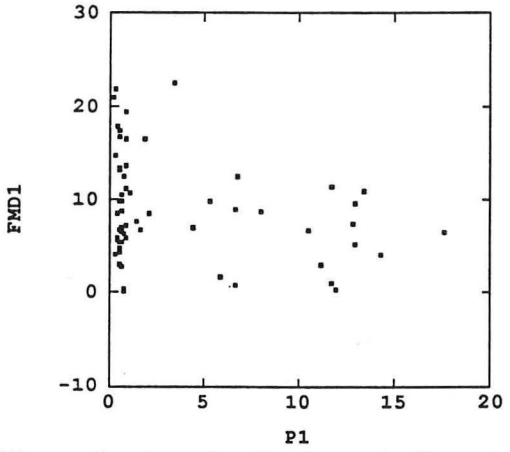


Figure 6: Relationship between flow-mediated vasodilation (FMD) and serum progesterone level (P1) (nmol/L) ($R = -0.261$, $p=0.027$). From reference 37.

VI. Endothelial dysfunction associated with hypercholesterolemia is reversible with LDL-cholesterol lowering

There is a large body of basic science literature demonstrating that LDL cholesterol, and particularly oxidized LDL, interferes with the L-arginine/nitric oxide pathway. For example, in an experiment quite similar to that of Furchgott and Zawadski, HE Andrews showed that LDL cholesterol blunts the normal acetyl choline-induced relaxation of vascular rings.[39] After the discovery of nitric oxide, Galle and colleagues showed that native and oxidized LDL both inhibit the release of nitric oxide from endothelial cells.[40] Excess oxidized LDL-cholesterol in the arterial wall leads to an overproduction of oxygen free radicals, such as superoxide anions which react with and subsequently degrade nitric oxide.[20,41] In addition, oxidized LDL has other important properties which inhibit the synthesis or effects of nitric oxide, such as impairment of nitric oxide synthase mRNA transcription,[42] inactivation of protein kinase C thus impairing synthesis of nitric oxide synthase [43] and down-regulating the inhibitory guanosine G protein subunit.[44] Finally, oxidized LDL leads to upregulation of nuclear factor-κB,[45] which promotes monocyte recruitment, inflammation, and thus further impairs nitric oxide function.

Working in a monkey model, Harrison and Heistad showed that dietary induced hypercholesterolemia leads to endothelial dysfunction and that this dysfunction is reversed by a low cholesterol diet.[46] These animal experiments led to a series of clinical trials of intermediate term (6-12 months) cholesterol lowering with endothelial function determined before and after therapy. Selwyn and Ganz's group showed that endothelial dysfunction could be reversed by a combination of LDL lowering and the administration of an antioxidant (probucol).[47] The Emory study led by Charles Treasure and Wayne Alexander showed that endothelial function could be improved by LDL lowering with an HMGCoA reductase inhibitor lovastatin.[48]

Our group studied the effect of gemfibrozil and niacin on endothelial function in a group of 100 patients with baseline HDL cholesterol level less than 40 mg/dl and LDL cholesterol less than 160 mg/dl. Although therapy was associated with a 27% improvement in HDL cholesterol and a 24% reduction in LDL cholesterol, we could not demonstrate an improvement in endothelial function. The two most important observations from this trial were first that endothelial function was normal in patients with this pattern of dyslipidemia who did not have a history of hypertension, and second that a history of hypertension was associated with a 50% reduction in flow-mediated vasodilation, even though the hypertension was treated to normal levels throughout the 2.5 years of the study.[49]

	Controls (n=47)	Treatment (n=53)	p value
Flow-mediated dilation (%)	6.3±7.3	6.9±6.5	0.67
Nitroglycerin-mediated dilation (%)	11.9±7.4	12.4±9.6	0.75

Table 5: Flow-mediated and nitroglycerin mediated dilation in subjects treated with niacin and gemfibrozil (treatment) and control subjects. From reference 49.

There is early evidence that high levels of triglycerides may adversely influence endothelial function. Vogel and colleagues measured endothelial function in healthy volunteers before and after a high- or low-fat breakfast, and showed that the fatty meal was associated with endothelial dysfunction that persisted for approximately 5 hours post-prandially.[50]

More recent studies strongly suggest that improvements in endothelial function associated with lowering LDL cholesterol occur too quickly to be ascribed to anatomical regression of coronary stenoses or to changes in atherosclerotic plaque composition. O'Driscoll showed that endothelial dysfunction is reversed after only 4 weeks of simvastatin therapy.[51] In a crossover study of patients with familial hypercholesterolemia, Stroes showed that endothelial function deteriorates within two weeks of discontinuation of lipid-lowering medications, improves with aggressive lipid lowering therapy within 12 weeks, and deteriorates again within 6 weeks off aggressive therapy.[52] Finally, Tamai showed that a single session of LDL-apheresis acutely improves endothelial function.[53]

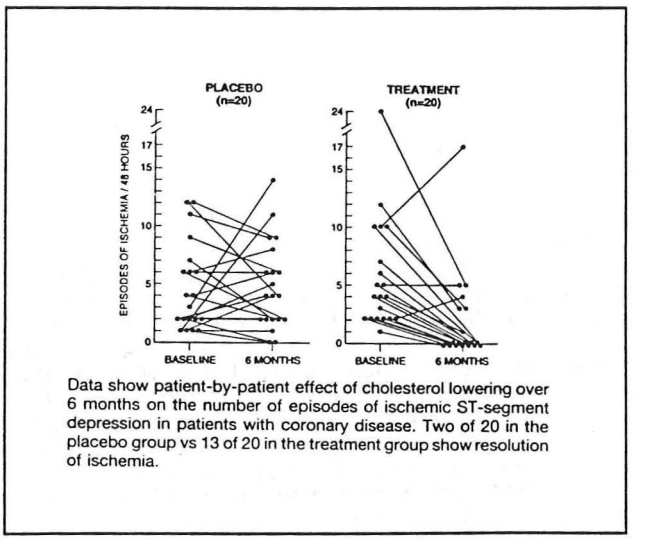
In sum, these data suggest that hypercholesterolemia, particularly high levels of LDL-cholesterol, is associated with endothelial dysfunction and that lowering LDL-cholesterol may lead to restoration of normal functioning long before anatomical changes are seen in the atherosclerotic plaque.

VII. LDL-cholesterol lowering in the treatment of myocardial ischemia

There is good evidence that inappropriate coronary vasoconstriction plays an important role in myocardial ischemia in patients with stable angina.[54] For example, Benhorin and colleagues demonstrated that the heart rate at which ischemia develops on an exercise test ("ischemic threshold") is significantly higher than the heart rate at which ischemia develops during daily life activities. In addition, activities that cause coronary

constriction such as mentally stressful situations and cigarette smoking have been demonstrated to cause myocardial ischemia in stable angina patients.

Several studies suggest that treatment of hypercholesterolemia can improve myocardial ischemia, perhaps by improving endothelial function and thereby preventing inappropriate coronary vasoconstriction. Kroon studied 42 hypercholesterolemic patients with quantitative angiography and exercise testing before and after 2 year treatment with simvastatin with or without bi-weekly LDL apheresis. There was no difference between the two treatment groups in regression of fixed lesions. However, in the patients undergoing LDL apheresis, there was an improvement in time to ischemia on exercise testing. Thus, these data demonstrate a functional improvement in ischemia in the absence of an anatomic improvement in stenosis severity.[55] Lance Gould measured changes in myocardial perfusion after dipyridamole using quantitative PET imaging before and after aggressive cholesterol lowering and showed improvement after only 3 months of therapy.[56] Finally, our group studied 40 patients with ischemia measured by the number of episodes of ST segment depression on ambulatory monitoring during daily life, and showed that such ischemia was markedly improved after 6 months of lipid-lowering therapy with lovastatin.[57] Ongoing studies are testing the hypothesis that the mechanism of these functional improvements are pathophysiologically linked to improvements in endothelial function.



Data show patient-by-patient effect of cholesterol lowering over 6 months on the number of episodes of ischemic ST-segment depression in patients with coronary disease. Two of 20 in the placebo group vs 13 of 20 in the treatment group show resolution of ischemia.

Figure 7: The effect of LDL-cholesterol lowering on myocardial ischemia during daily life. From reference 57.

VIII. Summary and future directions

Atherosclerosis is associated with dysfunction of the vascular endothelium, and such dysfunction may play an important role in the pathogenesis of atherosclerosis and in the development of myocardial infarction and ischemia. Hypercholesterolemia is associated with endothelial dysfunction in the absence of atherosclerosis, and treatment of hypercholesterolemia leads to rapid restoration of normal functioning. Finally, in patients with obstructive coronary atherosclerosis, treatment of hypercholesterolemia also leads to improvement in myocardial ischemia, and future studies will determine whether the mechanism of this improvement is pathophysiologically related to restoration of normal endothelial function.

IX. References

1. Topper J, Cai J, Anderson K, Falb D, Gimbrone M. Identification of endothelial genes differentially regulated by fluid mechanical forces. Circulation 1996;94:I-105.
2. Khan B, Harrison D, Olbrych M, Alexander R, Medford R. Nitric oxide regulates VCAM-1 gene expression and redox-sensitive transcriptional events in vascular endothelial cells. Circulation 1995;92:I-2417.
3. Cooke J, Singer A, Tsao P, etal. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 1992;90:1168-72.
4. Stamler J, Wentworth D, Neaton J. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986;256:2823.
5. Frick M, Elo O, Haapa K, et al. Primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia: Safety of treatment, changes in risk factors, and incidence of coronary heart disease. N Engl J Med 1987;317:1237.
6. Herd J, Ballantyne C, Farmer J, Ferguson J, Jones P, West M, Gould K, Gotto A. Effects of fluvastatin on coronary atherosclerosis in patients with mild to moderate cholesterol elevations (Lipoprotein and Coronary Atherosclerosis Study [LCAS]). Am J Cardiol 1997;80:278-86.
7. Sacks F, Pfeffer M, Moye L, Rouleau J, Rutherford J, Cole T, Brown L, Warnica J, Arnold J, Wun C-C, Davis B, Brauwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996;335:1001-9.
8. Sheperd J, Cobbe S, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301.
9. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease. Lancet 1994;344:1383.
10. Ambrose J, Tannenbaum M, Alexopoulos D, et al. Angiographic probession of coronary artery disease and the development of MI. J Am Coll Cardiol 1988;12:56-62.

11. Little W, Constantinexcu M, Applegate R, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? Circulation 1988;78:1157-66.
12. Hackett D, Davies G, Maseri A. Pre-existing coronary stenoses in patients with first MI are not necessarily severe. Eur Heart J 1988;9:1317-23.
13. Giroud D, Li J, Urban P, Meier B, Rutishauer W. Relation of the site of acute MI to the most severe coronary arterial stenosis at prior angiography. Am J Cardiol 1992;69:729-32.
14. Furchgott R, Zawadzki J. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;299:373-6.
15. Palmer R, Ferrige A, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524-6.
16. Palmer R, Ashton D, Moncada S. Vascular endothelial cells synthesize nitric oxide form L-arginine. Nature 1988;333:664-6.
17. Drexler H, Seiher A, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet 1991;338:1546-50.
18. Aji W, Ravalli S, Szabolcs M, Jiang X, Sciacca R, Michler R, Cannon P. L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. Circulation 1997;95:430-7.
19. Stamler J, Singel D, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. Science 1992;258:1898-1902.
20. Selwyn A, Kinlay S, Creager M, Libby P, Ganz P. Cell dysfunction in atherosclerosis and the ischemic manifestations of coronary artery disease. Am J Cardiol 1997;79(5A):17-23.
21. Ludmer P, Selwyn A, Shook T, Wayne R, Mudge GJ, Alexander R, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046-51.
22. Yeung A, Vekshtein V, Krantz D, Vita J, Ryan TJ, Ganz P, Selwyn A. The effect of atherosclerosis on the vasomotor response to coronary arteries to mental stress. N Engl J Med 1991;325:1551-6.
23. Nabel E, Selwyn A, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. J Am Coll Cardiol 1990;16:349-56.
24. Nabel E, Selwyn A, Ganz P. Mechanisms of flow-mediated vasodilation in human epicardial coronary arteries: Impairment by atherosclerosis. J Am Coll Cardiol 1990;16:349-356.
25. Nabel E, Ganz P, Gordon J, Alexander R, Selwyn A. Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. Circulation 1988;77:43-52.
26. Creager M, Cooke J, Mendelsohn M, Gallagher S, Coleman S, Loscalzo J, Dzau V. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. J Clin Invest 1990;86:228-234.
27. Celermajer D, Sorensen K, Gooch V, Spiegelhalter D, Miller O, Sullivan I, Lloyd J, Deanfield J. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111-15.
28. Joannides R, Haefeli W, Linder L, Richard V, Bakkali E, Thuillez C, Luscher T. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314-9.
29. Levine G, Frei B, Koulouris S, Gerhard M, Keaney J, Vita J. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1996;93:1107-1113.

30. Anderson T, Uehata A, Gerhard M, Meredith I, Knab S, Delagrange D, Lieberman E, Ganz P, Creager M, Yeung A, Selwyn A. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.
31. Sorensen K, Kristensen I, Celermajer D. Atherosclerosis in the human brachial artery. J Am Coll Cardiol 1997;29:318-22.
32. Okumura K, Yause H, Matsuyama K, Ogawa H, Morikami Y, Obata K, Sakaino N. Effect of acetylcholine on the highly stenotic coronary artery: Difference between the constrictor response of the infarct-related coronary artery and that of the noninfarct-related artery. J Am Coll Cardiol 1992;19:752-8.
33. Harris G, Green G, Personius B, Guidera S, Andrews TC. Brachial artery endothelial function in acute coronary syndromes. Submitted.
34. Tousoulis D, Davies G, Tentolouris C, Crake T, Toutouzas P. Coronary stenosis dilatation induced by L-arginine. Lancet 1997;349:1812-3.
35. Lieberman E, Gerhard M, Uehata A, Walsh B, Selwyn A, Ganz P, Yeung A, Creager M. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Int Med 1994;121:936-41.
36. Miller V, Vanhoutte P. Progesterone and modulation of endothelium-dependent response in canine coronary arteries. Am J Physiol 1991;261:R1022-7.
37. English J, Green G, Jacobs L, Andrews TC. Effect of the menstrual cucle on endothelium-dependent vasodilation of the brachial artery. Submitted.
38. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation 1995;92:3431-5.
39. Andrews H, Bruckdorfer K, Dunn R, Jacobs M. Low-density lipoprotein inhibits endothelium-dependent relaxation in rabbit aorta. Nature 1987;327:237-9.
40. Galle J, Mulsch A, Busse R, Bassenge E. Effects of native and oxideized low density lipoprotein on formation and inactivation of endothelium-derived relaxing factor. Arterio Thromb 1991;11:198-203.
41. Ohara Y, Pterson T, Harrison D. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91:2546-51.
42. Liao J, Shin W, Lee W, Clark S. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. J Biol Chem 1995;270:319-24.
43. O'Hara Y, Sayegh H, Yamin J, Harrison D. Regulation of endothelial constituitive nitric oxide synthase by protein kinase C. Hypertension 1995;25:415-20.
44. Liao J, Clark S. Regulation of G-protein subunit expression by oxidized low-density lipoprotein. J Clin Invest 1995;95:1457-63.
45. Marui N, Offermann M, Swerlick R, Kunsch C, Rosen C, Ahmand M, Alexander R, Medford R. Vascular cell adhesion moldecule-1 gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 1993;92:1866-74.
46. Harrison D, Armstrong M, Freiman P, Heistad D. Restoration of endotheliium-dependent relaxation by dietary treatment of atherosclerosis. J Clin Invest 1987;80:1808-11.
47. Anderson T, Meredith I, Yeung A, Frei B, Selwyn A, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488-93.
48. Treasure C, Klein J, Weintraub W, Talley J, Stillabower M, Kosinski A, Zhang J, Boccuzzi S, Cedarholm J, Alexander R. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481-7.
49. Andrews T, Whitney E, Green G, Kalenian R, Personius B. Effect of gemfibrozil±niacin±cholestyramine on endothelial function in patients with serum

low-density lipoprotein levels <160 mg/dl and high-density lipoprotein cholesterol levels < 40 mg/dl. Am J Cardiol 1997;80:In press.
50. Vogel R, Corretti M, Plotnick G. Effect of single high-fat meal on endothelial function in healthy subjects. Am J Cardiol 1997;79:350-4.
51. O'Driscoll G, Green D, Taylor R. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 1997;95:1126-31.
52. Stroes E, Koomans H, deBruin T, Rabelink T. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. Lancet 1995;346:467-71.
53. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis imrpves endothelium-dependent vasodilation in hypercholesterolemic humans. Circulation 1997;95:76-82.
54. Andrews T, Fenton T, Toyosaki N, Glasser S, Young P, MacCallum G, Gibson R, Shook T, Stone P. Subsets of ambulatory myocardial ischemia based on heart rate activity: Circadian distribution and response to anti-ischemic medication. Circulation 1993;88:92-100.
55. Kroon A, Aengevaeren W, van der Werf T, Uijen G, Reiber J, Bruschke A, Stalenhoef A. LDL-apheresis atherosclerosis regression study (LAARS). Effect of aggressive versus conventional lipid lowering treatment on coronary atherosclerosis. Circulation 1996;93:1826-35.
56. Gould K, Martucci J, Goldberg D, Hess M, Edens R, Latifi R, Dudrick S. Short-term cholesterol lowering decreases size and severity of perfusion abnormalities by positron emission tomography arfter dipyridamole in patients with coronary artery disease. Circulation 1994;89:1530-8.
57. Andrews T, Raby K, Barry J, Naimi C, Allred E, Ganz P, Selwyn A. The effect of cholesterol reduction on myocardial ischemia in patients with coronary disease. Circulation 1997;95:324-8.