Tr. J. of Medical Sciences
29 (1999) 117-123
© TÜBİTAK

Bülent SÖZMEN[1]
Fevziye UYSAL[2]
Derviş GAZİTEPE[1]
Leyla ASLAN[1]
Eser Yıldırım SÖZMEN[2]

A Novel Clinical and Laboratory Based Approach to Coronary Heart Disease: Relationship Between Angiography Findings, Antioxidant Enzymes and NO

Received: May 21, 1997

Department of Internal Medicine, Atatürk State Hospital, Yeşilyurt, İzmir-TURKEY
Department of Biochemistry, Ege University Faculty of Medicine, Bornova, İzmir-TURKEY

Abstract: Atherosclerosis is the most common manifestation of cardiovascular disease. However the exact mechanisms that take place in its pathogenesis have not yet been completely understood. Free radicals and lipid peroxidation may play an important role in the pathogenesis of atherosclerosis and endothelial dysfunction also may be an aggravating factor in this process. Unfortunately all of the previous studies have focused on the atherosclerotic tissue. Our aim was to establish a relationship between the antioxidant enzymes and plasma nitrate as an index of whole–body, endogenous nitric oxide production in coronary heart disease and also the relationship between these parameters and the severity of coronary disease. Thirty–three male patients referred to the Department of Cardiology for coronary angiography were considered for this study. Fifteen healthy men matched with patients for approximate age were selected as a control group. Plasma nitrite, nitrate levels, erythrocyte superoxide dismutase (SOD) and catalase activity were determined and serum lipid analysis was performed. Mean SOD and catalase activity in the patient group were significantly lower than in the controls ($P<0.05$, $P<0.01$, respectively). No significant difference was observed in nitrite or total nitrate levels. However nitrate values in patients were lower than in the controls ($P<0.05$). Although patients who were 55 or younger (n=14), had lower SOD and catalase activity than the control group ($P<0.01$, $P<0.01$), there was no significant difference between the nitrite, nitrate and total nitrate levels of these cases and the controls. In older patients (n=19), there was no significant difference in SOD, catalase, nitrite or total nitrate values when compared with the controls. In conclusion, hypercholesterolemia, increased oxygen free radicals, decreased antioxidant enzymes, increased ox–LDL and endothelial dysfunction caused by NO depletion are all intriguing mechanisms which act coherently in the progress of atherosclerosis. We proposed that the factors in the mechanism of atherosclerosis in younger patients is different from that in older ones. The insufficient antioxidant enzyme activity is a triggering factor to atherosclerosis. However it is not yet known if this decrease in antioxidant enzymes is a genetically determined primary factor or a secondary factor due to increased production of free radicals.

Key Words: NO, antioxidant enzymes, coromary heart disease.

Introduction

Atherosclerosis is the most common manifestation of cardiovascular disease. However the exact mechanisms that take place in its pathogenesis have not yet been completely understood. To date, a number of cardiovascular risk factors which include ageing, hypertension, hyperlipidemia, diabetes, ischemia and reperfusion have been identified (1) and these coronary risk factors have been shown to be associated with endothelial dysfunction in both experimental and clinical studies (2–4). It has been suggested that endothelial dysfunction also may be an aggravating factor in the atherosclerotic processes (4).

There are some controvesial results related with the production of nitric oxide (NO) in atherosclerosis. Some authors have reported low levels of NO in atherosclerotic arteries (5) while others have reported normal or high levels of NO but they proposed that the fraction was not active (6). Guerra et al. showed that atherosclerotic arteries release relatively lower amounts of active NO (5).

Abrams et al. suggested that endothelial dysfunction in humans is often caused by accelerated destruction of nitric oxide (NO), probably due to the presence of excess oxygen–free radical activity (7). Being a free radical species itself, NO is rapidly inactivated by superoxides (8, 9) which are in excess in atherosclerosis.

Darley–Usmar & Radomski proposed that there may be two processes regarding the effect of nitric oxide on endothelial cells (10): in normal physiological conditions, NO generation in more than SO anion and NO plays a scaevenging role in the prevention of free radical injury acting as an antioxidant. On the other hand, when free radical species overcome NO levels due to either an increase in SO anion production or a deficiency in NO production; a triggering mechanism is established which may play a role in the pathogenesis of atherosclerosis.

In fact, there is a lot of published work indicating that free radicals and lipid peroxidation may also play an important role in the pathogenesis of atherosclerosis (11). Lipid peroxidation is normally limited by a variety of antioxidant mechanisms including some enzymes such as superoxide dismutase and catalase and some vitamins such as alpha–tocopherol, ascorbic acid, retinol, etc. The relationship between the antioxidant enzymes and NO in the pathogenesis of atherosclerosis is still obscure.

In addition to these findings, it has also been shown that there is an impaired coronary blood flow in response to acetylcholine (ACh) in atherosclerosis (12, 13). Shiode et al. showed that the production of NO induced by ACh in coronary conductance and resistance vessels impaired in hypercholesterolemic patients and the impairment of conductance arteries was dependent on a decrease in NO production. However the authors did not determine the nitrite and/or nitrate levels (14) in these cases.

Unfortunately all of the aforementioned studies have focused on the atherosclerotic tissue. In this study, we investigated to focus on plasma NO levels in patients with atherosclerotic coronary heart disease and our aim was to establish any relationship between the antioxidant enzymes and plasma nitrate as an index of whole–body, endogenous nitric oxide production in coronary heart disease and also the relationship between these parameters and the severity of coronary atherosclerosis.

Patients and Methods

Patients:

Thirty–three male patients referred to the Ege University, Faculty of Medicine, Department of Cardiology for coronary angiography were considered for this study. All patients signed appropriate informed consent approved by the University Review Board. Patients with greater than 50% of a major vessel stenosed were diagnosed as having coronary artery disease and included in this study. Patients with previous myocardial infarction or valvular heart disease were excluded. Patients with unstable, severe or secondary hypertension, atrioventricular block exceeding first degree and not permanently paced, severe heart failure, hepatic failure, renal insufficiency or any severe or progressive disease were also excluded. All patients were in sinus, rhythm and were not being treated with any antihypertensive drug including diuretics. All of cases had typical angina (retrosternal chest pain on exercise, decreasing of the pain in ten minutes of rest, disappearance of the pain by sublingual nitrite in five minutes) and a significant ST–segment depression in the precordial leads on their ECG during exercise or at rest. There was no important concomitant disease.

Fifteen healthy men matched with patients for approximate age were selected as a control group. The control subjects had no history of cardiac or coronary disease and hypertension, without any intercurrent diseases or any other risk factors, and the ECG and physical examination were normal.

Coronary angiography was performed according to the Judkins technique. The severity of disease was evaluated according to an angiogram score, the method used by Gosta et al. (15). In this method the degree of occlusion of three major vessels and their major branches were added together and the result was noted as the patient's coronary score.

Blood parameters:

All patients stopped using drugs and the diet was restricted to foods including nitrite 5 days before the analysis. The fasting blood samples were put into tubes containing heparin. Nitrite and nitrate that were the end products of NO in plasma, SOD and catalase in erythrocyte and the lipid profile (Total Cholesterol, LDL, Triglyceride, HDL, VLDL) in serum were detected.

Nitrite–nitrate assay: Plasma nitrite levels were determined with a colorimetric method based on the Griess reaction (16). Nitrate levels were measured by using the enzymatic reduction of nitrate to nitrite with nitrate reductase from Aspergillus species (Boehringer Mannheim) (17) /*Serum triglyceride, total cholesterol and HDL–cholesterol levels* were determined with an Hitachi 705 autoanalyser and LDL–cholesterol levels were calculated using the Friedwald formula.

SOD activity: After separation of plasma, the packed erythrocytes were washed twice with 9 g/l NaCl solution and haemolysed with ice–cold water. SOD and catalase activity was determined immediately in haemolysates. The haemoglobin values of these haemolysates were determined with Drabkin's method. SOD activity was measured according to the method described by Misra & Fridovich based on the inhibition of autoxidation of epinephrine by SOD at 480 nm in a LKB Ultraspec–2 spectrophotometer. The activity of enzyme that causes 50% inhibition of epinephrine autoxidation was defined as 1 unit (18, 19).

Catalase activity: Catalase levels were determined as described by Aebi. The catalase mediated decomposition of H_2O_2 followed directly at 240 nm. One unit of catalase activity was defined as the level of enzyme required to decompose 1 µmol of H_2O_2 in 1 minute (20, 21).

Statistical analysis:

The results reported in this study represent the mean±SEM. Differences between the groups were evaluated using unpaired Student's t test and Kruskall–Wallis nonparametric test. A p value <0.05 was considered to be statistically significant.

Results

As it's seen from the Table 1, there was an increase in cholesterol levels (P<0.01) as well as trygliceride (P<0.01) and LDL cholesterol levels (P<0.01) and a decrease in HDL cholesterol levels (P<0.01) in the patient group compared with the controls. Mean SOD and catalase activity the patient group were significantly lower than controls (P<0.05, P<0.01, respectively). No significant difference were observed in nitrite and total nitrate levels. However nitrate values in patients were lower than in the controls (P<0.05).

Among the patient groups, when the cases with an angiogram score of 200 or higher (n=14) were compared with the controls, there was no difference in SOD values (Table 2). Catalase activity of patients with higher angiogram score were lower than the controls (P<0.05). We also couldn't find any difference in nitrite levels. On the other hand, serum nitrate and total nitrite levels of the patient group were significantly lower than the controls (P<0.05, P<0.05). Among the patient group when the cases with an angiogram score less than 200 (n=19) were evaluated, SOD and catalase activities were found to be lower than the controls (P<0.05, P<0.01) and there were no differences in nitrite, nitrate and total nitrate values between the patients and the controls.

We divided our patients into two subgroups according to their ages and the data is presented in Figure 1. Although patients who were 55 or younger (n=14), had lower SOD and catalase activity than the control group (P<0.01, P<0.01), there was no significant difference between the nitrite, nitrate and total nitrate levels of these cases and the controls. When older patients (n=19) were evaluated, there was no significant difference in SOD, catalase, nitrite and total nitrate values when compared with the controls. The only striking findings in this group was the depletion in nitrate values (P<0.05).

There was no difference in nitrite–nitrate levels and antioxidant enzyme activity between the patients at high risk (serum cholesterol levels<200 mg/dl) and low risk (serum cholesterol levels<200 mg/dl) according to cholesterol levels.

Table 1. The baseline characteristics of control and coronary heart disease groups. Data are given as mean±SEM. *p<0.01, **P<0.05.

	Control group n=15	CHD group n=33
Age	55.28±3.31	55.54±1.64
TAD (mmHg)	75.00±1.64	80.45±1.67
TAS (mmHg)	122.50±2.28	132.27±2.26
Triglyceride (mg/dl)	138.93±7.47	199.21±21.97*
Total cholesterol (mg/dl)	177.75±7.20	230.91±6.83*
LDL–cholesterol (mg/dl)	94.50±4.89	145.36±6.37*
HDL–cholesterol (mg/dl)	52.31±2.01	40.51±2.63*
SOD (U/g Hb)	2659.69±172.59	2089.09±206.55**
Catalase (U/g Hb)	12769.31±63494	10511.97±378.44*
Nitrite (µmol/l)	7.53±0.83	6.37±0.64
Nitrate (µmol/l)	24.46±1.99	19.52±1.55**
Total (nitrite+nitrate) (µmol/l)	31.73±1.89	27.93±1.69

	CS<200 n=19	Control n=15	CS≥200 n=14
Age	56.2±2.5	55.2±3.3	54.6±2.1
SOD (U/g Hb)	1939.7±267.8**	2659.6±172.6	2307.3±327.7
Catalase (U/g Hb)	10429.0±456.1*	12769.3±634.9	10610.5±647.8*
Nitrite (µmol/l)	6.3±1.1	7.53±0.83	6.5±0.6
Nitrate (µmol/l)	20.7±2.5	24.46±1.99	18.4±1.9**
Total NO (µmol/l)	28.3±2.9	31.73±1.89	26.4±1.6**
NSA	1.4±0.1	–	26.±0.1
CAS	122.3±11.1	–	316.2±27.2

Table 2. Results of patients according to coronary angiogram score. Data are given as mean ± SEM. * p<0.01, ** P<0.05 (comparisons were made versus control). Total NO means total nitrite, nitrate values; NAS, number of stenosed artery; CAS, coronary angiogram score.

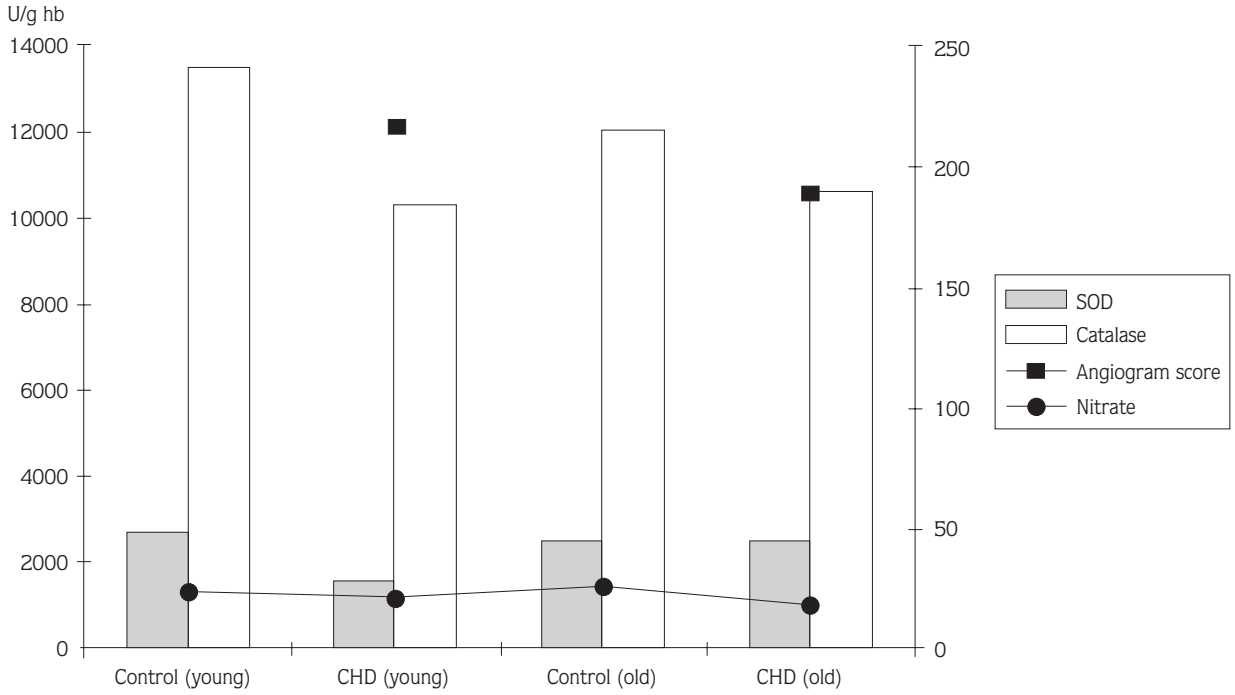

Figure 1. The relationship between antioxidant enzyme activities and angiogram score and nitrate. (CHD: Coronary heart disease).

Discussion

In this study we showed that patients with coronary heart disease have lower antioxidant enzyme activity than the controls. It is well known that reactive oxygen metabolites such as hydrogen peroxide and superoxide anion increase in otherosclerosis and hydrogen peroxide inactivates SOD (22) and superoxide anion radical inactivates catalase (23) and GSH–Px (24). In this study, a decrease in nitrate leves and no significant variation in nitrite levels were seen in the coronary heart disease group. Superoxide radicals from endothelial cells may favor the development of arterial vasospasm, not only by inactivation of NO (25) and inhibition of prostacyclin synthesis but also by a direct vasoconstrictor action on the vascular smooth muscle. There is another important effect of superoxide radicals on NO (26) The ONOO–formed as a product of the reaction between superoxide radicals and NO, can participate in the pathogenesis of the atherosclerotic lesion. Peroxynitrite may act as a relatively weak stimulus to guanylate cyclase activity in vascular smooth muscle cells and can initiate lipoprotein oxidation (26). Van der Vliet et al. investigated (27) the effect of peroxynitrite on plasma antioxidant

systems such as tocopherol, uric acid and ascorbate but not on antioxidant enzymes. They observed that these antioxidant defences were depleted after the addition of peroxynitrite to plasma. Recent investigations have been focused on the NO levels in atherosclerotic tissue. No experiments on nitrite–nitrate levels in plasma have been reported. Only Winlaw et al. (28) reported that there are no significant variations in nitrate levels in serum of patients with ischaemic heart disease: data based on a study of only 5 patients; although they found an increase in nitrite levels in patients with heart failure. Some explanations have been proposed for the depletion of NO in patients with risk factors for coronary heart disease. It may be due to a lower rate of synthesis of NO which may in turn result from substrate deficiency (29, 30) or a defect in the signal transduction pathways (31) or in the enzyme itself. Alternatively, Quyyumi et al. proposed that the depressed bioavailability of NO may be secondary to increased breakdown of normally produced NO by superoxide anions (6, 11). In addition, Ito et al. demonstrated that chronic oral treatment with L–NAME causes functional and structural changes in the coronary microvasculature but not in large epicardial coronary arteries in pigs in vivo (32). They suggested that defective NO synthesis plays an important role in mediating coronary microvascular disorders. They also showed that the presence of endothelial dysfunction of the coronary microcirculation in angina pectoris and normal coronary angiograms in which abnormal vasomotion in the coronary microcirculation contributes to myocardial ischemia. We were unable to determine whether there are differences in the magnitude of reduction of the bioavailabity of NO with the presence of risk factors such as hypertension, hypercholesterolemia, diabetes, smoking, or aging. In this study 6 patients with hypertension had the lowest nitrite–nitrate levels and lowest antioxidant ezymes (table 3). But the data was unchanged when these patients were excluded from this study.

It was remarkable to find a decrease in antioxidant enzymes in young patients. Coronary angiogram scoreS of the young patients were higher than those of the elderly but this difference was not significant. Based on this findings, we proposed that the factors in the mechanism of atherosclerosis in younger patients in different from those in older ones. The insufficient antioxidant enzyme activity is a triggering factor to atherosclerosis. However it is not yet known if this decrease in antioxidant enzymes is a genetically determined primary factor or a secondary factor due to increased production of free radicals. If the first assumption is correct, then determination of the antioxidant enzyme activities may be used to find the subjects at risk for atherosclerosis in future.

Table 3. The results related with antioxidant enzymes and NO status of hypertensive patients. Data are given as mean±SEM. *P<0.01, **P<0.05 (Comparisons were made versus control).

	Hypertensive patients (n=6)
SOD (U/g Hb)	1855.83±550.45*
Catalase (U/g Hb)	10823.00±1432.23**
Nitrite (µmol/l)	4.92±0.55*
Nitrate (µmol/l)	20.00±2.12**
Total (nitrite+nitrate) (µmol/l)	25.83±3.92**

Although it has been suggested that hypercholesterolemia increases the level of oxygen free radicals (33), there was no difference between the patients at high risk (serum cholesterol levels>200 mg/dl) and low risk (serum cholesterol levels<200 mg/dl), when we investigated the nitrite–nitrate levels and antioxidant enzyme activity in patient group according to cholesterol levels. On the other hand, Ohara et al. and Prasad et al. showed (11, 32) that superoxide anion production increased in endothelial cells from hypercholesterolemic vessels and these radicals (OFRs) would produce endothelial damage leading to the development and persistence of atherosclerosis. Leung et al. proposed that endothelial dysfunction associated with hypercholesterolemia is reversible with cholesterol reduction in man, even before angiographic evidence of atherosclerosis. The relations of total and LDL–cholesterol to endothelium–dependent vasomotor response observed in their study are also intriguing. Total and LDL cholesterol concentrations are significantly correlated with acetylcholine response at the follow–up but not at the base line (4). In accordance with this finding Lüscher et al. (2) proposed that the bioassayable EDRF release in coronary artery with hypercholesterolemia and atherosclerosis is clearly reduced. We found a significant positive correlation between the cholesterol levels and nitrite–nitrate levels and this correlation was clearer in older patients ($r=0.6$, $p<0.01$) and in patients with high coronary score ($r=0.5$, $P<0.05$). It is known that increases in the cholesterol levels, expecially in LDL–cholesterol levels cause oxidized–LDL and atherosclerosis. Lüscher et al. proposed that both native and oxidized–LDLs inactivate NO, and oxidized–LDL specifically interferes with the L–arginine pathway (1). We suggest that an elevation in nitrate levels correlated

with cholesterol elevation may be related with NO destruction by cholesterol. According to an attractive hypothesis that was proposed by Flavahan et al. (31), the G–protein would be inactivated in early atherosclerosis and hypercholesterolemia, yielding decreased NO formation in response to a certain level of stimulation of the endothelial surface receptors. This would be the first step towards endothelial dysfunction. Later when atherosclerosis manifests and/or hypercholesterolemia becomes more advanced. NO breakdown would be enhanced, possibly by increased formation of oxygen radicals. This hypothesis explains the NO depletion and/or NO elevation in different stages of atherosclerosis.

The SOD activity of patients whose coronary scores were below 200, was lower than that of patients whose coronary scores were higher than 200. Our interpretation was that the SOD activity is more affected in patients with lower angiogram scores due to higher mean cholesterol levels compared with the others (348.7±120 vs. 232.6±65). Although SOD activity was lower in patients with coronary scores below 200 and this value was different significantly compared with the controls, there was no significant difference (P>0.05) between patients with more and less atherogenesis.

In conclusion, it is obvious that in the stage of atherosclerosis if via oxygen radicals, there is an increased breakdown of NO which results with impaired endothelial function leading to critical alterations in vasomotor responses. Hypercholesterolemia, increased oxygen free radicals, decreased antioxidant enzymes and endothelial dysfunction caused by NO depletion are all intriguing mechanisms which act coherently in the progress of atherosclerosis. As it has been shown in our study, insufficient antioxidant enzyme activity and a decrease in total nitrite levels which might be independent triggering factors other than the well known risk factors such as age, sex, hypercholesterolemia and hypertension for atherosclerosis: therefore prospective studies are needed to confirm the hypothesis that antioxidant enzyme activity may be used to identify the subjects at risk for atherosclerosis.

Acknowledgement

The authors wish to thank to Ferhan Kulahcıoğlu Girgin, MD, PhD for helpful comments.

References

1. Lüscher TF, Tanner FC, Tschudi MR, Noll G, Endothelial dysfunction in coronary artery disese. Annu Rev Med 44: 395–418, 1993.
2. Vita JA, Treasure CB, Nabel EG et al, Coronary vasomotor response to acetyl choline relates to risk factors for coronary artery disease. Circulation 81: 491–7, 1990.
3. Jayakody L, Senaratne M, Thomson A, Kappagoda T, Endothelium–dependent relaxation in experimental atherosclerosis in the rabbit. Circ Res 60: 251–64, 1987.
4. Leung WH, Lau CP, Wong CK, Beneficial effect of cholesterol–lowering therapy on coronary endothelium–dependent relaxation in hypercholesterolemic patients Lancet 341 (12) 1496–1500, 1993.
5. Guerra R, Brotherton AFA, Goodwin PJ, Clark JR, Armstrong JL, Harrison DG, Mechanisms of abnormal endothelium–dependent vascular relaxation in atherosclerosis: Implications for altered autocrine and paracrine functions of EDRF. Blood Vessels 26: 300–14, 1989.
6. Minor RL, Myers PR, Guerra R, Bates JN, Harrison DG, Diet–induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. J Clin Invest 86: 2109–16, 1990.
7. Abrams, J, Beneficial actions of nitrates in cardiovascular disease. Am J Cardiol 77: 31C–37C, 1996.
8. Meredith IT, Yeung AC, Weidinger FF, Anderson TJ, Uehata A, Ryan TJ, Selwyn AP, Ganz P, Role of impaired endothelium–dependent vasodilation in ischemic manifestations of coronary artery disease. Circulation 87 (suppl V): V56–V66, 1993.
9. Gryglewski RJ, Palmer RMJ Moncada S, Superoxide anion is involved in breakdown of endothelium–derived relaxing factor. Nature 320: 454–6, 1986.
10. Darley–Usmar V, Radomski M, Free radicals in the vasculature: The good, the bad and the ugly. The Biochemist Oct/Nov: 15–8, 1994.
11. O'Hara Y, Peterson TE, Harrison DG, Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 91: 2541–51, 1993.
12. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ, Hypercholesterolemia attenuates endothelium–mediated vasodilation in man. J Clin Invest 86: 228–34, 1990.
13. Zeiher AM, Drexler H, Saurbier B, Just H, Endothelium–mediated coronary blood flow modulation in humans. J. Clin Invest 92: 652–62, 1993.

14. Shiode N, Nakayama K, Morishima N, Yamagata T, Matsuura H, Kajiyama G, Nitric oxide production by coronary conductance and resistance vessels in hypercholesterolemia patients. Am Heart J 131 (6): 1051–7, 1996.

15. Gosta H Dahlen, MD John R Guyton, Association of levels of lipoprotein, plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 74, (4): 758–65, 1986.

16. Green LC, Wagner DA, Glowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrite and nitrate in biological fluids. Anal Biochem, 126: 131–8, 1986.

17. Moshage H, Kok B, Huizenga JR, Jansen PLM, Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 41 (6): 892–6, 1995.

18. Misra HP & Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for Superoxide dismutase. J. Biol. Chem. 1972, 247: 3170–5.

19. Winterbourn C C, Hawkins R E Brian M & Carrel R W The estimation of red cell superoxide Dismutase activity. J. Lab. Clin. Med., 1975, 85, 337–341.

20. Aebi, H. Catalase in vitro. Methods in Enzymol., 1984, 105, 121–341.

21. Lück, H. Catalase. In: Methods of Enzymatic Analysis (Bergmeyer, H.U., eds.) Third Edition, Verlag Chemie Weinheim; 1983, vol. 3, p. 279.

22. Bray RC, Cockle SA, Martin–Fielden E, Roberts PB, Rotilio G, Calabrese L, Reduction and inactivation of superoxide dismutase by hydrogen peroxide. 139: 43–7, 1974.

23. Kono Y, Fridovich I, Superoxide radical inhibits catalase. J Biol Chem 257: 1571–8, 1982.

24. Blum J, Fridovich I, Inactivation of glutathione peroxidase by superoxide dismutase radical. Arch Biochem Biophys. 240: 500–8, 1985.

25. Chang KC, Chung SY, Chong WS, Suh JS, Kim SH, Noh HK, Seong BW, Ko KJ, Chun KW. Possible superoxide radical–induced alteration of vasucalr reactivity in aortas from streptozotocin–treated rats. J Pharmacol Exp Toxicol 266 (2): 992–1000, 1993.

26. Write CR, Brock TA, Chang LY, Crapo J, Briscoe P, Ku D, Bradley WA et al. Superoxide and peroxynitrite in atherosclerosis. Proc Natl Acad Sci USA, 91: 1044–8, 1994.

27. Van der Vliet A, Smith D, O'Neill CA, Kaur H, Darley–Usmar V, Cross CE, Halliwell B, Interactions of peroxynitrite with human plasma and its consituents: oxidative damage and antioxidant depletion. Biochem J 303: 295–301, 1994.

28. Winlaw DS, Smythe GA, Keogh AM, Schyvens CG, Spratt PM, Macdonald PS. Increased nitric oxide production in heart failure. The Lancet 344 (6): 373–4, 1994.

29. Quyyumi AA, Dakak N, Andrews NP, Husain S, Arora S, Gilligan DM, Panza JA, Cannon RO, Nitric oxide activity in the human coronary circulation. Impact of risc factors for coronary atherosclerosis. J Clin Invest 1995: 95: 1747–55.

30. Creager MA, Girerd XJ, Gallagher SH, Coleman S, Dzau VJ, Cooke JP, L–arginine improves endothelium–dependent vasodilation in hypercholesterolemic humans. J Clin Invest 90: 1248–53, 1992.

31. Flavahan NA, Atherosclerosis or lipoprotein induced endothelial dysfunction: potential mechanisms underlying reduction in EDRF/nitric oxide activity. Circulation 85: 1927–38, 1992.

32. Ito A, Egashira K, Kadokami T, Fukumoto Y, Takayanagi T, Nakaike R, Ruga T, Sueishi K et al. Chronic inhibition of endothelium–derived NO synthesis causes coronary microvascular structural changes and hyperreactivity to serotonin in pigs. Circulation 92: 2636–44, 1995.

33. Prasad K, Kalra J, Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. Am Heart J, 125: 958–71, 1993.