Biosciences, Biotechnology Research Asia Vol. **6**(2), 665-670 (2009)

Non-high density lipoprotein cholesterol, lipid ratios and risk of cardiovascular diseases

P. SAMPATH KUMAR[1] and A. VIJAYA ANAND[2]

[1]Department of Biochemistry, SASTRA University, SRC, Kumbakonam (India).
[2]Department of Biochemistry, M.I.E.T. Arts and Science College, Tiruchirappalli (India).

(Received: July 26, 2009; Accepted: August 21, 2009)

ABSTRACT

Cardiovascular disease (CVD) is multifactorial in etiology. No other life–threatening disease is as prevalent or expensive to society as CVD. Hence it is essential to diagnose the complications with multi markers and inexpensive methods. Atherogenic dyslipidemia, metabolic syndrome, non-high-density lipoprotein (non–HDL) cholesterol and lipid ratio offers the benefit of being an aggregate measure and currently believed to contribute to atherosclerosis. Therefore the present study was aimed to investigate the role atherogenic dyslipidemia, metabolic syndrome, non–HDL cholesterol and the various lipid ratios as an individual marker of cardiovascular events. Two hundred patients were recruited for the study, of which hundred belongs to control and hundred were had CVD (test group). The result showed that the test group have elevated levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) cholesterol, non-HDL cholesterol and various lipid ratios promotes CVD. Similarly, decreased levels of high-density lipoprotein (HDL) cholesterol were associated with the development of CVD. Atherogenic dyslipidemia and metabolic syndrome were also positively associated with CVD. These are more sophisticated methodology than that used in routine clinical practice can identify these multiple interrelated abnormalities.

Key words: Atherogenic dyslipidemia, Metabolic syndrome, Non-HDL cholesterol, Lipid ratio.

INTRODUCTION

India is experiencing an alarming increase in cardiovascular disease (CVD), which seems to be linked to changes in lifestyle and diet, rapid urbanization, and possibly an underlying genetic component. The World Health Organisation (WHO) estimates that, by 2010, 60 percent of the world's cardiac patients will be in India. About 50 percent of CVD–related deaths occur among people younger than 70, compared with about 22 percent in the West. Between 2000 and 2030, about 35 percent of all CVD deaths in India will occur among those aged 35 to 64, compared with only 12 percent in the United States and 22 percent in China (Leeder *et al.,* 2004).

While the measurement of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol are recommended in most current cardiovascular screening algorithms (Conroy *et al.,* 2003), recent guidelines have emphasized the importance of non-high density lipoprotein (non–HDL) cholesterol as a predictor of cardiovascular risk (Executive summary of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), while others have strongly advocated the use of specific lipid ratios such as TC to HDL cholesterol, LDL to HDL cholesterol, triglycerides (TG) to HDL cholesterol and non-HDL cholesterol and HDL cholesterol (Natarajan *et al.,* 2003; Kinosian *et al.,* 1994; Grover *et al.,* 1995). The aim of the present study is to focus the clinical utility of atherogenic dyslipidemia, metabolic syndrome, non–HDL cholesterol and the lipid ratios (TC to HDL cholesterol, LDL to HDL cholesterol, TG to HDL cholesterol, non-HDL to HDL cholesterol,) as an individual marker of cardiovascular events.

MATERIAL AND METHODS

Patients

The total number of patients included in this study was 200, all registered for a medical check-up at the Ramakrishna Nursing Home, Trichy, between April 2006 to September 2007. At the time of admission or entrance all patients responded to a standardized questionnaire covering many personal details (such as smoking habit, alcohol intake, physical activity, food habit, family history, and medical information) organised by trained interviewers. The study population consisted of 100 patients (test group) with a mean age of 59.4±8.2 years, the control group included 100 patients with mean age of 54.3±6.0 years.

Characterization of the study subjects

According to NCEP ATP III standard guidelines (Executive Summary of The Third Report of The National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), hypercholesterolemia and hypertriglyceridemia were defined as TC and TG levels of >200 mg/dL and >150 mg/dL, respectively. Low-HDL cholesterolemia was defined as HDL cholesterol level of <40 mg/dL. LDL hypercholesterolemia was defined as >100 mg/dL. Atherogenic dyslipidemia was defined as having hypercholesterolemia, hypertriglyceridemia, and/or low-HDL cholesterolemia. According to the NCEP criteria, an individual may be diagnosed to have metabolic syndrome if he or she has three or more of the following: obesity, hypertriglyceridemia, low-HDL cholesterolemia, hypertension and diabetes.

Biochemical parameters and Assay

Samples for the analysis of lipid profile were obtained in the fasting state. The venous blood samples were drawn into pyrogen-free blood collection tubes without additive. TC and TG were assayed by routine enzymatic methods using an auto analyser. HDL cholesterol was measured using the same enzymatic method after precipitation of the plasma with phosphotungstic acid in the presence of magnesium ions. For cost reasons, LDL cholesterol values have long been estimated using the Friedewald formula: [TC] " [total HDL cholesterol] " 20% of the TG value = estimated LDL cholesterol. The VLDL cholesterol is estimated as one-fifth of the TG. Non-HDL cholesterol value has calculated as TC " HDL cholesterol. The value of TC to HDL cholesterol ratio, LDL to HDL cholesterol, TG to HDL cholesterol and non-HDL to HDL cholesterol ratios were calculated by TC/HDL cholesterol, LDL/HDL cholesterol, TG/HDL cholesterol and non-HDL/HDL cholesterol respectively.

Statistical Analysis

Statistical analysis was performed with SPSS 12 statistical software package. Data were recorded on a pre-designed proforma and managed on spreadsheet. Descriptive statistics for quantitative variables were computed by mean and standard deviation. Means in the two groups were compared by Student's t-test. In this study, $p<0.05$ has been considered as statistically significant.

RESULTS

The study sample in control consisted of 58 males and 42 females and in test group 68 males and 32 females. Baseline characteristics of the test group (Table 1) were identified a predominantly male cohort (68%), with a relatively high percentage of smokers (48%), obesity (8%), diabetes (47%) and hypertension (49%) when compare with control. The percentage of hypercholesterolemia (44%), hypertriglyceridemia (53%), low–HDL cholesterolemia (65%), high–LDL cholesterolemia (50%), atherogenic dyslipidemia (21%) and metabolic syndrome (32%) were higher in test group when compare with control.

Table 2 summarises the baseline mean levels of blood pressure and lipid data in the control and test group. The mean values of sugar ($p < 0.001$), TC ($p < 0.001$), TG ($p < 0.003$), HDL cholesterol ($p < 0.001$), LDL cholesterol ($p < 0.001$), VLDL cholesterol ($p < 0.004$), non-HDL cholesterol ($p < 0.001$), TC to HDL cholesterol ($p < 0.001$), TG to HDL cholesterol ($p < 0.001$), LDL to HDL cholesterol ($p < 0.001$) and non-HDL to HDL cholesterol ($p < 0.001$) in test group was higher than in control. There were a significant increase found between test group and control in systolic BP ($p<0.03$) and diastolic BP ($p<0.02$).

Table 1: Clinical characteristic of the study subjects

	Control (n=100)	Test group (n=100)
Age	54.3±6.0	59.4±8.2
Elders e" 65 years (%)	4	21
Sex M/F	58/42	68/32
Family History of CHD (%)	Nil (0)	11
Food Habit – Veg/Non-Veg (%)	91/9	93/7
Cigarette Smoking – Ever/Never (%)	21/79	48/52
Alcohol Consumption – Ever/Never (%)	9/91	10/90
BMI (%)	2	12
Obesity (%)	2	8
Physical Activity – Low or Lack (%)	72	87
Diabetes (%)	20	47
Hypertension (%)	32	49
Hypercholesterolemia (%)	14	44
Hypertriglyceridemia (%)	33	53
Low-HDL cholesterolemia (%)	21	65
High-LDL cholesterolemia (%)	10	50
Atherogenic Dyslipidemia (%)	1	21
Metabolic Syndrome (%)	3	32

Table 2: Baseline mean levels of the blood pressure and biochemical parameters examined in serum samples of all patients

	Control	Test group
Systolic BP	123.5±11.3	127.3±14.7
Diastolic BP	81.8±7.8	84.4±8.9
Glucose	111.6±18.1	134.6±46.8
Total Cholesterol	165.3±29.9	202.0±41.4
Triglycerides	140.4±67.3	175.6±86.2
High-density lipoprotein cholesterol	42.1±8.3	36.9±6.1
Low-density lipoprotein cholesterol	95.2±25.6	137.4±41.9
Very Low-density lipoprotein cholesterol	28.2±13.7	35.1±16.9
Non-HDL cholesterol	123.3±27.1	164.9±39.9
TC to HDL cholesterol	4.0±0.8	5.6±1.2
LDL to HDL cholesterol	2.4±0.8	3.8±1.2
TG to HDL cholesterol	3.4±1.7	4.8±2.5
Non-HDL to HDL cholesterol	3.0±0.8	4.5±1.3

DISCUSSION

Atherogenic dyslipidemia, metabolic syndrome, non-HDL cholesterol and lipid ratios were positively associated with CVD. The lipid triad occurs commonly in persons with premature coronary heart disease (CHD) (Austin *et al.,* 1988; Austin *et al.,* 1990), hence the designation atherogenic lipoprotein phenotype or atherogenic dyslipidemia. Typical characteristics of persons with atherogenic dyslipidemia are obesity, abdominal obesity, insulin resistance, and physical inactivity (National Institutes of Health, 1998; National Institutes of Health, 1998). Many persons with type 2 diabetes have atherogenic dyslipidemia (Kreisberg, 1998; Verges, 1999; Durrington, 1999). In epidemiological studies in high-risk populations, the contributions of individual components of atherogenic dyslipidemia to CHD risk cannot reliably be dissected from the sum of lipid risk factors. Although there is evidence that each component of the lipid triad—low HDL cholesterol, small LDL, and remnant lipoproteins—is individually atherogenic, the relative quantitative contribution of each cannot be determined. For this reason, it is reasonable to view the lipid triad as a whole as a "risk factor."

From a population viewpoint, the increasing prevalence of the metabolic syndrome threatens to partially reverse the reduction in CHD risk that has resulted from a decline in serum LDL cholesterol levels in the United States population, which has occurred over the past three decades. The metabolic syndrome and its associated risk factors have emerged as a coequal partner to cigarette smoking as contributors to premature CHD (U.S. Department of Health and Human Services, 1996; Wilson *et al.,* 1998; National Institutes of Health, 1998; National Institutes of Health, 1998; Assmann *et al.,* 1998; Eckel and Krauss, 1998). In addition, the insulin resistance accompanying the metabolic syndrome is one of the underlying causes of type 2 diabetes (Groop, 1999; Cavaghan *et al.,* 2000). For these reasons, ATP III places increased emphasis on the metabolic syndrome as a risk enhancer.

Non–HDL cholesterol offers the benefit of being an aggregate measure that includes the concentrations of *all* lipoproteins currently believed to contribute to atherosclerosis. By providing an inclusive measure of all atherogenic particles, there is a strong degree of biologic plausibility for the hypothesis that non–HDL cholesterol is a superior predictor of CVD. Not surprisingly, as TG increase, non–HDL cholesterol correlates with apo B much better than LDL cholesterol (Abate *et al.,* 1993; Ballantyne *et al.,* 2001). Several groups encouraged use of non–HDL cholesterol long before supporting longitudinal epidemiologic data was published (Garg and Grundy, 1990; Frost and Havel, 1998).

Two recent studies from the Framingham Cohort Study (FCS) confirm what has been learned about non–HDL cholesterol. First, Liu *et al.,* (2006) found that after multivariate adjustment, there was no residual association between LDL cholesterol and risk for CHD after accounting for non–HDL cholesterol, whereas a strong positive and graded association between non–HDL cholesterol and risk for coronary disease after accounting for LDL remained. More recently, (Ingelsson *et al.,* 2007) reported improved discrimination, better model calibration statistics, and a significant association between non–HDL cholesterol and CHD after adjusting for other risk factors. In their model, the association between LDL cholesterol and CHD was not significant. Non–HDL cholesterol also appears to be a superior predictor of subclinical atherosclerosis.

Lipid parameters can be combined into ratios that reflect the proportion of atherogenic to antiatherogenic lipids and lipoproteins. With regard to lipid ratios, the present study data are consistent with prior reports that the ratio of TC to HDL cholesterol, LDL to HDL cholesterol, non-HDL to HDL cholesterol and TG to HDL cholesterol, (Castelli *et al.,* 1983; Grover *et al.,* 1995; Natarajan *et al.,* 2003; Blake *et al.,* 2002; Dobiasova, 2004) are strongly associated with incident cardiovascular events independently.

Perhaps the most widely used ratios are LDL to HDL cholesterol and TC to HDL cholesterol. Retrospective analysis of the Helsinki Heart Study (HHS) revealed that LDL to HDL cholesterol values >5 were associated with increased coronary risk (Manninen *et al.,* 1992), whereas an analysis of 5-year data from the Program on the Surgical Control of the Hyperlipidemias (POSCH) study found that the highest hazard ratios were for LDL to HDL cholesterol, with each 1-unit increment associated with a 1.2-fold increase in CHD risk (Buchwald *et al.,* 2001).

The ability of the TC to HDL cholesterol ratio to predict development of CHD has been evaluated by statistical tests that compared this ratio with other lipid measures (Kinosian *et al.,* 1994). The TC to HDL cholesterol ratio was superior to LDL to HDL cholesterol in the Lipid Research Clinics Coronary Primary Prevention trial (LRC-CPPT) cohort, an advantage that may be due to the inclusion of potentially atherogenic VLDL cholesterol (a surrogate for TG) in the numerator of the TC to HDL cholesterol ratio. In addition, analysis of the association between TC to HDL cholesterol and 8-year CHD risk among Framingham men and women revealed a continuous increase in risk with increasing ratio (Kinosian *et al.,* 1994). Thus, on the basis of the data in this study, as well as other nested case-control studies that have found the ratio of TC to HDL cholesterol to perform favorably. However, the present investigation showed that, high TG to HDL cholesterol ratio was as strong a lipid predictor of CHD as the widely used TC to HDL cholesterol ratio. Thus, the ratio of TG to HDL cholesterol is likely to be the result of metabolic interactions, which may confer greater risk than the isolated factor in either (Lianqun *et al.,* 2006). As a result of this interrelation between TG and HDL cholesterol, recent focus on high TG-low HDL cholesterol abnormality has grown considering risk assessment and drug therapy for CHD (Gaziano *et al.,* 1997; Rizos and Mikhailidis, 2002). Nevertheless, few data are available at this time regarding the relation of the non-HDL to HDL cholesterol ratio to CHD risk.

CONCLUSION

Non-HDL cholesterol shows a significant correlation with CVD and it has been useful to identify high-risk individuals. Cholesterol ratio is a simple approach for lipid risk assessment. This ratio reflects two powerful components of risk. A high

TC, TG and LDL cholesterol is a marker for atherogenic lipoproteins, whereas low HDL cholesterol correlates with the multiple risk factors of the metabolic syndrome and probably imparts some independent risk. A final advantage of non–HDL cholesterol and the various lipid ratios are that it can be readily calculated from the values obtained on a routine lipid profile.

ACKNOWEDGMENTS

Authors are thankful to Alhaj. Er. A. Mohamed Yunus, Correspondent, M.I.E.T. Institutions, Trichy, whose permission officiated as a key to this research.

REFERENCES

1. Leeder, S.R., Raymond, S.U., Greenberg, H., Lui, H., Esson, K. A race against time: the challenge of cardiovascular disease in developing economies. The Center for Global Health and Economic Development. New York: Columbia University (2004).
2. Conroy, R.M., Pyorala, K., Fitzgerald, A.P., Sans, S., Menotti, A., De Backer, G., De Bacquer, D., Ducimetière, P., Jousilahti, P., Keil, U., Njolstad, I., Oganov, R.G., Thomsen, T., Tunstall-Pedoe, H., Tverdal, A., Wedel, H., Whincup, P., Wilhelmsen, L., Graham, I.M; SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J*. **24**: 987-1003 (2003).
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *J Am Med Assoc* **285**: 2486-2497 (2001).
4. Natarajan, S., Glick, H., Criqui, M., Horowitz, D., Lipsitz, S.R., Kinosian, B. Cholesterol measures to identify and treat individuals at risk for coronary heart disease. *Am J Prev Med*. **25**: 50-57 (2003).
5. Kinosian, B., Glick, H., Garland, G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. *Ann Intern Med*. **121**: 641-647 (1994).
6. Grover, S.A., Coupal, L., Hu, X.P. Identifying adults at increased risk of coronary disease. How well do the current cholesterol guidelines work?. *JAMA*. **274**: 801-806 (1995).
7. Stampfer, M.J., Sacks, F.M., Salvini, S., Willett, W.C., Hennekens, C.H. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med*. **325**: 373-381 (1991).
8. Brown, B.G., Zhao, X.Q. Lipid therapy to stabilize the vulnerable atherosclerotic plaque: new insights into the prevention of cardiovascular events. In: Grundy SM, ed. Cholesterol-lowering therapy: evaluation of clinical trial evidence. *New York: Marcel Dekker, Inc.*, **249**-72 (2000).
9. Grundy, S.M. Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome. *Am J Cardiol.* **81**: 18B-25B (1998).
10. Krauss, R.M. Atherogenicity of triglyceride-rich lipoproteins. *Am J Cardiol.* **81**: 13B-17B (1998).
11. Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., Willett, W.C., Krauss, R.M. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA.* **260**: 1917-21 (1988).
12. Austin, M.A., King, M.C., Vranizan, K.M., Krauss, R.M. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation.* **82**: 495–506 (1990).
13. National Institutes of Health. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults-the evidence report. NIH Pub. No. 98-4083. Bethesda, MD: National Heart, Lung and Blood Institute, 228 pages (1998).
14. National Institutes of Health. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults -the evidence report. *Obesity Res.* **6** (suppl 2): 51S-209S (1998).
15. Kreisberg, R.A. Diabetic dyslipidemia. *Am J Cardiol.* **82**: 67U-73U (1998).
16. Verges, B.L. Dyslipidaemia in diabetes mellitus: review of the main lipoprotein abnormalities and their consequences on the development of atherogenesis. *Diabetes Metab.* **25** (suppl 3): 32-40 (1999).
17. Durrington, P.N. Diabetic dyslipidaemia.

Baillière's Clin Endocrinol Metab. **13**:265-78 (1999).

18. U.S. Department of Health and Human Services. Physical activity and health: a Report of the Surgeon General. Atlanta, Georgia: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, 278 pages (1996).
19. Wilson, P.W.F., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., Kannel, W.B. Prediction of coronary heart disease using risk factor categories. *Circulation.* **97**: 1837-47 (1998).
20. Assmann, G., Cullen, P., Schulte, H. The Munster Heart Study (PROCAM): results of follow-up at 8 years. *Eur Heart J.* **19** (suppl A): A2-A11 (1998).
21. Eckel, R.H., Krauss, R.M; for the AHA Nutrition Committee. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. *Circulation.* **97**: 2099-100 (1998).
22. Groop, L.C. Insulin resistance: the fundamental trigger of type 2 diabetes. *Diabetes Obes Metab.* **1** (suppl 1): S1-S7 (1999).
23. Cavaghan, M.K., Ehrmann, D.A., Polonsky, K.S. Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. *J Clin Invest.* **106**: 329-33 (2000).
24. Abate, N., Vega, G.L., Grundy, S.M. Variability in cholesterol content and physical properties of lipoproteins containing apolipoprotein B-100. *Atherosclerosis.* **104**: 159-71 (1993).
25. Ballantyne, C.M., Olsson, A.G., Cook, T.J., Mercuri, M.F., Pedersen, T.R., Kjekshus, J. Influence of low high-density lipoprotein cholesterol and elevated triglyceride on coronary heart disease events and response to simvastatin therapy in 4S. *Circulation*. **104**: 3046–51 (2001).
26. Garg, A., Grundy, S.M. Management of dyslipidemia in NIDDM. *Diabetes Care*. **13**: 153–169 (1990).
27. Frost, P.H., Havel, R.J. Rationale for use of non-highdensity lipoprotein cholesterol rather than low-density lipoprotein cholesterol as a tool for lipoprotein cholesterol screening and assessment of risk and therapy. *Am J Cardiol.* **81**: 26B-31B (1998).
28. Liu, J., Sempos, C.T., Donahue, R.P., Dorn, J., Trevisan, M., Grundy, S.M. Non–high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. *Am J Cardiol.* **98**:1363-1368 (2006).
29. Ingelsson, E., Schaefer, E.J., Contois, J.H., McNamara, J.R., Sullivan, L., Keyes, M.J., Pencina, M.J., Schoonmaker, C., Wilson, P.W., D'Agostino, R.B., Vasan, R.S. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. *JAMA*. **298**: 776–785 (2007).
30. Castelli, W.P., Abbott, R.D., McNamara, P.M. Summary estimates of cholesterol used to predict coronary heart disease. *Circulation.* **67**: 730-734 (1983).
31. Blake, G.J., Otvos, J.D., Rifai, N., Ridker, P.M. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation.* **106**: 1930-1937 (2002).
32. Dobiasova, M. Atherogenic index of plasma [log (triglycerides/HDLcholesterol)]: theoretical and practical implications. *Clin Chem.* **50**: 1113-1115 (2004).
33. Manninen, V., Tenkanen, L., Koskinen, P., Huttunen, J.K., Manttari, M., Heinonen, O.P., Frick, M.H. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study: implications for treatment. *Circulation*. **85**: 37-45 (1992).
34. Buchwald, H., Boen, J.R., Nguyen, P.A., Williams, S.E., Matts, J.P. Plasma lipids and cardiovascular risk: a POSCH report. Program on the surgical control of the hyperlipidemias. *Atherosclerosis.* **154**: 221-7 (2001).
35. Lianqun Jiaa, Shiyin Longb, Mingde Fua, Bingyu Yana, Ying Tianb, Yanhua Xuc, Lantu Goua (2006). Relationship between total cholesterol/high-density lipoprotein cholesterol ratio, triglyceride/high-density lipoprotein cholesterol ratio, and high-density lipoprotein subclasses. *Metabolism Clinical and Experimental*. **55**: 1141-1148 (2006).
36. Gaziano, J.M., Hennekens, C.H., O'Donnell, C.J., Breslow, J.L., Buring, J.E. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. *Circulation*. **96**: 2520-2525 (1997).
37. Rizos, E., Mikhailidis, D.P. Are high-density lipoprotein and triglyceride levels important in secondary prevention: impressions from the BIP and VA-HIT trials. *Int J Cardiol*. **82**: 199-207 (2002).