High-Density Lipoprotein Subfractions In Normolipidemic Individuals Without Clinical Atherosclerosis Lipoprotein Subfractions in an Adult Population

Fabio L. Sodr,^{1,2} Vera S. Castanho,^{1,2} Lucia N. Castilho,^{1,2} Silvia de Barros-Mazon,¹ and Eliana C. de Faria^{1,2*}

¹*Departamento de Patologia Clnica da Faculdade de Cincias Mdicas Universidade Estadual de Campinas, So Paulo, Brasil*

²*Ncleo de Medicina e Cirurgia Experimental, Universidade Estadual de Campinas, So Paulo, Brasil*

This study evaluated the serum concentrations of lipids, lipoproteins, apolipoproteins, and high-density lipoprotein (HDL) subfractions in Brazilian adults. We analyzed the distribution of lipids in HDL₂ and HDL₃ in a normolipidemic population without evidence of established cardiovascular disease (CVD). A total of 93 males and 92 females, healthy and normolipidemic, volunteered to be submitted to a clinical examination, a blood collection, and to answer a questionnaire aimed at determining signs and symptoms of atherosclerotic disease. Their fasting plasma lipid, lipoproteins, apolipoproteins, and the cholesterol and triglyceride concentrations in HDL₂ and HDL₃, isolated by microultracentrifugation, were determined by enzymatic-colorimetric methods. The interpercentile intervals (2.5–97.5) for the population were

established as being 5–18 mg/dL in men and 4–28 mg/dL in women for HDL₂ cholesterol (HDL₂chol) and 1–57 mg/dL in men and 2–61 mg/dL in women for HDL₃ cholesterol (HDL₃chol). HDL₂ triglyceride levels (HDL₂Tg) in men were 1–26 mg/dL and in women 2–28 mg/dL; moreover, the HDL₃ triglyceride (HDL₃Tg) intervals were established as 4–46 mg/dL for both sexes. The determination of reference ranges for lipids in HDL subfractions in populations without clinical atherosclerosis, is an useful tool for metabolic, diagnostic, and therapeutic approaches. We determined the intervals for HDL₂chol, HDL₃chol, HDL₂Tg, and HDL₃Tg. There were variations with sex and/or age for HDL₂chol, HDL₃chol, and HDL₂Tg in the studied population. *J. Clin. Lab. Anal.* 20:113–117, 2006. 2006 Wiley-Liss, Inc.

Key words: high-density lipoprotein subfractions; cholesterol; triglycerides; sex; age

INTRODUCTION

As in economically developed countries worldwide, Brazil has a very high frequency of cardiovascular disease, although a decrease in the mortality rate from CHD may now be observed, as shown in a recent study (1). In Campinas, an urban area in the state of So Paulo, the age-classified mortality rates for ischemic disease are similar to those obtained for the North American population. The most frequent cause of death is cardiovascular disease, representing 30.5% of the deaths notified in 2002 and 2003 (2).

The great clinical interest in the evaluation of plasma lipoproteins is generated by its importance in the determination of CHD risk, in the therapeutic follow-up of dyslipidemia, and in prevention of CHD (3).

Prospective studies demonstrate that the risk for coronary disease is directly related to low-density lipoprotein cholesterol (LDLchol) and inversely related to high-density lipoprotein cholesterol (HDLchol) levels in plasma (4,5). An increase of 10 mg/dL in HDLchol levels is associated with a 42% reduction in the risk for CHD (4–7).

Grant sponsor: Fundo de Apoio ao Ensino e Pesquisa da Universidade Estadual de Campinas So Paulo, Brazil.

*Correspondence to: Dr. Eliana Cotta de Faria, C.P. 6111, CEP 13084-971, Baro Geraldo, Campinas, SP, Brasil.

E-mail: cotta@fcm.unicamp.br

Received 14 January 2005; Accepted 5 December 2005

DOI 10.1002/jcla.20111

Published online in Wiley InterScience (www.interscience.wiley.com).

Using multivariate analysis, studies carried out by the Lipid Research Clinics Program Follow-Up Study demonstrated that the HDLchol is a variable with strong predictive value for mortality by coronary heart disease (CHD). Independently of cholesterol levels (including values below 200 mg/dL), the CHD risk was inversely associated with the level of HDLchol and the total-cholesterol/HDLchol ratio identified people at high risk. According to the same analysis, HDLchol was shown to be a factor having twice the predictive power than LDLchol for CHD risk (8,9).

The mechanisms responsible for the antiatherogenic properties of HDL are still not completely elucidated and are certainly not restricted to its plasmatic concentration. The heterogeneity of HDL particles, in terms of density (HDL₂, HDL₃), size (HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c}), and protein content of apolipoprotein (apo AI or apo AI plus apo AII) should be considered specially in patients with increased risk for CHD. Therefore, the analysis of the composition of HDL subfractions could be a better predictor of risk of coronary disease than the total HDL quantification. Recent studies correlate concentrations of different HDL subfractions with the risk of coronary and extracoronary disease (10–18) but this issue is not yet clear.

Until now there have been very few reports in the literature on the determination of the reference intervals for lipids in HDL subfractions in adults. Therefore the objective of this work was to determine the reference ranges of cholesterol and triglycerides in HDL subfractions, by sex and age, in an adult population without evidence of established cardiovascular disease.

MATERIALS AND METHODS

Subjects and Lipoprotein Profile Evaluation

A total of 185 healthy individuals, selected by consecutive sampling, 93 men and 92 women, aged from 20 to 86 years participated in this study. They were as well normolipidemic according to NCEP III's (19) criteria and presented serum levels up to borderline for total cholesterol (Tchol), up to near or above optimal for LDLchol, and desirable for triglyceride (Tg).

The individuals were examined at the Dyslipidemia Clinic of the University Hospital -UNICAMP for anthropometric measurements and for information on the presence of cardiovascular disease. Established cardiovascular disease (CVD) was defined as the occurrence of one or more of myocardial infarction, coronary artery bypass grafting and percutaneous transluminal coronary angioplasty, stroke, transient ischemic attack, and peripheral arterial disease; these individuals were excluded from the study.

Blood samples were collected in ethylene diaminetetra acetic (EDTA)-containing tubes (following a 12-hr fast) for the lipoprotein profile evaluation (Tchol, LDLchol, HDLchol, Tg, apolipoprotein AI and B100) and to obtain HDL (HDL₂ and HDL₃) subfractions. Total cholesterol and Tg serum levels were quantified by enzymatic-colorimetric methods in the Hitachi-917 analyzer (Roche Diagnostics, Mannheim, Germany). HDLchol was enzymatically determined in the supernatant after sodium phosphotungstate/MgCl₂ ApoB100-containing lipoprotein precipitation and LDLchol by Friedewald's equation. Apolipoprotein AI and B100 (Apo AI and B100) were quantified by nephelometry Array 360 (Beckman Instruments, Palo Alto, CA).

HDL subfraction isolation was achieved by the microultracentrifugation (20) technique. Following Apo B100-containing lipoprotein precipitation, 90 μ L of the supernatant containing total HDL was transferred to polyethylene tubes, with 45 μ L of KBr solution ($d = 1.346$ g/mL) added for density adjustment to 1.125 g/mL. HDL₂ was isolated after ultracentrifugation for 3 hr and 30 min at 4°C, 20 psi, 100,000g, in an Airfuge microcentrifuge rotor A100-30 (Beckman Instruments, Palo Alto, CA). Thirty microliters of the supernatant (corresponding to the HDL₂) were removed for cholesterol and triglyceride quantification. The remaining infranatant (105 μ L), corresponding to the HDL₃ fraction, was submitted to the same measurements (chol and Tg).

The study protocol was approved by the Ethics Committee of the School of Medical Sciences, UNICAMP.

Statistical Analysis

The normal distribution of the data was tested by the Kolmogorov-Smirnov test. Logarithmic transformation was applied to Tchol, HDL₃Tg, and ApoB100. For the comparison tests for sex and age groups (≤ 39 , 40–59, and ≥ 60 years) the analysis of variance (ANOVA) monofactorial was used as well as Mann-Whitney or Kruskal-Wallis tests. Correlation between the HDL subfractions and anthropometric variables were verified by the Spearman test. All statistical analyses were considered significant when P values were ≤ 0.05 .

RESULTS

According to NCEP III's criteria (19) serum lipids, lipoproteins, and body-mass index (BMI) were within optimal and/or desirable limits. The Apo AI and B100 values (Table 1) are in agreement with the manufacturer's reference ranges: 73 to 169 mg/dL and 58 to 138 mg/dL, respectively.

TABLE 1. Clinical and Biochemical Parameters Categorized by Sex, Age, and in the Total Population[†]

Groups/ parameters (n)	Sex		Age			Total population (185)
	Male (93)	Female (92) ^a	≤ 39 years (122) ^a	40–59 years (45)	≥ 60 years (18)	
Age (years)	35 ± 18	39 ± 14	27 ± 5	49 ± 6	74 ± 8	37 ± 16
BMI (kg/m ²)	23 ± 2	23 ± 3	23 ± 2	24 ± 3	24 ± 3	23 ± 3
Tchol (mg/dL)	162 ± 28	178 ± 35*	159 ± 29**	191 ± 31	194 ± 29	170 ± 33
LDLchol (mg/dL)	100 ± 23	107 ± 27*	97 ± 24**	119 ± 24	115 ± 23	104 ± 26
HDLchol (mg/dL)	42 ± 11	51 ± 11*	44 ± 11**	51 ± 12	55 ± 12	47 ± 12
Tg (mg/dL)	104 ± 46	109 ± 51	103 ± 51	110 ± 48	117 ± 37	107 ± 49
Apo AI (mg/dL)	135 ± 23	150 ± 25*	137 ± 24**	151 ± 24	151 ± 29	143 ± 25
Apo B100 (mg/dL)	77 ± 20	102 ± 34*	86 ± 33**	101 ± 27	91 ± 21	90 ± 31

[†]Data expressed as mean ± SD; (n) number of individuals.

^aMann-Whitney and ANOVA tests. ****P* < 0.05, sex and age comparison, respectively.

BMI, body mass index.

TABLE 2. Cholesterol and Triglycerides in HDL Subfractions by Sex, Age and in the Total Population[†]

Parameters (n)	Sex		Age			Total population (185)
	Male (93)	Female (92) ^a	≤ 39 years (122) ^a	40–59 years (45)	≥ 60 years (18)	
HDL ₂ chol (mg/dL)	11 ± 3	14 ± 7*	13 ± 5	14 ± 10	11 ± 4	12 ± 6
HDL ₂ Tg (mg/dL)	9 ± 6	12 ± 8*	14 ± 7	12 ± 8	13 ± 5	11 ± 7
HDL ₃ chol (mg/dL)	28 ± 16	33 ± 18*	42 ± 10**	35 ± 15	43 ± 10	30 ± 17
HDL ₃ Tg (mg/Dl)	16 ± 8	19 ± 12	16 ± 6	20 ± 12	18 ± 8	18 ± 10

[†]Data expressed as mean ± SD; (n) number of individuals.

^aMann-Whitney and ANOVA tests. ****P* < 0.05, sex and age comparison, respectively.

When the population was analyzed by age, significant higher values for Tchol, LDLchol, HDLchol, ApoAI, and Apo B100 serum levels were found in individuals older than 40 years of age, in agreement with the literature (21) (Table 1).

Surprisingly, when the data were analyzed by sex, Tchol, LDLchol were higher in women than in men, as well as ApoAI and Apo B100, but no BMI differences were found (Table 1). The subgroup with BMI ≥ 25 kg/m² presented higher concentrations of total cholesterol, LDL-chol, Lp(a), HDLchol and HDL₃chol as compared to individuals with BMI < 25 kg/m².

HDLchol concentrations were significantly increased amongst women and increased with age reaching a plateau by their forties (Table 1). The older women did not present higher Tchol or LDLchol values as compared to men in the same age group (data not shown).

Cholesterol and triglyceride contents of HDL subfractions (HDL₂ and HDL₃) are represented in Table 2. A significant difference was observed in HDL₂chol, HDL₂Tg, and HDL₃chol, which were greater in women as compared to men; HDL₃chol was over two times higher than HDL₂chol in both sexes. There were no

significant differences in HDL₃Tg concentrations irrespective of sex or age. Age differences were observed only for HDL₃chol, with lower values in the middle aged group (Table 2).

The interpercentile interval (2.5–97.5) for HDL₂chol was 5 to 18 mg/dL in men and 4 to 28 mg/dL in women. The same intervals for HDL₃chol of the studied population were established as being 1–57 mg/dL for men and 2–61 mg/dL for women. For HDL₂Tg in men the interval was 1–26 mg/dL and in women it was 2–28 mg/dL; moreover the HDL₃Tg intervals were established as 4–46 mg/dL for both sexes.

Age correlated positively with HDL₂chol (*r* = 0.156), HDL₃chol (*r* = 0.275), and HDL₃Tg (*r* = 0.147). The BMI did not correlate significantly with any one of the variables tested.

DISCUSSION

In the present study, cholesterol concentrations in HDL subfractions are similar to some population-control studies that evaluated cardiovascular risk (11,22–24), although they differ from others (13,25–28). There is much controversy in the literature

concerning the role of HDL subfractions against atherosclerosis. Bakogianni et al. (29) consider HDL₂ more protective but others describe increased protection by HDL₃ including protection against LDL oxidation (30–32).

Our data are in agreement with Hartung et al. (24), which showed an HDL₃chol mean concentration of 39 mg/dL in sedentary women with a mean age of 41 years. Similar values were described by Gardner et al. (33), who demonstrated values significantly higher in women when compared to men in the same age group. Moreover, coherent results were obtained in Meilahn et al.'s study (22), carried out in women with an average age of 50.6 years, mean BMI of 25.9 kg/m², whose HDL₃chol value was 39.5 mg/dL. We found statistically significant sex differences in serum HDL₂chol, HDL₂Tg, and HDL₃chol concentrations, as described by Gardner et al. (33).

However, Shepherd et al. (34) showed that HDL₂ and HDL₃ are substantially different in lipid and protein composition, and that HDL₂chol concentrations are higher in women than in men. The increased HDL₃chol in women, demonstrated in the present work, could be a protective factor against atherosclerosis in this sex. In contrast with our study, HDL₃chol concentrations were not different between the sexes in Shepherd et al.'s (34) study.

The variability of results found in the literature regarding cholesterol concentrations in HDL subfractions may be due to small sample sizes, differences in methodologies (ultracentrifugation, electrophoresis, or selective precipitation), or to characteristics variations in the studied populations, such as BMI, age range, sex, or lifestyle.

Evidence from controlled clinical trials indicates that modifications in lifestyle are determinant for concentrations of HDL, HDL₂chol, HDL₃chol and Apo AI (10,22,34). Physical activity and alcohol consumption increase the two subfractions; physical activity affects predominantly HDL₂chol (10,22,25,35), while alcohol consumption interferes with HDL₃chol (14,36,37). Obesity and insulin resistance are associated with low HDLchol and HDL₂chol levels and a high rate of HDL₃chol (33). The exogenous estrogen predominantly increases HDL₂chol levels, while testosterone may diminish them (23,33).

In climacterium, when cardiovascular diseases are important cause of death, women present a decline in HDLchol and an increase in LDLchol levels with consequent increased risk (28). Moreover, it is known that HDLchol levels present a greater impact on the mortality rate and on cardiovascular disease evaluation risk in women than in men (38). Therefore, in this period, cholesterol evaluation in HDL subfractions

could contribute to define lifestyle alterations, precocious use of medication or more rigorous control of already existing pathologies.

This study introduced cholesterol and triglyceride reference ranges for HDL subfractions in a normolipidemic adult population, without evidence of established CVD. Moreover, these reference limits in a population without overt atherosclerosis, are important guides in the evaluation of patients' CHD risk. Further studies on the chemical composition of HDL in populations presenting atherosclerosis are necessary to fully understand the clinical meaning of these parameters in atherosclerosis.

ACKNOWLEDGMENTS

We thank Mrs. Mirian Danelon and Mr. Helymar da Costa Machado for their excellent technical and statistical support.

REFERENCES

1. Mansur AP, Favarato D, Souza MFM, et al. Trends in death from circulatory diseases in Brazil between 1979 and 1996. *Arq Bras Cardiol* 2001;76:497–510.
2. Secretaria Municipal da Saúde da Cidade de Campinas: <http://www.campinas.sp.gov.br/saude/dados/mortalidade/2.htm>.
3. Mille NE, Hammet F, Salttissi S, et al. Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. *Br Med J* 1981;282:1741–1743.
4. Castelli WP. Lipids, risk factors and ischemic heart disease. *Atherosclerosis* 1996;124(Suppl):S1–S9.
5. Kannel WB. Contributions of the Framingham Study to the conquest of coronary artery disease. *Am J Cardiol* 1988;62:1109–1112.
6. Kannel WB. Metabolic Risk Factors for Coronary Heart Disease in Women: Perspective from the Framingham Study. *Am Heart J* 1987;114:413–419.
7. Giannini SD. Lipoproteínas como Fatores de Risco em Mulheres. *Rev Soc Cardiol Estado de São Paulo* 1996;6:681–689.
8. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and non contraceptive estrogen use in women: Results from the Lipid Research Clinics Program Follow-Up Study. *Circulation* 1987;75:1102–1109.
9. Godsland IF, Wynn V, Crook D, Miller EN. Sex, plasma lipoprotein, and atherosclerosis: prevailing assumptions and outstanding questions. *Am Heart J* 1987;114:1467–1503.
10. Nye ER, Carlson K, Kirstein P, Rösser S. Changes in high-density lipoprotein subfractions and other lipoprotein induced by exercise. *Clinica Chimica Acta* 1981;113:51–57.
11. Ballantyne FC, Clark RS, Simpson HS, Ballantyne D. High-density and low density subfractions in survivors of myocardial infarction and in control subjects. *Metabolism* 1982;31:433–437.
12. Marques-Vidal P, Ruidavets JB, Cambou JP, Cambien F, Chap H, Perret B. Distribution, fatty acid composition and apolipoprotein A-I immunoreactivity of high density lipoprotein subfractions in myocardial infarction. *Atherosclerosis* 1995;112:29–38.
13. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and risk of myocardial infarction. *N Engl J Medicine* 1991;325:373–381.
14. Sich D, Saïdi Y, Giral P, et al. Hiperalphalipoproteinemia: characterization of a cardioprotective profile associating increased

- high-density lipoprotein 2 levels and decreased hepatic lipase activity. *Metabolism* 1998;47:965–973.
15. Laakso M, Pyörälä K. Adverse effects of obesity on lipid and lipoprotein levels in insulin-dependent and non-insulin-dependent diabetes. *Metabolism* 1990;39:117–122.
 16. Atger V, Giral P, Simon A, et al. High-density lipoprotein subfractions as markers of early atherosclerosis. *Am J Cardiol* 1995;75:127–131.
 17. Ohta T, Saku K, Takata K, Nagata N, Maung KK, Matsuda I. Fractional esterification rate of cholesterol in high-density lipoprotein (HDL) can predict the particle size of low density lipoprotein and HDL in patients with coronary heart disease. *Atherosclerosis* 1997;135:205–212.
 18. Mowat BF, Skinner ER, Wilson HM, Leng GC, Fowkes FGR, Horrobin D. Alterations in plasma lipids, lipoproteins and high-density lipoprotein subfractions in peripheral arterial disease. *Atherosclerosis* 1997;131:161–166.
 19. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. 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 (Adults Treatment Panel III). *JAMA* 2001;285:2486–2497.
 20. Eyre J, Hammett F, Miller NE. A micro-method for the rapid ultracentrifugal separation of human plasma high-density lipoprotein subfractions, HDL2 and HDL3. *Clin Chim Acta* 1981;114:225–231.
 21. Lipid Research Clinics Program Epidemiology Committee. Plasma lipid distribution in selected North American populations: the Lipid Research Clinics Program Prevalence Study. *Circulation* 1980;60:427–439.
 22. Meilahn EN, Kuller LH, Matthews KA, Wing RR, Caggiula AW, Stein EA. Potential for increasing high-density lipoprotein cholesterol subfractions HDL2-cholesterol and HDL3-cholesterol, and apolipoprotein A-I among middle-age women. *Prev Med* 1991;20:462–473.
 23. Asscherman H, Gooren LJG, Megens JAJ, Nauta J, Kloosterboer HJ, Eikelboom. Serum testosterone level is the major determinant of the male-female differences in serum levels of high-density lipoprotein (HDL) cholesterol and HDL2-cholesterol. *Metabolism* 1994;43:935–939.
 24. Hartung GH, Reeves RS, Foreyt JP, Patsch W, Gotto AM. Effect of alcohol intake and exercise on plasma high-density lipoprotein cholesterol subfractions and apolipoprotein A-I in women. *Am J Cardiol* 1986;58:148–151.
 25. Haffner SM, Stern MP, Hazuda HP, et al. Do upper-body and centralized adiposity measure different aspects of regional body-fat distribution? Relationship to non insulin-dependent diabetes mellitus, lipids, and lipoproteins. *Diabetes* 1987;36:54–59.
 26. Haffner SM, Applebaum-Bowden D, Wahl P, et al. Epidemiological correlates of high-density lipoprotein subfractions, apolipoproteins AI, AII and D, and lecithin cholesterol acyl transferase: effects of smoking, alcohol, and adiposity. *Arteriosclerosis* 1985;5:169–177.
 27. Fulton-Keroe DL, Eckel RH, Shetterly SM, Hamman RF. Determinants of total high-density lipoprotein cholesterol and high-density lipoprotein subfraction levels among Hispanic and non-Hispanic white persons with normal glucose tolerance: the San Luis Valley Diabetes Study. *J Clin Epidemiol* 1992;45:1191–1200.
 28. Abbott RD, Wilson PWF, Kannel WB, Castelli WP. High-density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction: the Framingham Study. *Arteriosclerosis* 1988;8:207–211.
 29. Bakogianni MC, Kalofoutis CA, Skenderi KI, Kalofoutis AT. Clinical evaluation of plasma high-density lipoprotein subfractions (HDL₂, HDL₃) in non-insulin-dependent diabetics with coronary artery disease. *J Diabetes Complications* 2001;15:265–269.
 30. Drexel H, Amann FW, Rentsch K, et al. Relation of the level of high-density lipoprotein subfractions to the presence and extent of coronary artery disease. *Am J Cardiol* 1992;70:436–440.
 31. Sakuma N, Yoshikawa M, Hibino T, et al. HDL3 exerts a more powerful antiperoxidative and protective effect against peroxidative modification of LDL than HDL2 does. *J Nutr Sci Vitaminol (Tokyo)* 2002;48:278–282.
 32. Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. *Arterioscler Thromb Vasc Biol* 2003;23:1881–1888.
 33. Gardner CD, Tribble DL, Young DR, Ahn D, Fortmann MD. Population frequency distributions of HDL, HDL₂, and HDL₃ cholesterol and apolipoprotein A-I and B in healthy men and women and associations with age, gender, hormonal status and sex hormone use: the Stanford Five City Project. *Prev Med* 2000;31:335–345.
 34. Shepherd J, Packard CJ, Stewart JM, Vallance BD, Lawrie TDV, Morgan HG. The relationship between the cholesterol content and subfraction distribution of plasma high-density lipoproteins. *Clin Chim Acta* 1980;10:57–62.
 35. Sunami Y, Motoyama M, Kinoshita F, et al. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol in healthy elderly subjects. *Metabolism* 1999;48:984–988.
 36. Spat-Douglas T, Keyser ER. Exercise intensity: its effect on the high-density lipoprotein profile. *Arch Phys Med Rehabil* 1999;80:691–695.
 37. Sillanaukee P, Koivula T, Jokela H, Pitkääjärvi T, Seppä K. Alcohol consumption and its relation to lipid-based cardiovascular risk factors among middle-aged women: the role of HDL₃ cholesterol. *Atherosclerosis* 2000;152:503–510.
 38. Legato MJ. Dyslipidemia, gender, and the role of high-density lipoprotein cholesterol: implications for therapy. *Am J Cardiol* 2000;86(Suppl):15L–18L.