Multiple Lipid Scoring System for Prediction of Coronary Heart Disease Risk: Application to African Americans

Charles J. Everett, PhD; Arch G. Mainous III, PhD; Richelle J. Koopman, MD, MS; and Vanessa A. Diaz, MD, MS

Charleston, South Carolina

Financial support: This study was funded in part through grant #2 D54 HP-00023 from the Health Resources and Services Administration. The authors have no financial interest in the results of this study. The Framingham Heart Study-Offspring Study and the Atherosclerosis Risk in Communities (ARIC) Study are conducted and supported by the National Heart, Lung and Blood Institute (NHLBI) in collaboration with the Framingham Heart Study-Offspring Study and ARIC Study investigators, respectively. This manuscript was prepared using limited access data sets obtained by the NHLBI and does not necessarily reflect the opinions or views of the Framingham Heart Study-Offspring Study, the ARIC Study or the NHLBI.

Background: Clinicians often obtain a panel of lipids but then only use low-density-lipoprotein (LDL) cholesterol to make clinical decisions. We previously described the multiple lipid measure, a strategy that integrates information about seven lipid measures. Our current inquiry uses the multiple lipid measure to create a scoring system and validates that system in a second cohort.

Methods and Results: A scoring system that uses total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol and triglycerides was developed and tested. African-American participants of the Atherosclerosis Risk in Communities (ARIC) Study were used to validate the multiple lipid measure score. For nonsmokers, scores ≥2 had a hazard ratio of 4.25 (95% CI 1.92–9.40) compared to reference scores of ≤-3 in adjusted survival analysis predicting incident coronary heart disease risk in the ARIC. The best conventional single lipid measure for nonsmokers was LDL cholesterol. Compared to LDL cholesterol <100 mg/dl, those with LDL cholesterol ≥160 mg/dl had a hazard ratio of 2.31 (95% CI 1.13–4.75). For current smokers, the best conventional lipid measure was the total cholesterol/HDL cholesterol ratio, which was similar in predictive ability to the multiple lipid measure score. However, the multiple lipid measure score predicted an additional 10% of the cohort at risk compared to the total cholesterol/HDL cholesterol ratio.

Conclusions: The use of the multiple lipid scoring system improves the assessment of incident coronary heart disease risk and may have utility for clinicians in integrating lipid values.

Key words: epidemiology ■ coronary disease ■ lipids ■ risk factors

 From the Department of Family Medicine at the Medical University of South Carolina, Charleston, SC. Send correspondence and reprint requests for *J Natl Med Assoc.* 2006;98:1740–1745 to: Dr. Charles J. Everett, Department of Family Medicine, Medical University of South Carolina, 295 Calhoun St., PO Box 250192, Charleston, SC 29425; phone: (843) 792-3413; fax: (843) 792-3598; e-mail: everettc@musc.edu

INTRODUCTION

Abnormal lipid levels are recognized as risk factors for coronary heart disease.[1] Emphasis is placed on measuring low-density-lipoprotein (LDL) cholesterol levels, as LDL reduction is thought to be the primary mode by which statins reduce coronary artery disease risk.[2] However, total cholesterol, high-density-lipoprotein (HDL) cholesterol and triglycerides are also known to affect risk for coronary heart disease. The National Cholesterol Education Program Adult Treatment Panel III (ATP III) report[1] uses a hierarchy of lipid measures to make a risk assessment. However, the ATP-III report looks at each individual lipid marker essentially separately to determine risk.

While clinicians making use of the ATP-III guidelines measure a lipid panel typically consisting of the above measures, they usually use mostly LDL cholesterol to make decisions. However, clinicians may encounter patients with for example, high LDL cholesterol and very high HDL cholesterol, which would lead to conflicting assessments of risk and potentially inappropriate treatment decisions. In a previous attempt to address this problem, Assmann[3] used a different hierarchical scheme that included using information gleaned from two lipid measures together to establish risk. This system combined total cholesterol and HDL cholesterol in a ratio as an indicator of myocardial infarction risk. Cross-sectional analyses of the Framingham Offspring Study have shown both apolipoprotein B and apolipoprotein A-I to be coronary heart disease risk factors.[4-7] Sniderman[8,9] reviewed five prospective epidemiological studies[10-14] and placebo groups in two clinical trials,[15,16] and concluded that apolipoprotein B is a stronger predictor of cardiovascular disease than LDL

cholesterol and that the ratio of apolipoprotein B/ apolipoprotein A-I is superior to total cholesterol/ HDL cholesterol as an index of risk.

Strategies using combinations of lipids beyond two-variable ratios have been relatively uncommon. However, we think the use of information from the entire standard panel of measured lipids might prove a better indicator of future risk. We previously[17] used principal component analysis[18] to summarize variation among seven lipid measures in the Framingham Offspring Study. Principal component analysis allows for the reduction of many variables into a few variables, or a single new variable, that contains as much information as possible from the original data set. Called the multiple lipid measure (MLM), an overall measure combining information from the seven lipids was compared to conventional lipid measures and was found to be a superior predictor of coronary heart disease risk.[17] Our present study takes the MLM and develops a scoring system that uses total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. The scoring system is then tested using the African-American participants of the Atherosclerosis Risk in Communities (ARIC) Study. Our analyses of the ARIC Study indicated there was a substantial interaction among participants' lipid levels, smoking status and incident coronary heart disease; hence, we analyzed nonsmokers and current smokers separately. This interaction is due to the fact African Americans who smoke and have desirable lipid profiles have very high incidence of coronary heart disease.

METHODS

Development of the Multiple Lipid Measure Score

The MLM, previously described,[17] was simplified and turned into a scoring system to be used with a standard lipid panel composed of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. Mathematically, substitutions were made replacing apolipoprotein A-I with HDL cholesterol, apolipoprotein B with LDL cholesterol, and VLDL cholesterol with triglycerides in the formula describing the MLM. As part of the original principal component analysis,[17] each variable was standardized to a mean of 0, plus one standard deviation equal to +1 and minus one standard deviation equal to -1. Scores were assigned by scaling weighted standardized values of each variable (weighted standardized value = factor loading x standardized value). One unit of each score was equal to 0.5 units of a weighted standardized value. The total score for the model was the sum of the scores for the component lipid measures and can range from -7–+6. A total score of 0 represents an average condition but does not indicate the absence of risk.

Data Set Used for Test of Model—ARIC Study

Longitudinal analysis of the public use ARIC Study was accomplished using the first examination cycle (1986–1989) as the baseline and following the cohort through the end of 1998. Our cohort consisted of 3,311 African-American participants, 45–64 years old in 1986–1989, with a mean follow-up of 9.5 years. Persons with coronary heart disease at baseline were excluded from our analyses. Patients with triglycerides ≥400 mg/dl were also excluded because LDL cholesterol could not be calculated for these persons.

Medical history, physical examination and laboratory analyses done in 1986–1989 were used to characterize participants. Blood pressure measurements were taken three times, and the average of the second and third used in our analyses. Participants with systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or that used antihypertension medication were classified as having hypertension. To define diabetes status, we used a definition current in 1986–1989. Persons with fasting blood glucose >140 mg/dl or that

Table 1. Scoring of lipids for multiple lipid measure score

Total Cholesterol (mg/dl)	Score	HDL Cholesterol (mg/dl)	Score
≥238	1	≥74	-2
≥186 and <238	0	≥59 and <74	-1
<186	-1	≥44 and <59	0
		<44	1
LDL Cholesterol (mg/dl)	**Score**	**Triglycerides (mg/dl)**	**Score**
≥174	2	≥179	2
≥147 and <174	1	≥121 and <179	1
≥120 and <147	0	≥82 and <121	0
≥93 and <120	-1	≥56 and <82	-1
<93	-2	<56	-2

Multiple lipid measure (MLM) score: total cholesterol score + HDL cholesterol score + LDL cholesterol score + triglycerides score. The MLM score ranges from -7–6.

were being treated with insulin or oral hypoglycemic agents were categorized as having diabetes. The use of antilipemic agents recorded at the first, second (1990–1992), third (1993–1995) or fourth examination (1996–1998) was noted and a time-dependent covariate created to describe treatment with antilipemic agents. At the baseline examination in 1986–1989, 45 African-American participants were using cholesterol-lowering medications, and 1,007 were using medications that secondarily affect cholesterol. Current smoking status was defined by answers to the questions: "Have you ever smoked cigarettes? (more than 400 cigarettes in a lifetime)" and "Do you now smoke cigarettes?"

Measurement of Lipids and Outcome—ARIC Study

Total plasma cholesterol, HDL cholesterol, triglycerides, apolipoprotein A-I and apolipoprotein B were determined at baseline.[19] Only persons who had fasted for ≥12 hours prior to their blood being drawn were included in our study. HDL cholesterol was determined after precipitation of LDL cholesterol and VLDL cholesterol with dextran sulfate-magnesium.[20,21] Total cholesterol, HDL cholesterol and triglycerides were measured by automated enzymatic procedures.[22,23] LDL cholesterol was calculated using the Friedewald equation.[24] Both apolipoprotein A-I and apolipoprotein B were measured using radioimmunoassay procedures.[25-27]

The cohort was followed through 1998 for the development of incident coronary heart disease. Incident coronary heart disease was defined by combinations of chest pain, ECG changes, cardiac enzyme levels and surgical revascularization.

Analysis—ARIC Study

For survival analyses, we used the statistical package SAS (Statistical Analysis System, Research Triangle Park, NC). The 964 current smokers and 2,347 nonsmokers were analyzed separately. All survival analyses used gender, age, hypertension, diabetes status and treatment with antilipemic agents as covariates. MLM scores were classified into four ascending groups: ≤-3, (-2 or -1), (0 or 1) and ≥2 for nonsmokers, and as ≤0 and ≥1 for current smokers. Only two categories were used for the current smokers because there were fewer of them. For comparison, survival analyses were computed using total cholesterol, HDL cholesterol, LDL cholesterol, total cholesterol/HDL cholesterol ratio, apolipoprotein B and apolipoprotein B/apolipoprotein A-I ratio to predict incident coronary heart disease risk in the ARIC cohort. Total cholesterol/HDL cholesterol was chosen because it is widely used and is valid when a nonfasting blood sample is analyzed.[3] The MLM score and alternative lipid measures and ratios of two lipids were compared by considering hazard ratios and the number cases, person-years and persons in a risk category.

RESULTS

In preliminary analyses, we observed a substantial interaction between participants' lipid levels, smoking status and incident coronary heart disease. Once smoking was accounted for, we observed a slight interaction between participants' lipid levels and gender, and no interaction between participants' lipid levels and age. As smoking was the most important interaction we observed, we analyzed nonsmokers and current smokers separately, with gender and age handled as covariates.

The MLM score was calculated by summing component lipid scores, as given in Table 1. The HDL cholesterol score decreases as HDL cholesterol increases because HDL cholesterol is protective with respect to coronary heart disease. Total cholesterol contributes to the model by modifying the final result when >1 of the other variables is elevated. Possible MLM scores range from -7–6.

Adjusted survival analyses of the MLM score are shown in Table 2. For nonsmokers, scores of 0 or 1 had a

Table 2. Adjusted Cox regressions using the multiple lipid measure score to predict incident coronary heart disease risk[1]

Multiple Lipid Measure	Hazard Ratio	95% CI	Number of Cases	Number of Person-Years	Number of Persons
Nonsmoker					
MLM Score					
≤-3	1.00	—	7	5,480	556
-2 and -1	2.35	0.98–5.59	19	5,306	548
0 and 1	2.39	1.02–5.60	23	5,383	556
≥2	4.25	1.92–9.40	57	6,560	687
Current Smoker					
MLM Score					
≤0	1.00	—	46	5,368	586
≥1	1.60	1.06–2.41	48	3,436	378

1: Adjusted for gender, age, hypertension, diabetes status and antilipemic agents

hazard ratio of 2.39 (95% CI 1.02–5.60), and scores ≥2 had a hazard ratio of 4.25 (95% CI 1.92–9.40) compared to reference scores of ≤-3. For current smokers, scores ≥1 had a hazard ratio of 1.60 (95% CI 1.06–2.41) compared to reference scores of ≤0. The nonsmoker reference category had an unadjusted incident coronary heart disease rate of 1.28 cases per 1,000 person-years. For current smokers, the reference category had a substantial higher unadjusted incident coronary heart disease rate of 8.57 cases per 1,000 person-years.

Survival analyses using more conventional lipid measures, and selected ratios of two lipids are shown in Tables 3 and 4. The total cholesterol/HDL cholesterol ratio and apolipoprotein B/apolipoprotein A-I ratio were better predictors of coronary heart disease risk than total cholesterol alone or HDL cholesterol alone for both groups. For nonsmokers, the best conventional lipid measure was LDL cholesterol. Compared to LDL cholesterol <100 mg/dl, LDL cholesterol ≥160 mg/dl had a hazard ratio of 2.31 (95% CI 1.13–4.75). For current smokers, LDL cholesterol was not as predictive, and the best conventional lipid measure was the total cholesterol/HDL cholesterol ratio with values >5 having a hazard ratio of 1.65 (95% CI 1.09–2.51) compared to values ≤5.

For nonsmokers, all of the single measures and two-variable ratios tested have hazard ratios that are lower than that of the MLM score. For current smokers, the MLM score produces results that are similar to the total cholesterol/HDL cholesterol ratio, apolipoprotein B, and the apolipoprotein B/apolipoprotein A-I ratio. However, for current smokers, the MLM score identifies an additional 100 persons—out of 964—at increased risk compared to the total cholesterol/HDL cholesterol ratio.

DISCUSSION

Utilizing information provided by multiple lipid indicators can substantially enhance coronary heart disease risk assessments over currently used strategies. In clinical practice, physicians and other healthcare providers measure lipid panels in patients to make a decision about the patient's risk for coronary heart disease and about initiating or changing therapy based on that risk. While a whole suite of fasting lipids may be measured, in general, the clinician, as directed by clinical guidelines (reference ATP III), may focus primarily on the information about the LDL to make this decision. The lipid panel may reveal lipid values that support conflicting decisions, such as the patient with a high LDL

Table 3. Adjusted Cox regressions using different lipid measures to predict incident coronary heart disease risk of non-smokers[1]

	Hazard Ratio	95% CI	Number of Cases	Number of Person Years	Number of Persons
Total Cholesterol (mg/dl)					
<200	1.00	—	25	8,543	876
≥200	1.82	1.16–2.85	81	14,186	1,471
HDL Cholesterol (mg/dl)					
≤40	1.80	1.16–2.77	32	3,300	348
≥40	1.00	—	74	19,430	1,999
LDL Cholesterol (mg/dl)					
<100	1.00	—	9	3,857	395
≥100 and <130	1.62	0.75–3.48	24	6,093	629
≥130 and <160	1.76	0.84–3.71	30	6,380	658
≥160	2.31	1.13–4.75	43	6,399	665
Total Cholesterol/HDL Cholesterol Ratio					
≤5	1.00	—	54	17,379	1,781
>5	2.25	1.52–3.33	52	5,350	566
Apolipoprotein B (mg/dl)					
Quartile 1 & 2	1.00	—	29	11,246	1,140
Quartile 3	1.99	1.22–3.25	36	5,750	601
Quartile 4	2.00	1.24–3.24	41	5,733	606
Apolipoprotein B/Apolipoprotein A-I Ratio					
<0.85	1.00	—	55	17,500	1,783
≥0.85 (fourth quartile)	2.13	1.44–3.17	51	5,229	564

1: Adjusted for gender, age, hypertension, diabetes status and antilipemic agents

but also a very high HDL. Using the multiple lipid scoring system synthesizes information from the multiple lipids measured in a lipid panel and identifies more people at risk for coronary heart disease.

Common combinations of lipid values can be readily assessed using the multiple lipid scoring system. African-American patients who have normal triglyceride levels and elevated total cholesterol and LDL cholesterol can be scored and an overall assessment of incident coronary heart disease risk made. The HDL cholesterol level of the patients would be particularly important in these cases.

Sharrett et al.[13] tested a series of models by gender in the ARIC cohort, including one with five lipid measures. Our results differ from those of Sharrett et al. because we included patients who used antilipemic agents, and developed a time-dependent covariate to describe antilipemic agent use, where as Sharrett et al. excluded these patients from their analyses. Shai et al.[28] used multivariate models to evaluate multiple lipid parameters in the Nurses' Health Study and found HDL cholesterol-related ratios to be the best predictors of coronary heart disease. In our study, the total cholesterol/HDL cholesterol ratio and apolipoprotein B/apolipoprotein A-I ratio were not as predictive of coronary heart disease risk as the MLM score for nonsmoker African Americans. Our study improves on the approach of Sharrett et al. and Shai et al. by using a scoring system based on principal component analysis.

There are limitations to this study. First, we made comparisons using factor loadings based on lipid levels of the Framingham Offspring Study. The Framingham Offspring Study is limited to the population of one community and does not include minorities. However, the concordant results, in the African-American participants of the ARIC study, support the generalizability of the MLM beyond one cohort. Second, other lipid assessments (e.g., LDL particle size) that have demonstrated associations with coronary heart disease risk were not included in the MLM score. However, the selected markers are commonly available to clinicians and therefore were chosen for investigation to try and improve the ultimate practical utility of the multiple lipid scoring system. Third, other control variables could have been included such as body mass index, education, exercise, fibrinogen, von Willebrand factor, white blood cell count, prehypertension and heart rate.[29,30]

There are some strengths of this study that are worth

Table 4. Adjusted Cox regressions using different lipid measures to predict incident coronary heart disease risk of current smokers[1]

	Hazard Ratio	95% CI	Number of Cases	Number of Person Years	Number of Persons
Total Cholesterol (mg/dl)					
<200	1.00	—	39	4,100	448
≥200	1.15	0.76–1.74	55	4,704	516
HDL Cholesterol (mg/dl)					
<40	1.49	0.96–2.32	29	1,934	216
≥40	1.00	–	65	6,870	748
LDL Cholesterol (mg/dl)					
<100	1.00	—	17	1,989	222
≥100 and <130	1.13	0.61–2.11	24	2,626	281
≥130 and <160	1.42	0.76–2.65	24	1,965	217
≥160	1.48	0.81–2.71	29	2,223	244
Total Cholesterol/HDL Cholesterol Ratio					
≤5	1.00	—	56	6,309	686
>5	1.65	1.09–2.51	38	2,495	278
Apolipoprotein B (mg/dl)					
Quartile 1 & 2	1.00	—	41	4,614	505
Quartile 3	1.12	0.66–1.89	21	1,999	217
Quartile 4	1.60	1.00–2.55	32	2,190	242
Apolipoprotein B/Apolipoprotein A-I Ratio					
<0.85	1.00	—	56	6,290	685
≥0.85 (fourth quartile)	1.60	1.06–2.43	38	2,514	279

1: Adjusted for gender, age, hypertension, diabetes status and antilipemic agents

noting. First, the study successfully develops a scoring system using one well-designed cohort and applies it to individuals in another large, robust and well-designed prospective cohort. Second, the multiple lipid scoring system was more predictive of coronary heart disease in nonsmokers than other commonly used lipid risk assessments in a cohort with follow-up sufficient to indicate coronary heart disease.

In conclusion, a scoring system integrating information from multiple lipid assessments is more predictive of coronary heart disease in African-American nonsmokers than other widely used lipid markers and will help clinicians synthesize information about the MLMs obtained in a lipid panel. This practical, office-based scoring system using multiple lipids has benefits for risk assessment above contemporary lipid measures in African-American patients and should be incorporated into lipid panel laboratory reporting.

REFERENCES

1. 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). *JAMA*. 2001;285:2486-2497.

2. Pedersen TR. Pro and con: low-density lipoprotein cholesterol lowering is and will be the key to the future of lipid management. *Am J Cardiol.* 2001; 87(suppl):8B-12B.

3. Assmann G. Pro and con: high-density lipoprotein, triglycerides, and other lipid subfractions are the future of lipid management. *Am J Cardiol.* 2001;87(suppl):2B-7B.

4. Schaefer EJ, Lamon-Fava S, Ordovas JM, et al. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study. *J Lipid Res.* 1994;35:871-882.

5. Schaefer EJ, Lamon-Fava S, Cohn SD, et al. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *J Lipid Res.* 1994;35:779-792.

6. Contois JH, McNamara JR, Lammi-Keefe CJ, et al. Reference intervals for plasma apolipoprotein A-I determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem*. 1996;42:507-514.

7. Contois JH, McNamara JR, Lammi-Keefe CJ, et al. Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem.* 1996;42:515-523.

8. Sniderman AD, Furberg CD, Keech A, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. *Lancet.* 2003;361:777-780.

9. Sniderman AD, St-Pierre AC, Cantin B, et al. Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk. *Am J Cardiol.* 2003;91:1173-1177.

10. Walldius G, Jungner I, Holme I, et al. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS Study): a prospective study. *Lancet.* 2001;358:2026-2033.

11. Lamarche B, Moorjani S, Lupien PJ, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec Cardiovascular Study. *Circulation.* 1996;94:273-278.

12. Moss AJ, Goldstein RE, Marder VJ, et al. Thrombogenic factors and recurrent coronary events. *Circulation.* 1999;99:2517-2522

13. Talmud PJ, Hawe E, Miller GJ, et al. Nonfasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. *Arterioscler Thromb Vasc Biol.* 2002;22:1918-1923.

14. Sharrett AR, Ballantyne CM, Coady SA, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation.* 2001;104:1108-1113.

15. Gotto AM, Whitney E, Stein EA, et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation.* 2000;101:477-484.

16. Simes RJ, Marschner IC, Hunt D, et al. Relationship between lipid levels and clinical outcomes in the Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) Trial: to what extent is the reduction in coronary events with pravastatin explained by on-study lipid levels? *Circulation.* 2002;105:1162-1169.

17. Everett CJ, Mainous AG III, Koopman RJ, et al. Predicting coronary heart disease risk using multiple lipid measures. *Am J Cardiol.* 2005;95:986-988.

18. Dunteman GH. Principal components analysis. Sage University Paper series on Quantitative Applications in the Social Sciences, series no. 07-069. Newbury Park, CA: Sage Publications; 1989:1-96.

19. Brown SA, Hutchinson R, Morrisett J, et al. Plasma lipid, lipoprotein cholesterol, and apoprotein distributions in selected US communities: the Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb.* 1993;13: 1139-1158.

20. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem*. 1982;28:1379-1388.

21. Wahlefeld AW, Gruber W. Regent for the improved liplytic efficiency. *J Clin Chem Clin Biochem*. 1984;22:165-174.

22. Siedel J, Hagele E, Ziegenhorn J, et al. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem*. 1983;29:1075-1080.

23. Nagele U, Hagele E, Sauer G, et al. Reagent for the improved liplytic efficiency. *J Clin Chem Cin Biochem*. 1984;22:165-174.

24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.

25. Brown SA, Rhodes CE, Dunn K, et al. Effect of blood collection and processing on radioimmunoassay results for apolipoprotein A-I in plasma. *Clin Chem*. 1988;34:920-924.

26. Schonfeld G, Lees R, George PK, et al. Assay of total plasma apolipoprotein concentration in human subjects. *J Clin Invest*. 1974;53: 1458-1467.

27. Brown SA, Epps DF, Dunn JK, et al. Effect of blood collection and processing on radioimmunoassay results for apolipoprotein B in plasma. *Clin Chem*. 1990;36:1662-1666.

28. Shai I, Rimm EB, Hankinson SE, et al. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. *Circulation.* 2004;110:2824-2830.

29. Kshirsagar AV, Carpenter M, Bang H, et al. Blood pressure usually considered normal is associated with an elevated risk of cardiovascular disease. *Am J Med*. 2006;119:133-141.

30. King DE, Everett CJ, Mainous AG III, et al. Long-term prognostic value of resting heart rate in people with prehypertension. *Am J Hypertension* (in press). ■