RESEARCH PAPER

Oxidative Stress and Anti-oxidative Defense in Schoolchildren Residing in a Petrochemical Industry Environment

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Objective: To evaluate the possible relationship between industrial air pollution and oxidative stress in schoolchildren by comparing parameters from children residing in two nearby localities with contrasting environmental conditions.

Participants: 42 schoolchildren (12-15 years) from Pancevo (site of Serbia's largest petrochemical installation) formed the exposed group. 82 schoolchildren from Kovacica village, located 30 km north of Pancevo, formed the non-exposed group.

Methods: Oxidative stress status, anti-oxidative defense parameters, paraoxonase-1 status, lipid status, glucose concentration and leukocyte counts were compared in two groups.

Results: The children from Pancevo showed higher level of oxidative stress demonstrated by an elevated malondialdehyde concentration (P <0.001) and decreased superoxide dismutase activity (P<0.01) in comparison to the non-exposed group.

Conclusions: The results suggested a relationship between the presence of air pollutants and increased oxidative stress in schoolchildren residing in an industrial environment.

Key words: *Air pollution, Anti-oxidative defense, Cardiovascular disease, Environment, Oxidative stress.*

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Many epidemiological studies have demonstrated air pollution as a risk factor for respiratory illnesses, malignancies and cardiovascular diseases (CVD)(1-4). Environmental air contains a range of pollutants, many of which are free radicals or have the ability to drive free radical reactions. Exposure to these pollutants gives rise to oxidative stress (OS), which appears to initiate responses particularly dangerous to children(5-7). An early increase of OS may be responsible for predisposition to premature atherosclerosis leading to development of CVD in later life(8-10). There is a paucity of data evaluating the effect of air pollution on OS and anti-oxidative defense (AOD) parameters in healthy children. It is known that the Serbian males have increased risk for early development of CVD and its consequences(11). We planned this study with the objective of evaluating the contribution of environmental air pollutants on OS status parameters and on protective antioxidative high-density lipoprotein (HDL) function in Serbian schoolchildren.

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METHODS

Study population and sample size

The study population consisted of 124 healthy schoolchildren (aged between 12 and 15 years)

scheduled for a regular health check in April and May 2007. Forty two (34%) children were from Pancevo where Serbia's largest petrochemical facilities and oil refineries are situated. These individuals were labeled as 'exposed group' (EG). The living conditions in Pancevo are characterized by a high concentration of chemical industries, and the close proximity of industrial zones to residential areas(12). Eighty two children from Kovacica village, located 30 km north of Pancevo, formed the 'non-exposed group' (NEG). The main occupation of parents in the municipality of Kovacica is agriculture. Dietary habits are similar for both locations.

Sample size was determined according to Hopkins(13), using the formula $N=32/ES^2$ where ES is the smallest effect size worth detecting (smallest difference worth noticing divided by standard deviation, expressed in the same units). The ES for our study was 0.55. With Type I error of 0.05 and Type II error of 0.2, the sample size was calculated as 119 with unequal number of subjects in two groups (39+80). Informed consent was obtained from children and their parents prior to enrolment in the study. The institutional review committee of the Faculty of Pharmacy, University of Belgrade and committees of both Health Centers' approved the study protocol.

Air pollution monitoring in Pancevo

Air pollution in Pancevo was determined according to standard operating procedures(14) from the air pollution monitor network. The mean concentrations of benzene, total suspended particles, polycyclic aromatic compounds and benzopyrenes were more than the maximum allowable concentrations (MAC) on most days in three years preceding the survey, whereas the levels of sulphur dioxide, nitrogen dioxide, ammonia, toluene and total precipitated matter were below the MAC on all days during this period. Air pollution data from Kovacica were not available as it is not monitored because of its distance from industries.

Blood sampling and analysis

Venous blood was drawn from the antecubital vein after overnight fasting and samples were stored at – 80° C in aliquots, until analysis. For malondialdehyde (MDA) determination, butylated hydroxytoluene (BHT, 0.05% w/v) was immediately added to the plasma (0.2 mL) before storage, and for the measurement of superoxide anion (O_2-), plasma from heparinized blood samples was used immediately. Leukocyte count was determined by a combination of flow impedance and light absorbance using a Pentra 60C+ analyser (Horiba ABX, Montpelier, France). Glucose, total cholesterol (TC) and triglycerides (TG) were assayed by routine enzymatic methods using an ILab 300+ analyser (Instrumentation Laboratory, Milan, Italy) and Randox Laboratories (Armdore, UK) reagents. High-density lipoprotein cholesterol (HDL-C) was measured using the same method (as above) after precipitation of the plasma with phosphotungstic acid in the presence of magnesium ions. Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured by immunoturbidimetry using the ILab 600 analyser and Dialab (Vienna, Austria) reagents. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula(15). The plasma concentration of lipoprotein(a) [Lp(a)] was measured using immunoturbidimetry (BIOKIT, Barcelona, Spain). The lipid tetrad index (LTI) was calculated using the formula [TC × TG × Lp(a)]/HDL-C(16) and the lipid pentad index (LPI) was calculated using the formula [TC × TG × Lp(a) × apoB]/apoA(17).

Parameters of oxidative stress

To determine the OS index, we used the thiobarbituric acid-reacting substances (TBARs) assay that measures the quantity of the malondialdehyde (MDA)-TBA 1:2 adduct described previously by Girotti, *et al*.(18). The rate of nitroblue tetrazolium reduction was used to measure the level of O_2-, as previously described(19). Plasma superoxide dismutase (SOD) activity was measured according to a previously published method(20). The method for plasma lipid hydroperoxide (LOOH) determination is based on the oxidation of Fe^{2+} to Fe^{3+} under acidic conditions(21). The plasma advanced oxidation protein product (AOPP) concentration was determined using standard method(22).

Determination of Paraoxonasi-1 (PON1) status involved the measurement of PON1 activity towards two substrates [paraoxon (POase activity) and diazoxon (DZOase activity)] and the subsequent assessment of $PON1_{192}$ activity phenotype. Rates of POase and DZOase activity were measured spectrophotometrically using a UV/VIS Ultrospec III spectrophotometer (Pharmacia LKB, Cambridge, UK) in serum according to the method described by Richter and Furlong(23,24). The $PON1_{192}$ phenotype (QQ, QR or RR) was predicted after examination of the two-dimensional plot of diazoxon vs. paraoxon hydrolysis rates and also by calculating the DZOase/POase activity ratio(24).

Statistical methods

The differences between the groups with continuous variables were statistically tested using the Student's t test for normally distributed variables. As the distributions of TG, Lp (a), LTI, LPI, HDL-C, POase, O_2-, MDA, AOPP and the O_2- /SOD ratio were skewed, logarithmic transformation of the values was performed before statistical comparisons. The distribution of LOOH concentrations was not normally distributed even after logarithmic transformation so they were compared using the Mann-Whitney test. Because obesity can influence the OS status(25), the BMI (body mass index) was used for analysis of covariance in order to determine the adjusted means. Categorical variables and phenotype distributions between the study groups were compared using the Chi-square test. Two-tailed *P* values less than 0.05 were considered statistically significant. All analyses were conducted using MedCalc® (Mariakerke, Belgium) version 9.3.90.

RESULTS

Basic demographic and biochemical characteristics of the two examined groups are shown in ***Table* I.** 22 subjects in exposed group (EG) and 37 in non-exposed group (NEG) were males. The mean age and body mass index of children in NEG were significantly greater than those in EG. There were no significant differences in the basic biochemical

TABLE I BASIC DEMOGRAPHIC CHARACTERISTICS AND BIOCHEMICAL PARAMETERS OF THE STUDY GROUPS

Parameters	Exposed (*n*=42), mean (SD)	Non-exposed (*n*=82), mean (SD)	*P* value
Age (years)	12 (0.22)	14.24 (1.08)	< 0.0001
BMI (kg/m^2)	18.6 (3.35)	21.54 (3.49)	< 0.0001
TC (mmol/L)	4.33 (0.78)	4.34 (0.73)	0.93
TG (mmol/L)*	0.69 (0.59-0.79)	0.62 (0.57-0.68)	0.22
LDL-C (mmol/L)	2.68 (0.63)	2.71 (0.62)	0.78
HDL-C (mmol/L)*	1.27 (1.18-1.36)	1.29 (1.23-1.35)	0.71
apoA (g/L)	1.76 (0.21)	1.67 (0.25)	0.64
apoB (g/L)	0.89 (0.20)	0.89 (0.23)	0.87
Lp a (mg/L)*	9.36 (6.48-13.5)	8.73 (7.16-10.65)	0.72
TC/HDL-C	3.43 (0.65)	3.39 (0.68)	0.79
LDL-C/HDL-C	2.16 (0.64)	2.15 (0.64)	0.97
apoB/apoA	0.51 (0.15)	0.53 (0.14)	0.32
LPI (mg/dL)*	4.6×10^3 [(3.0-7.0)$\times 10^3$]	4.0×10^3 [(3.1-5.2) $\times 10^3$]	0.59
LTI (mg/dL)*	1.9×10^2 [(1.3-2.8) $\times 10^2$]	1.6×10^2 [(1.2- 2.0) $\times 10^2$]	0.35
Leukocyte $X10^9$/L	6.61 (1.62)	7.02(1.83)	0.22
Glucose (mmol/L)	4.8 (0.41)	4.83(0.47)	0.79

*BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol;HDL-C, high-density lipoprotein cholesterol; apoA, apolipoprotein A-I; apoB, apolipoproteinB; Lp(a), lipoprotein(a); LPI, lipid pentad index; LTI, lipid tetrad index; * Mean values derived from log normal distribution given as geometric mean values (95% CI).*

parameters between the two groups.

The EG exhibited a higher level of OS (demonstrated by an elevated MDA concentration) when compared to the NEG (***Table* II**). SOD activity was significantly lower in the EG compared with the NEG (P=0.043). DZOase and POase activities showed no significant differences between the two groups, although in the EG both enzymatic activities were lower. The adjusted MDA concentration in the EG was significantly higher than that in the NEG (P=0.002).

There was no statistically significant difference in the $PON1_{192}$ phenotypes between the two groups (QQ_{EG} *vs*. QQ_{NEG} 0.49 *vs*. 0.47; QR_{EG} *vs*. QR_{NEG} 0.42 *vs*. 0.43 and RR_{EG} *vs* RR_{NEG} 0.09 *vs*. 0.10, P=0.832). In order to further analyze changes in PON activities, we categorized children from both groups according to their $PON1_{192}$ phenotype and compared corresponding POase and DZOase activities. Distinct PON1 phenotype activity was always lower in the EG compared to the NEG. The highest statistical significance was found in the RR phenotype subgroup (***Table* III**).

DISCUSSION

The increase in CVD in Serbian adults(11) and the fact that risk factors are established at a young age indicate the necessity to investigate possible contributors in young populations. We evaluated the potential influence of outdoor air pollution on OS in schoolchildren in two nearby localities having distinct living conditions(12).

Total suspended particles (TSP) of smaller size have been shown to penetrate the alveolar epithelium causing local inflammation and OS. The systemic inflammatory response to particulate air pollution and its relationship to adverse coronary events in

TABLE II OXIDATIVE STRESS STATUS AND ANTI-OXIDANT DEFENSE PARAMETERS IN THE STUDY GROUPS

Parameters	Exposed (n=42), mean (SD)	Non-exposed (n=82), mean (SD)	P value
O_2^-, (îmol/min/L)*	189 (168-213)	207 (193-223)	0.17
MDA (µmol/L) *	1.25 (1.12-1.39)	0.99 (0.92-1.06)	< 0.001
AOPP (µmol/L) *	14.0 (11.8-16.7)	13.1 (12.4-13.9)	0.40
LOOH (µmol/L)†	0.01(0.01-1.57)	0.01 (0.01-0.77)	0.25
SOD (U/L)	98 (25)	114 (27)	< 0.01
O_2^-/SOD**	2.03 (1.76-2.34)	1.87 (1.70-2.05)	0.30
POase (IU/L)*	309 (248-385)	381 (320 - 455)	0.15
DZOase IU/L	11083 (4511)	11429 (4416)	0.69

*O_2^-, superoxide anion; MDA, malondialdehyde; AOPP, plasma advanced oxidation protein product; LOOH, lipid hydroperoxide; SOD, superoxide dismutase; O_2^-/SOD, OS index; POase, PON1 activity towards paraoxon; DZOase, PON1 activity towards diazoxon. * Mean values derived from lognormal distribution given as geometric mean values (95% CI); †Values presented as median (interquartile range),P value for Mann-Whitney test.*

TABLE III PON1 ACTIVITY TOWARD PARAOXON AND DIAZOXON ACCORDING TO PON1 ACTIVITY PHENOTYPES

	POase activity, Geometric mean (95% CI)		P	DZOase activity, (mean±SD)		P
	EG	NEG		EG	NEG	
QQ	170 (151-192)	186 (161-215)	0.070	12901±3529	11631±4831	0.411
QR	542 (437-672)	647 (567-740)	0.070	10340±4572	10970±4028	0.287
RR	643 (334-1239)	1093 (837-1426)	0.028	4972±2565	7757±1920	0.058

POase, PON1 activity towards paraoxon; DZOase, PON1 activity towards diazoxon; QQ, QR,RR, phenotype subgroups for $PON1_{192}$ isoenzyme.

WHAT IS ALREADY KNOWN

- Oxidative stress and weakened antioxidative defense are factors in cardiovascular disease pathogenesis and carcinogenesis.

WHAT THIS STUDY ADDS

- Healthy children living near petrochemical industries in Serbia have increased oxidative stress and decreased anti-oxidant defense mechanism.

patients with coronary artery disease and in the healthy population are well established(4,6,25). Polycyclic aromatic hydrocarbons (PAH) can induce OS indirectly through its biotransformation by liver enzymes to generate redox active quinones which act as catalysts for free radical production(5,6). *In vitro* reactive oxygen species (ROS) formation has been shown to be highly correlated with PAH content in air(6). The oxidation of membrane lipids, one of the primary events during oxidative cellular damage, can be assessed by measuring plasma MDA concentrations, a late-stage OS biomarker of injured cells and subcellular structures(26,27). Our finding that increased MDA concentrations in children belonging to the EG was consistent with a study by Zalata, *et al.*(28).

In our study, the LOOH level, a marker of early damage to cellular membranes, lipoproteins and other lipid containing molecules(21,29) did not significantly differ between the two groups. However, an elevated MDA concentration(10) and a tendency for AOPP to increase(30) in the EG indicated long term (chronic) exposure to air pollution in Pancevo.

Protective HDL function is primarily via the enzyme paraoxonase 1 (PON1) which hydrolyzes lipid peroxides in human atherosclerotic lesions(31-33). PON1 has a common coding region polymorphism, a glutamine (Q) to arginine (R) substitution at position 192, which is associated with a number of pathophysiological conditions such as CVD and some others(31). It is well-documented that $PON1_{192RR}$ isoform is less capable for antioxidative protection than $PON1_{192QR}$ or $PON1_{192QQ}$ isoforms(32). The activities of PON1 and SOD were analyzed as indicators of the AOD status. Significantly lower plasma SOD activity, which indicated a reduced ability of SOD to remove ROS, was noted in EG. We detected reduced PON1 activity in children exposed to air pollution, although this difference did not reach statistical significance. Lower POase and DZOase activities in children from the EG was particularly evident and reached statistical significance in $PON1_{192RR}$ risk phenotype carriers.

A limitation of our study was the absence of air pollution related data in the Kovaica village, which we assumed as non-exposed group. It would have been interesting to see whether the air pollution levels are actually less at a distance of 30 Km from industries but the absence of monitoring in this village precluded this analysis. Children from NEG had significantly greater BMI and they were significantly older than children from EG but they were not in the state of OS. Greater BMI and older age could lead to higher OS parameters and lower AOD parameters values(34). On the contrary, children from EG, even younger and thinner, were in OS state.

In conclusion, there is an enhanced oxidative stress and a fall in anti-oxidant defense in children exposed to industrial pollution. Increase in serum MDA concentration and decrease in plasma SOD activity in the exposed children could be an indirect response of cells to increased oxidative challenge attributable to air pollution. These findings support the view that air pollution increases oxidative stress and suggest a metabolic self-defense adaptation mechanism of antioxidant systems following exposure to air contaminants.

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Contributors: AV performed experimental work, statistically analyzed data and wrote the manuscript. JKS, DK and AS performed experimental work. SS, JKS and NBS participated in study design and statistical analysis. DK, MD, SB and BA helped in data collection. SS, JKS, NBS and ZJI critically revised the manuscript.

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REFERENCES

1. Ballester F, Rodríguez P, Iñíguez C, Saez M, Daponte A, Galán I, *et al*. Air pollution and cardiovascular admissions association in Spain: results within the EMECAS project. J Epidemiol Commun Hlth 2006; 60: 328-336.
2. Health aspects of air pollution. Results from the WHO project "Systematic Review of Health Aspects of Air Pollution in Europe": Report of a WHO Working Group. Copenhagen, WHO Regional Office for Europe, 2004. Available from: URL: http://www.euro.who.int/document/E83080.pdf. Accessed on December 3, 2007.
3. Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, *et al*. Lung cancer, cardiopulmonary mortality and long-term exposure to fine particulate air pollution. JAMA 2002; 287: 1132-1141.
4. Brook RD, Frankin B, Cascio W, Hong Y, Howard G, Lipsett M, *et al*. Air pollution and cardiovascular disease. A statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 2004; 109: 2655–2671.
5. Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 2003; 60: 612-616.
6. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, *et al*. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Persp 2003; 111: 455-460.
7. Schwartz J. Air pollution and children's health. Pediatrics 2004; 113: 1037-1043.
8. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torrko N, *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA 2003; 290: 2277–2283.
9. Landrigan PJ, Trasande L, Thorpe LE, Gwynn C, Lioy PJ, D'Alton ME, *et al*. The National Children's Study: A 21-year prospective study of 100 000 American children. Pediatrics 2006; 118: 2173-2186.
10. Ece A, Gürkan F, Kervancýoðlu M, Kocamaz H, Güneb A, Atamer Y, *el al*. Oxidative stress, inflammation and early cardiovascular damage in children with chronic renal failure. Pediatr Neurol 2006: 21: 545-552.
11. Vukomirovic, D. Causes of deaths. *In*: Statistical Yearbook of Serbia 2005, Statistical Office of the Republic of Serbia, Belgrade, Serbia, 2005. p. 90.
12. Djordjevic D, Solevic T. Final report on investigation of cause and degree of air pollution by harmful and dangerous material on territory of Pancevo city. Ministry of Science and Environmental Protection, Republic of Serbia. 2004. pp. 1-80. Available from: URL: http://www.rnp.co.yu/rnp/ekologija/analiza_stanja_lat.jsp. Accessed on December 14, 2007.
13. Hopkins WG. Estimating sample size for magnitude-based inferences, Sportscience 2006; 10: 63-70.
14. Law About the Border Values, Methods of Measuring Emission, Establishing Criteria for Measuring Sites and Recording Data 54/92, 30/99, 19/2006 (In Serbian Language). Ministry of Environment and Spatial Planning. Available from: URL:http://www.ekoserb.sr.gov.yu/index.php?option=com_remository&Itemid=89&func=startdown&id=614. Accessed on November 29, 2007.
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
16. Enas EA, Dhawan J, Petkar S. Coronary artery disease in Asian Indians: lessons learnt and the role of lipoprotein(a). Indian Heart J 1997; 49: 24-34.
17. Das B, Daga MK, Gupta SK. Lipid Pentad Index: A novel bioindex for evaluation of lipid risk factors

for atherosclerosis in young adolescents and children of premature coronary artery disease patients in India. Clin Biochem 2007; 40: 18-24.

18. Girotti MJ, Khan N, McLellan BA. Early measurement of systemic lipid peroxidation products in plasma of major blunt trauma patients. J Trauma 1991; 31: 32-35.
19. Auclair C, Voisin E. Nitroblue tetrazolium reduction. In: Greenwald RA, editor. CRC Handbook of Methods for Oxygen Radical Research. Boca Raton, Florida: CRC Press; 1985. pp. 123-132.
20. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170-3175.
21. Jiang ZY, Wollard ACS, Wolff SP. Lipid hydroperoxides measurement of Xylenol Orange, comparison with the TBA assay and iodometric method. Lipids 1991; 26: 853-856.
22. Witko-Sarsat V, Friedlander M, Capeille're-Blandin C Nguyen-Khoa T, Nguyen AT, Zingraff J, *et al.* Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49: 1304.
23. Richter R, Furlong CE. Determination of paraoxonase (pon1) status requires more than genotyping. Pharmacogenetics 1999; 9: 745-753.
24. Kotur-Stevuljevic J, Spasic S, Stefanovic A, Zeljkovic A, Bogavac-Stanojevic N, Kalimanovska-Ostric D, *et al*. Paraoxonase-1 (PON1) activity, but not PON1Q_{192}R phenotype, is a predictor of coronary artery disease in a middle-aged Serbian population. Clin Chem Lab Med 2006; 44: 1106-1113.
25. Routledge HC, Ayres JG. Air pollution and the heart. Occup Med 2005; 55: 439-447.
26. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma-malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem 1997; 43: 1209-1214.
27. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005; 15: 316-328.
28. Zalata A, Yahia S, El-Bakary A, Elsheikha HA. Increased DNA damage in children caused by passive smoking as assessed by comet assay and oxidative stress. Mutat Res 2007; 629: 140-147.
29. Stocker R, Keaney JF. Role of Oxidative Modifications in Atherosclerosis. Physiol Rev 2004; 84: 1381-1478.
30. Witko-Sarsat V, Friedlander M, Khoa TN, Capeille're-Blandin C, Nguyen AT, Canteloup S, *et al*. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure**.** J Immunol 1998; 161: 2524-2532.
31. Durrington PN, Mackness MJ. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21: 473-480.
32. Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, *et al*. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation 2000; 101: 2510-2517.
33. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN, *et al*. Human paraoxonases (PON1, PON2 and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res 2005; 46: 1239-1247.
34. 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.