Distinguished Paper From the 2003 North American Primary Care Research Group Meeting

The Combined Effect of Transferrin Saturation and Low Density Lipoprotein on Mortality

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Background and Objectives: *Evidence suggests that cardiovascular disease (CVD) is accelerated by the oxidation of low-density lipoprotein (LDL) in the presence of iron. This study examined whether adults with elevated iron, as measured by transferrin saturation (TS), and elevated LDL are at an increased risk for mortality.* Methods: *This is a cohort study of the adult US population using the National Health and Nutrition Examination Survey 1976–1980 (NHANES II) merged with the NHANES II Mortality Study in 1992. Multivariate Cox regression was performed to determine hazard ratios (HR) for CVD and all-cause mortality for high (>55%) or low (<55%) levels of TS and high (>160mg/dl) or low (<160mg/dl) levels of LDL.* Results: *An elevated LDL alone did not significantly increase CVD mortality or all-cause mortality in the adjusted model. Individuals with elevated LDL and elevated TS had a statistically significant increase in both CVD mortality and all-cause mortality (HR=5.74 and 3.53, respectively) compared to the low LDL and low TS group.* Conclusions: *The results of this study indicate an increased risk associated with the combination of elevated LDL and elevated TS, which suggests that iron-mediated oxidation of LDL may be a significant factor in the progression of CVD.*

(Fam Med 2004;36(5):324-9.)

Total low-density lipoprotein (LDL) levels above 100mg/dl are related to the development of cardiovascular disease (CVD).[1] Treating elevated LDL reduces CVD mortality, both in persons with known CVD and in persons who do not yet have CVD.[1] However, the reduction in mortality is not as great as initially hypothesized.[2] One potential explanation for this discrepancy is that total LDL, the form of LDL usually measured, may not correlate with CVD risk as well as oxidized LDL. A mounting body of evidence is demonstrating that oxidized LDL is a major culprit in CVD progression.[3-7] Indeed, several studies have demonstrated that there are elevated levels of oxidized LDL in both the serum and the atherosclerotic plaques of individuals with extensive CVD.[5,8,9]

There have also been investigations into the role of iron in CVD, but those studies have had conflicting results.[10-13] The reason for these conflicting results may be that these studies did not account for oxidation of LDL, which may be a necessary cofactor in iron-induced atherogenesis. Animal research has shown marked interactions between iron and LDL in the development of atherosclerosis.[16-18] In particular, Araujo et al demonstrated that rabbits with both hypercholesterolemia and elevated iron levels had significantly more aortic atherosclerosis than rabbits that were merely hypercholesterolemic.[17]

Although oxidation of LDL in the presence of iron is a well-described phenomenon in humans,[14,15] little data exist on the clinical outcomes of humans with concomitant hypercholesterolemia and elevated body iron stores. Only one study, conducted on eastern Finnish men, showed worse outcomes for patients with both hypercholesterolemia and high ferritin than either risk factor alone.[19] However, this study used ferritin as a marker for iron levels, and ferritin levels can fluctuate considerably in the presence of illness or inflammatory processes. In addition, the sample population in the Finnish study had very high rates of heart disease and iron overload. Therefore, results of the Finnish study may not be generalizable to patients in the United States.

Serum transferrin saturation (TS) is a widely used marker for iron overload conditions like hemochroma-

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tosis and African iron overload disease and is considered by many experts to be a gold standard for assessing iron overload.[20]

Although few individuals are diagnosed with iron overload conditions, the prevalence of elevated serum TS ranges from 1%–6% in the adult US population.[21] Recent data have suggested that having elevated serum TS >55% carries an increased all-cause mortality risk.[22] Data also show that high TS combined with high red meat intake is associated with increased mortality risk.[23]

This study examined, in a 12-year cohort representative of the US population, CVD and all-cause mortality in adults with both elevated TS and elevated LDL levels.

Methods

We conducted an analysis of the cohort available in the second National Health and Nutrition Examination Survey 1976–1980 (NHANES II), merged with the NHANES II Mortality Study of 1992. The NHANES II is a nationwide probability sample with adjusted sampling weights for 76 age-sex-income groups designed to closely reflect the target population at the midpoint of the survey. The NHANES II Mortality Study (NH2MS) is a prospective cohort study that passively followed a subset of participants in the NHANES II. The NH2MS cohort is comprised of adults who were ages 30–75 at the time of their NHANES II examination (n=9,252). Due to the clustered sampling design performed in the NHANES, it is possible to use NHANES data to make population estimates for the entire adult US population.

Due to inadequate fasting times with resultant triglyceride levels >400, many participants (n=4,576) could not have accurate LDL calculations made. Although this resulted in exclusion of some subjects, the NCHS provides special population weights that allow accurate population estimates from the individuals who did have fasting triglyceride levels.

The NH2MS searched national databases containing information about mortality and causes of death. Mortality status was ascertained by computerized matching to national databases and evaluation of the resulting matches. Persons not found to be deceased are assumed alive for analytic purposes. The cohort was terminated in December 1992, at which point all persons had been followed for a minimum of 154 months. Primary cause of death and up to 20 secondary causes are listed by the *International Classification of Diseases, Ninth Edition* (ICD-9).

Patients who were previously diagnosed with hemochromatosis (n=2) were excluded from the study. The final analysis included 3,410 patients who, after applying population weights, represent more than 71 million Americans. No subjects in the sample were pregnant.

Variables

Low-density Lipoprotein. The NHANES II presented values for total cholesterol, high-density lipoprotein (HDL), and triglycerides. LDL was calculated using a standard formula: LDL=total cholesterol - HDL - (triglycerides/5). This formula is considered inaccurate for triglyceride levels >400 mg/dl.[25] As noted, therefore, these individuals with triglyceride levels >400 mg/dl were also excluded from the analysis. An LDL level was considered elevated if it was >160 mg/dl, matching the definition of high LDL from the third report of the National Cholesterol Education Program III.[1]

Transferrin Saturation. In the NHANES II, serum TS was calculated by dividing serum iron level by total iron-binding capacity. Although a variety of different levels of TS have been suggested as indicating iron overload, TS>55% has been shown in the NHANES I to carry an elevated mortality risk.[22] Therefore, we defined elevated serum TS as levels greater than 55%.

Mortality. Mortality was analyzed as both all-cause mortality and CVD mortality. CVD mortality was determined by whether it was identified as the primary or any of the secondary causes of death. ICD-9 codes 391–448.9 were used to define CVD mortality.

Control Variables. Control variables available in the NHANES II data were age, race, gender, poverty status, and education. Three age categories were defined (30–50, 51–64, and >64). Race follows the NHANES II designations (white, black, Hispanic, other). The poverty income ratio (PIR) is also calculated. PIR is an index based on income and number of household members. Poverty was defined as a PIR<1.0.

Because of our focus on coronary artery disease, we also adjusted for smoking and body mass index (BMI). Smoking was a self-reported current status. BMI was calculated from height and weight measurements taken at baseline, with values over 30 defining obesity.

In addition, the Charlson Comorbidity Index (CCI) was calculated to adjust for comorbid illnesses, including diabetes, hypertension, CVD, cerebrovascular disease, dementia, pulmonary disease, connective tissue diseases, ulcers, liver disease, hemiplegia, renal disease, tumors, leukemia, lymphomas, and acquired immunodeficiency syndrome. This index is a validated tool for predicting mortality in longitudinal studies.[26] The index was calculated using self-reported answers at baseline in the NHANES survey.

Analysis

We classified the population into four groups based on normal and elevated TS and low or high LDL levels. For the survival analyses, we used sampling weights to calculate prevalence estimates for the civilian,

non-institutionalized US population. Because of the complex sampling design of the survey, we performed all analyses with SUDAAN. Using the population estimates calculated by SUDAAN, as recommended by NCHS, we generated Kaplan-Meier survival curves to graphically show the unadjusted relationship between the four groups for both all-cause and CVD mortality. Cox proportional hazards analyses were conducted for survival time for all-cause and CVD mortality and the TS/LDL groups, adjusted for age, race, sex, poverty status, education, BMI, smoking status, and score on the CCI. In these models, survival time was a continuous variable measured in 1-month increments up to 154 months from the date of the NHANES II examination. A log-log plot of the relative hazards by time was used to confirm the assumption of proportional hazards.

Results

These results are derived from a study sample of 3,410 people in the NHANES, which provides a population estimate of more than 71 million Americans. The data showed that 27.34% of the population had LDL cholesterol >160mg/dl, and 1.64% had TS >55%. Females comprised 54.35% of the cohort. Demographic information for the four groups is shown in Table 1.

In agreement with previous studies, Table 2 shows that in a model unadjusted for control variables, LDL was associated with modestly higher CVD mortality but no difference in all-cause mortality. Similarly, when analyzed alone, TS is associated with higher all-cause mortality but not CVD mortality (Table 2). When combined together, however, the group with both elevated LDL and TS had a greater unadjusted risk of both all-cause and CVD mortality. Figures 1 and 2 show the unadjusted Kaplan-Meier survival curves for all-cause mortality and CVD mortality respectively.

Results of the Cox proportional hazards analysis are shown in Table 3. Persons with both elevated LDL and TS showed a significantly greater hazards ratio for all-cause and CVD mortality than persons with both normal LDL and TS or elevated LDL without elevated TS.

Due to a small absolute sample size in the highest risk category and wide confidence interval, an unweighted survival analysis was performed, with no control for design effect. This allowed an examination of the unweighted cohort to determine if the weighting of some small groups may have affected the results. The unweighted cohort survival analysis remained significant for both CVD mortality (P=.02) and all-cause mortality (P=.04) for the high LDL/high TS group.

Table 1

Population Characteristics (Ages 30–75) From the NHANES II

	Total	*Low LDL Low TS*	*Low LDL High TS*	*High LDL Low TS*	*High LDL High TS*
Population	71,420,298 (100%)	50,805,515 (71.1%)	1,089,486 (1.5%)	19,368,351 (27.2%)	156,946 (0.2%)
Age group					
30–50	54.8%	59.0%	71.0%	43.1%	44.2%
51–64	30.1%	27.1%	19.6%	38.4%	41.2%
>64	15.1%	13.9%	9.4%	18.5%	14.6%
Race					
White	76.6%	75.7%	76.9%	79.2%	54.6%
Black	8.7%	9.0%	10.0%	8.0%	0.0%
Hispanic	4.2%	4.8%	0.0%	3.0%	0.0%
Other	10.4%	10.5%	13.1%	9.7%	45.3%
Gender					
Male	45.7%	45.4%	48.5%	45.9%	66.5%
Female	54.4%	54.6%	51.5%	54.1%	33.5%
Poverty income ratio					
<1.0	12.6%	13.4%	7.0%	10.7%	16.6%
≥1.0	87.4%	86.6%	93.0%	89.3%	83.4%
Smoker					
No	60.6%	60.3%	44.4%	62.4%	40.1%
Yes	39.4%	39.7%	55.6%	37.6%	59.9%
Body mass index					
<30	85.3%	85.7%	94.3%	83.8%	100.0%
≥30	14.7%	14.3%	5.7%	16.2%	0.0%

LDL—low-density lipoprotein
TS—transferrin saturation

Discussion

The most important finding of this study is that elevated LDL combined with elevated TS is strongly predictive of CVD mortality over a 12-year interval. Notably, elevated LDL with high TS was associated with CVD and all-cause mortality. However, elevated LDL without elevated TS was not associated with a higher mortality rate when compared with having low LDL and low TS. The results of our study were significant even after controlling for demographic factors, BMI, smoking status, and comorbidities at baseline.

The finding of a greater risk with the combination of elevated TS and elevated LDL in this study corroborates the findings that have been demonstrated in animal models. The

Table 2

Unadjusted Risk and 95% CIs for Mortality According to Levels of LDL and TS

	All-cause Mortality		*CVD Mortality*	
	Hazards Ratio	*95% CI*	*Hazards Ratio*	*95% CI*
LDL<160	1.00		1.00	
LDL>160	1.09	0.88–1.35	1.40	1.07–1.85
TS<55	1.00		1.00	
TS>55	1.67	1.01–2.76	1.57	0.82–3.02
LDL<160/TS<55	1.00		1.00	
LDL<160/TS>55	1.46	0.80–2.63	1.31	0.71–2.42
LDL>160/TS<55	1.08	0.87–1.35	1.39	1.05–1.84
LDL>160/TS>55	3.81	1.22–11.87	5.21	1.01–26.99

CVD—cardiovascular disease
CI—confidence interval
LDL—low-density lipoprotein
TS—transferrin saturation

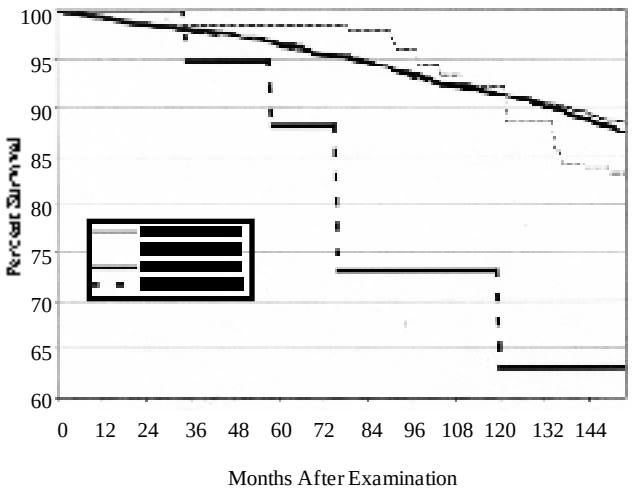
results of this study have important implications for CVD risk assessment among persons with elevated LDL levels. If our theory, that high levels of iron hasten the progression of CVD through the oxidation of LDL, is supported in subsequent research, then oxidized LDL levels may become a key clinical tool. Elevated LDL may be a less specific indicator of CVD risk than oxidized LDL, just as elevated total cholesterol is a less specific indicator than elevated LDL. Currently, laboratory measures of oxidized LDL are not extensively available. If the associations among TS, LDL, and mortality are confirmed in future studies, testing of persons with high LDL for TS levels may be indicated. This study suggests that improved prevention of CVD might be attained by aggressively targeting people with both elevated LDL and elevated TS.

Another important implication of the results of this study is that treatment of iron overload (as reflected by elevated TS) may be a strategy for reducing all-cause mortality in people with high LDL. It is possible that avoiding large sources of dietary iron, such as red meat, may help reduce CVD risk in persons with elevated LDL. Ascherio et al showed in a previous study that the intake of red meat was associated with an increased risk of fatal myocardial infarction over a 4-year period.[12] Potential treatments for lowering TS, such as therapeutic phlebotomies, have not been studied in humans with elevated LDL.

A third issue that is raised by these findings is the role of antioxidants. Can treatment with antioxidants prevent oxidation of LDL and lower CVD risk? Studies of antioxidant therapy have thus far yielded disappointing results. While some epidemiologic data suggest that vitamin E might help prevent heart disease through its antioxidant properties, clinical trials showed no apparent benefit from vitamin E.[27,28] Similarly, a recent systematic review conducted by the US Preventive Services Task Force recommended against the use of vitamins in the prevention of CVD.[29] However, the studies in this review did not account for TS levels and,

Figure 1

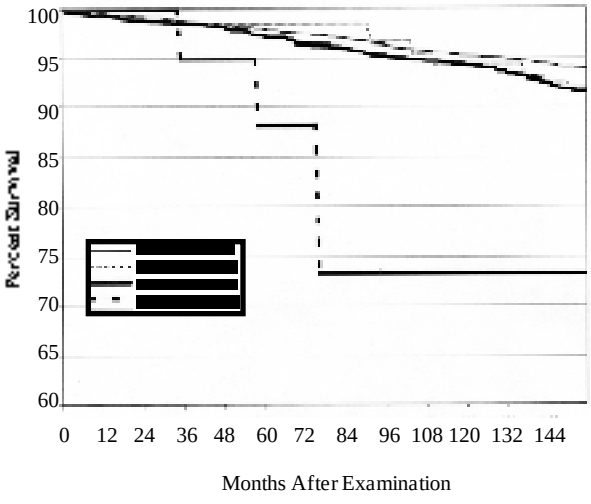
Kaplan-Meier Survival Curves for All-cause Mortality



LDL—low-density lipoprotein
TS—transferrin saturation

Figure 2

Kaplan-Meier Curves for Cardiovascular Disease Mortality



LDL—low-density lipoprotein
TS—transferrin saturation

Table 3

Adjusted Rates of Mortality

	All-cause Mortality		*CVD Mortality*	
	Hazards Ratio	*95% CI*	*Hazards Ratio*	*95% CI*
Age category				
30–50	1.00		1.00	
51–64	3.23	2.23–4.68	3.93	2.05–7.53
>64	7.80	5.54–10.97	11.43	6.05–21.58
Race				
White	1.00		1.00	
Black	0.99	0.70–1.41	1.33	0.92–1.92
Hispanic	0.66	0.44–0.99	0.32	0.14–0.73
Other	0.80	0.50–1.28	0.72	0.46–1.15
Gender				
Male	1.00		1.00	
Female	0.53	0.45–0.62	0.44	0.35–0.56
Poverty income ratio				
<1.0	1.00		1.00	
>1.0	0.65	0.51–0.82	0.67	0.51–0.89
Current smoker				
No	1.00		1.00	
Yes	2.04	1.66–2.52	2.06	1.62–2.62
Body mass index				
<30	1.00		1.00	
>30	1.15	0.85–1.55	1.04	0.70–1.57
Charlson Comorbidity Index	1.18	1.12–1.25	1.25	1.18–1.33
LDL<160/TS<55	1.00		1.00	
LDL<160/TS>55	1.66	0.86–3.21	1.49	0.90–2.48
LDL>160/TS<55	0.91	0.72–1.14	1.17	0.86–1.58
LDL>160/TS>55	3.53	1.08–11.58	5.74	1.04–31.54

CVD—cardiovascular disease
CI—confidence interval
LDL—low-density lipoprotein
TS—transferrin saturation

Controlling for age, race, gender, body mass index, smoking status, Charlson Comorbidity Index, and poverty income ratio

therefore, any positive antioxidant effects may have been masked by not focusing on those with high TS levels.

Limitations

There are several limitations to this study. First, the presence or absence of comorbid diseases was determined by self-report. Second, there is the potential for confounding, though the use of control variables should minimize this possibility. Third, TS is not a direct measure of iron levels and, therefore, could be affected by both false-positive or false-negative results. Additionally, some researchers may argue that ferritin would be a better indicator of iron status than TS. However, ferritin was not measured due to its tendency to show false-positive results during times of illness and inflammation, while this does not occur with TS.[20]

The strengths of this study, however, offset these limitations to a substantial degree. Particular strengths include the use of a large sample from a national cohort and the 12-year follow-up period. The NHANES data and follow-up cohort also allowed us to adjust for important factors that might have confounded the relationship between elevated LDL, elevated TS, CVD death, and all-cause mortality. Finally, the use of all-cause mortality as an outcome is a more unbiased endpoint than disease-specific mortality.[30]

Conclusions

Our study points to the greater risk arising from the combined states of elevated TS and elevated LDL and the relatively lower mortality risk of elevated LDL without elevated TS. The findings were maintained after controlling for other CVD risk factors. These results have important implications for further research into the value of screening for iron overload in people with elevated LDL. Further research is needed to confirm the importance of iron overload as a cofactor with LDL in the risk of CVD, and if the relationship is confirmed, to determine the optimal treatment strategy.

Acknowledgment: This study was funded in part through grant 1D12HP00023-03 from the Health Resources and Services Administration and the Delaware Department of Health and Social Services Division of Public Health #PSC0432.

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References

1. 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) Final Report. Circulation 2002;106(25):3143-421.
2. Pignone M, Phillips C, Mulrow C. Use of lipid lowering drugs for primary prevention of coronary heart disease: meta-analysis of randomized trials. BMJ 2000;321(7267):983-6.
3. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320(14):915-24.
4. Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low-density lipoprotein hypothesis. Atherosclerosis 1998;141(1998):1-15.
5. Ehara S, Ueda M, Naruko T, et al. Elevated levels of oxidized low-density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation 2001;103:1955-60.
6. Iuliano L, Micheletta F, Violi F. Low-density lipoprotein oxidation. Ital Heart J 2001;2(12):867-72.
7. Sherer Y, Tenenbaum A, Praprotnik S, et al. Coronary artery disease but not coronary calcification is associated with elevated levels of cardiolipin, beta-2-glycoprotein-I, and oxidized LDL antibodies. Cardiology 2001;95(1):20-4.

8. Vasankari T, Ahotupa M, Toikka J, et al. Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: lower levels of oxidized LDL with statin therapy. Atherosclerosis 2001;155(2):403-12.
9. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. Circulation 2001;103:1930-2.
10. Miller M, Hutchins GM. Hemochromatosis, multiorgan hemosiderosis, and coronary artery disease. JAMA 1994;272(3):231-3.
11. Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and risk of coronary heart disease. N Engl J Med 1994;330(16):1119-24.
12. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation 1994;86:969-74.
13. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis. Circulation 1997;96(10):3300-7.
14. Smith C, Mitchinson MJ, Aruoma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl-radical generation by contents of human atherosclerotic lesions. Biochem J 1992;286:901-5.
15. Heinecke JW, Rosen H, Chait A. Iron and copper promote modification of low-density lipoprotein by human arterial smooth muscle cells in culture. J Clin Invest 1984;74(5):1890-4.
16. Ponraj D, Makjanic J, Thong PS, Tan BK, Watt F. The onset of atherosclerotic lesion formation in hypercholesterolemic rabbits is delayed by iron depletion. Federation of European Biochemical Societies 1999;459:218-22.
17. Araujo JA, Romano EL, Brito BE, et al. Iron overload augments the development of atherosclerotic lesions in rabbits. Arterioscler Thromb Vasc Biol 1995;15:1172-80.
18. Lee T, Shiao M, Pan C, Chau L. Iron-deficient diet reduces atherosclerotic lesions in ApoE-deficient mice. Circulation 1999;99:1222-9.
19. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. Circulation 1992; 86(3):803-11.
20. McCullen MA, Crawford DHG, Hickman PE. Screening for hemochromatosis. Clin Chim Acta 2002;315:169-86.
21. Looker AC, Johnson CL. Prevalence of elevated serum transferrin saturation in adults in the United States. Ann Intern Med 1998;129:940-5.
22. Mainous AG III, Gill JM, Pearson WS, Carek PJ. Elevated serum transferrin saturation and mortality. Annals of Family Medicine 2004;2(2):133-8.
23. Mainous AG III, Wells BJ, Carek PJ, Gill JM, Geesey ME. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. Annals of Family Medicine 2004;2(2):139-44.
24. National Center for Health Statistics—McDowell A, Engel A, Massey J, Maurer K. Plan and operation of the Second National Health and Nutrition Examination Survey, United States, 1976–1980. (DHHS publication no. PHS81-1317, Vital and Health Statistics, ser.1, no.15) Washington, DC: US Government Printing Office, 1981.
25. Warrick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald Equation is adequate for classifying patients on the basis of nationally recommended cutpoints. Clin Chem 1990;36:15-9.
26. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
27. Hodis HN, Mack WJ, LaBree L, et al. VEAPS Research Group. Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). Circulation 2002;106(12): 1453-9.
28. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000;342(3):154-60.
29. US Preventive Services Task Force. Routine vitamin supplementation to prevent cancer and cardiovascular disease: recommendations and rationale. Ann Intern Med 2003;139:51-5.
30. Black WC, Haggstrom DA, Welch HG. All-cause mortality in randomized trials of cancer screening. J Natl Cancer Inst 2002;94:167-73.