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Original Contribution

Apolipoprotein E Genotype, Plasma Cholesterol, and Cancer: A Mendelian Randomization Study

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Observational studies have shown an association between low plasma cholesterol levels and increased risk of cancer, whereas most randomized clinical trials involving cholesterol-lowering medications have not shown this association. Between 1997 and 2002, the authors assessed the association between plasma cholesterol levels and cancer risk, free from confounding and reverse causality, in a Mendelian randomization study using apolipoprotein E (*ApoE*) genotype. *ApoE* genotype, plasma cholesterol levels, and cancer incidence and mortality were measured during a 3-year follow-up period among 2,913 participants in the Prospective Study of Pravastatin in the Elderly at Risk. Subjects within the lowest third of plasma cholesterol level at baseline had increased risks of cancer incidence (hazard ratio (HR) = 1.90, 95% confidence interval (CI): 1.34, 2.70) and cancer mortality (HR = 2.03, 95% CI: 1.23, 3.34) relative to subjects within the highest third of plasma cholesterol. However, carriers of the *ApoE2* genotype ($n = 332$), who had 9% lower plasma cholesterol levels than carriers of the *ApoE4* genotype ($n = 635$), did not have increased risk of cancer incidence (HR = 0.86, 95% CI: 0.50, 1.47) or cancer mortality (HR = 0.70, 95% CI: 0.30, 1.60) compared with *ApoE4* carriers. These findings suggest that low cholesterol levels are not causally related to increased cancer risk.

apolipoproteins E; cholesterol; genetics; neoplasms; random allocation

Abbreviations: ApoE, apolipoprotein E; CI, confidence interval; HR, hazard ratio; LDL, low density lipoprotein; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SE, standard error.

Investigators in numerous observational studies have reported an association between low plasma cholesterol levels and increased risk of cancer (1–6). This has led to concerns that treatments or lifestyle changes that lower cholesterol levels might increase cancer risk. However, these observed associations between low plasma cholesterol and increased risk of cancer might originate from reverse causality or confounding. For example, low plasma cholesterol levels might be caused by a hypocholesterolemic effect of cancer in preclinical stages (7). In that case, subjects with cancer would have an abnormally low cholesterol level because of the cancer, not vice versa (reverse causality). Furthermore, confounding factors such as age, smoking, and alcohol use might also explain some of the observed associations. Most randomized clinical trials have shown that cholesterol-lowering medications (statins) have no effect on cancer risk (8–12), although some exceptions have been reported (13, 14). However, the length of these trials was limited, and the answer to the question of whether a lifelong low plasma cholesterol level increases cancer risk has remained elusive.

An alternative epidemiologic method, Mendelian randomization, overcomes the problem of reverse causality and confounding, since it is based on Mendel's law that inheritance of 1 trait is independent of inheritance of other traits (15). This means that the association between a genetically determined phenotypic trait and a disease is unlikely to be caused by reverse causality or confounding, provided

that the presence of the genotype that causes the trait does not influence the subject's lifestyle or environment. This condition will usually be fulfilled as long as subjects are unaware of their genotype.

In 1986, one of us (M. B. K.) first suggested investigating the causality of the relation between plasma cholesterol and cancer by investigating the relation between apolipoprotein E (*ApoE*) genotype and cancer risk (16). ApoE is involved in the clearance of lipoproteins from plasma, and differences in its amino acid sequence produce differences in plasma cholesterol levels within a population. Three independent alleles of the *ApoE* gene occur frequently. They give rise to the isoforms E2, E3, and E4, with 1 cysteine residue being replaced by arginine from E2 to E3 and another one from E3 to E4. Plasma cholesterol levels rise from E2 to E3 to E4. Therefore, if low cholesterol levels promote tumor growth, then subjects with the E2/E2 or E2/E3 phenotype should have the highest risk of cancer. Our method of analysis (16) constituted the first instance of what would later be named Mendelian randomization (17). In the cholesterol-and-cancer debate, it has never (to our knowledge) been put to the test. Here we report on the association between the *ApoE* genotype, plasma cholesterol levels, and cancer risk in an elderly cohort.

MATERIALS AND METHODS

Participants

Study participants came from the placebo group of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the trial protocol and study results have been published elsewhere (13, 18). A short outline is provided here.

PROSPER was a multicenter randomized, placebo-controlled trial designed to assess whether treatment with pravastatin decreased the risk of major vascular events in elderly persons. Between December 1997 and May 1999, we screened and enrolled subjects in the United Kingdom (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70–82 years were recruited if they had preexisting vascular disease or had increased risk of such disease because of smoking, hypertension, or diabetes. Subjects with a history of malignancy within 5 years prior to the trial were not eligible to participate. A total of 5,804 subjects were randomly assigned to receive pravastatin ($n =$ 2,891) or placebo ($n = 2{,}913$). In the current study, all analyses were performed in subjects with placebo allocation ($n = 2{,}913$) so that a possible effect of pravastatin on cancer could not affect the results. The primary outcome studied was the combined endpoints of fatal coronary heart disease, nonfatal myocardial infarction, and clinical stroke, either fatal or nonfatal. Other study endpoints were transient ischemic attack, disability, cognitive function, and cancer incidence and mortality. Information on all deaths was received through postmortem reports, death certificates, hospital records, general practitioners' records, and/or interviews of family members or witnesses. All endpoints were adjudicated by a study endpoint committee. The mean duration of follow-up was 3.2 years (range, 2.8–4.0).

Measurements

Plasma cholesterol levels were measured twice at fasting visits during the placebo run-in phase according to the Lipid Research Clinics protocol (19) in a central laboratory which was standardized through the US Centers for Disease Control and Prevention network. The second measurement taken during the placebo run-in phase was used as the baseline measurement. During the follow-up of the PROSPER study, lipid and lipoprotein measurements were again performed after 3, 6, 12, 24, and 36 months. ApoE phenotype was determined in plasma samples by Western blotting, following the method of Havekes et al. (20). Subjects were classified according to the presence of the E2, E3, or E4 bands on gel blots. The gel phenotyping method showed very high concordance (>95%) with genotype testing by allele-specific oligonucleotide assay; therefore, we considered this measurement a measurement of *ApoE* genotype (21).

Statistical analysis

For the association with *ApoE* genotype, participants were divided into 3 categories: *E2+* (genotypes *E2/2* and *E2/3*), *E3/3* (the most frequent genotype), and *E4+* (genotypes *E3/4* and *E4/4*). Subjects with the *ApoE2/4* genotype ($n = 59$) were excluded from all analyses. The plasma cholesterol levels measured at baseline were divided into 3 equal strata representing low (<5.22 mmol/L), intermediate (5.22–6.02 mmol/L), and high (>6.02 mmol/L) levels. The association between *ApoE* genotype and plasma cholesterol level was assessed by linear regression. The cross-sectional associations between *ApoE* genotypes, plasma cholesterol levels, and potential confounders were assessed using the linear-by-linear association test for categorical variables and using linear regression for continuous variables. Hazard ratios with 95% confidence intervals for cancer incidence and cancer mortality were calculated using Cox proportional hazards models. Subjects who died of causes other than cancer, subjects who withdrew consent, and subjects who were lost to follow-up were censored at the date of death or the last date of follow-up. In all adjusted analyses, we corrected for the potential confounders sex, age, current smoking, alcohol use, history of hypertension, diabetes, and myocardial infarction.

All analyses were performed with SPSS statistical software (version 12.0.1; SPSS, Inc., Chicago, Illinois). *P* values lower than 0.05 were considered statistically significant.

RESULTS

The mean age of the participants was 75.3 years, and 52% were female (Table 1). The mean duration of follow-up of study subjects was 3.2 years (range, 2.8–4.0) for participants who did not die or withdraw consent. Of the 2,913 subjects allocated to placebo, apoE phenotyping was available for 2,794 (95.9%). The category *E2+* contained 332 (12%) subjects, *E3/E3* 1,768 (63%) subjects, and *E4+* 694 (25%) subjects. Translated into genotypes, the frequencies were in Hardy-Weinberg equilibrium. The genotype frequencies

Table 1. Baseline Characteristics of Participants in the Placebo Arm ($n = 2{,}913$) of the Prospective Study of Pravastatin in the Elderly at Risk, 1997–2002

Characteristic	Mean (SD)	No.	%
Continuous variables			
Age, years	75.3 (3.4)		
Body mass index[a]	26.8 (4.3)		
Cholesterol level, mmol/L			
Total cholesterol	5.7 (0.9)		
LDL cholesterol	3.8 (0.8)		
HDL cholesterol	1.3 (0.4)		
Categorical variables			
Female sex		1,505	52
Current smoker		805	28
Diabetes		320	11
Hypertension		1,793	62
Apolipoprotein E genotype[b]			
E2+		332	12
E3/E3		1,768	65
E4+		635	23

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; SD, standard deviation.

[a] Weight (kg)/height $(m)^2$.

[b] Apolipoprotein E genotype was measured in 2,735 participants.

between the 3 countries were not significantly different ($P = 0.15$).

The association between the *ApoE* genotypes and plasma lipoprotein levels is depicted in Figure 1. As expected, *ApoE2/E2* carriers had the lowest plasma cholesterol levels (mean = 5.26 mmol/L (standard error (SE), 0.25)), *ApoE3/3* carriers had intermediate levels (mean = 5.66 mmol/L (SE, 0.02)), and subjects with the *ApoE4/E4* genotype had the highest levels (mean = 5.97 mmol/L (SE, 0.12)). The

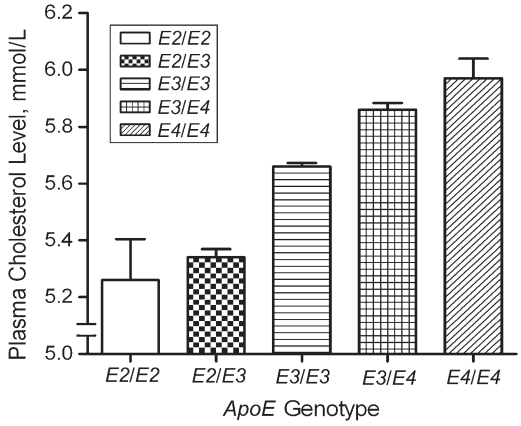

Figure 1. Association between apolipoprotein E (*ApoE*) genotype and mean plasma cholesterol level, Prospective Study of Pravastatin in the Elderly at Risk, 1997–2002. Bars, standard error.

P value for trend over the 5 categories was statistically significant ($P < 0.01$).

We divided participants into 3 equal strata representing low, intermediate, and high plasma cholesterol levels and compared various characteristics of the subjects in these 3 groups. *ApoE* genotype, sex, alcohol use, current smoking, history of diabetes, history of myocardial infarction, and history of hypertension were all significantly different over strata of cholesterol level (Table 2; all *P*'s < 0.01). As expected, when we divided the subjects into the 3 *ApoE* genotype groups, total cholesterol levels increased significantly over strata of *ApoE* genotype ($P < 0.01$). We found the same trend for low density lipoprotein (LDL) cholesterol levels, with *ApoE2* carriers having the lowest LDL cholesterol level and *ApoE4* carriers the highest ($P < 0.01$). For high density lipoprotein cholesterol, the trend was reversed: *ApoE2+* carriers had the highest levels and *ApoE4+* carriers the lowest ($P = 0.01$). However, no other characteristic was significantly different between subjects with different *ApoE* genotypes (all *P*'s > 0.07).

During follow-up, there were 199 subjects who developed cancer and 91 subjects who died of it. Results for the association between plasma cholesterol level and *ApoE* genotype, on the one hand, and cancer incidence and cancer death, on the other hand, are shown in Table 3. The group with low total cholesterol levels had an increased risk of cancer incidence compared with the group with intermediate cholesterol levels (hazard ratio (HR) = 1.45, 95% confidence interval (CI): 1.05, 2.01; $P = 0.02$) or high cholesterol levels (HR = 1.90, 95% CI: 1.34, 2.70; $P < 0.01$). After adjustment for potential confounders, subjects with low cholesterol levels still had an increased risk of incident cancer compared with subjects with intermediate levels (HR = 1.35, 95% CI: 0.97, 1.89; $P = 0.08$) or subjects with high levels (HR = 1.70, 95% CI: 1.16, 2.50; $P = 0.01$). Results were similar for LDL cholesterol: Subjects with low LDL cholesterol levels had an increased risk of incident cancer compared with subjects with higher LDL cholesterol levels. Moreover, subjects with incident cancer had cholesterol levels that decreased significantly more prior to cancer diagnosis than subjects without incident cancer (mean change = −0.23 mmol/L (SE, 0.05) vs. −0.13 mmol/L (SE, 0.01); $P = 0.05$); this finding remained significant after adjustment for sex, age, and country ($P = 0.04$).

The association between *ApoE* genotype and cancer incidence presented a different picture (Table 3). *E2+* carriers, who had the lowest cholesterol levels, had no increased risk of cancer incidence compared with the *E3/E3* subjects (HR = 0.90, 95% CI: 0.41, 1.81; $P = 0.67$) or *E4+* carriers (HR = 0.91, 95% CI: 0.53, 1.54; $P = 0.72$).

A similar trend was seen for cancer mortality as for cancer incidence (Table 3). Subjects with low levels of plasma cholesterol had an increased risk of cancer mortality compared with subjects with intermediate (HR = 2.10, 95% CI: 1.27, 3.50; $P < 0.01$) and high (HR = 2.03, 95% CI: 1.23, 3.34; $P = 0.01$) cholesterol levels. When we adjusted this association for the potential confounders, the results did not change. In the association with *ApoE* genotype and cancer mortality, we found that *ApoE2* carriers, who had the lowest plasma cholesterol levels, had similar risks of cancer

Table 2. Association Between Apolipoprotein E Genotype, Plasma Cholesterol Level, and Various Characteristics in Subjects Treated With Placebo ($n = 2,913$), Prospective Study of Pravastatin in the Elderly at Risk, 1997–2002

	Plasma Cholesterol Level[a]							Apolipoprotein E Genotype						
	Low ($n = 978$)		Intermediate ($n = 967$)		High ($n = 968$)		*P* Value[b]	*E2+* ($n = 332$)		*E3/E3* ($n = 1,768$)		*E4+* ($n = 635$)		*P* Value[b]
	Mean (SE)	No. (%)	Mean (SE)	No. (%)	Mean (SE)	No. (%)		Mean (SE)	No. (%)	Mean (SE)	No. (%)	Mean (SE)	No. (%)	
Lipoprotein profile														
Cholesterol level, mmol/L														
Total cholesterol	4.72 (0.01)		5.62 (0.01)		6.67 (0.02)		NA	5.34 (0.05)		5.66 (0.02)		5.87 (0.04)		<0.01
LDL cholesterol	3.01 (0.01)		3.75 (0.01)		4.59 (0.02)		NA	3.33 (0.04)		3.80 (0.02)		4.00 (0.03)		<0.01
HDL cholesterol	1.19 (0.01)		1.29 (0.01)		1.35 (0.01)		NA	1.31 (0.02)		1.28 (0.01)		1.24 (0.01)		0.01
Apolipoprotein E4 carrier[c]		151 (17)		229 (25)		255 (28)	<0.01	NA		NA		NA		NA
Demographic factors														
Age, years	75.2 (0.10)		75.2 (0.11)		75.5 (0.11)		0.10	75.3 (0.19)		75.4 (0.08)		75.0 (0.13)		0.07
Education, years	15.1 (0.07)		15.1 (0.06)		15.1 (0.06)		0.77	15.1 (0.11)		15.1 (0.05)		15.0 (0.07)		0.78
Body mass index[d]	26.9 (0.14)		26.7 (0.14)		26.9 (0.14)		0.78	27.0 (0.22)		26.9 (0.10)		26.7 (0.17)		0.57
Alcohol use, units/week[e]	5.5 (0.29)		5.6 (0.32)		4.1 (0.24)		<0.01	5.3 (0.47)		5.2 (0.21)		4.4 (0.30)		0.10
Female sex		300 (31)		512 (53)		693 (72)	<0.01		163 (49)		928 (53)		325 (51)	0.74
Current smoker		310 (32)		266 (28)		229 (24)	<0.01		94 (28)		484 (27)		162 (26)	0.30
Disease history														
Vascular disease		431 (44)		424 (44)		404 (42)	0.30		134 (40)		784 (44)		273 (43)	0.64
Hypertension		559 (57)		594 (61)		640 (66)	<0.01		197 (59)		1,101 (62)		399 (63)	0.35
Diabetes		149 (15)		101 (10)		70 (7)	<0.01		45 (14)		187 (11)		62 (10)	0.10
Stroke or transient ischemic attack		103 (11)		109 (11)		109 (11)	0.61		34 (10)		193 (11)		77 (12)	0.33
Myocardial infarction		167 (17)		136 (14)		96 (10)	<0.01		54 (16)		231 (13)		89 (14)	0.54

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; NA, not applicable; SE, standard error.

[a] Plasma cholesterol values were divided into 3 equal strata representing low, intermediate, and high levels.

[b] *P* values for categorical variables were assessed with the linear-by-linear association test; *P* values for continuous variables were assessed with linear regression.

[c] Apolipoprotein E genotype was measured in 2,735 participants.

[d] Weight (kg)/height $(m)^2$.

[e] Alcohol intake was quantified in terms of usual weekly alcohol intake, in alcohol units, for the previous month.

Table 3. Association Between Apolipoprotein E Genotype, Plasma Cholesterol Level, and Cancer Risk in Subjects Treated With Placebo ($n = 2,913$), Prospective Study of Pravastatin in the Elderly at Risk, 1997–2002

	Plasma Cholesterol Level[a]						Apolipoprotein E Genotype					
	Low vs. Intermediate[b]			Low vs. High[b]			*E2*+ vs. *E3*/*E3*[b]			*E2*+ vs. *E4*+[b]		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Crude model												
Cancer incidence	1.45	1.05, 2.01	0.02	1.90	1.34, 2.70	<0.01	0.90	0.41, 1.81	0.67	0.91	0.53, 1.54	0.72
Cancer mortality	2.10	1.27, 3.50	<0.01	2.03	1.23, 3.34	0.01	0.86	0.56, 1.45	0.69	0.74	0.33, 1.68	0.47
Adjusted model[c]												
Cancer incidence	1.35	0.97, 1.89	0.08	1.70	1.16, 2.50	0.01	0.88	0.55, 1.41	0.59	0.86	0.50, 1.47	0.59
Cancer mortality	2.16	1.28, 3.64	<0.01	1.93	1.12, 3.34	0.02	0.85	0.40, 1.79	0.67	0.70	0.30, 1.60	0.39

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a] Plasma cholesterol values were divided into 3 equal strata representing low, intermediate, and high levels.
[b] The second category was the reference category.
[c] Results were adjusted for sex, age, smoking, alcohol use, and history of hypertension, diabetes, and myocardial infarction.

mortality as *ApoE3*/*E3* carriers (HR = 0.86, 95% CI: 0.56, 1.45; $P = 0.69$) and *ApoE4* carriers (HR = 0.74, 95% CI: 0.33, 1.68; $P = 0.47$).

DISCUSSION

In this study, we assessed the association between plasma cholesterol level and cancer risk, free of confounding and reverse causality, using the method of Mendelian randomization. We found that subjects, when grouped by their baseline cholesterol levels, had an increased cancer risk if their cholesterol levels were lower. This risk remained even after adjustment for potential confounders. However, when we categorized subjects according to their *ApoE* genotypes, which also resulted in groups with significantly different cholesterol levels, no increased risk of cancer was observed between groups. These findings suggest that low levels of cholesterol are not causally related to an increased risk of cancer.

If cholesterol is causally related to an increased risk of cancer, we would have found similar results for the association between plasma cholesterol level and cancer risk as for the association between *ApoE* genotype and cancer risk. When we grouped subjects on the basis of their cholesterol level, those in the low-cholesterol group had an increased risk of cancer. However, when we grouped subjects according to *ApoE* genotype, subjects in the *ApoE2*+ group had no increased risk of cancer, despite their significantly lower level of cholesterol. We were planning to formally test with statistical software using Mendelian randomization whether the 2 different methods gave different results. However, to our knowledge, this is only possible for continuous outcome data. Since we had dichotomous outcome data, we were unable to formally test whether the 2 methods actually yielded different results. When we adjusted the association between plasma cholesterol level and cancer for a wide range of potential confounders, including age, sex, current smoking, alcohol use, diabetes, myocardial infarction, and history of hypertension, we still found a significant association between low cholesterol level and higher risk of cancer. Therefore, we think that the association between low plasma cholesterol level and increased cancer risk is more likely to be due to reverse causality, and less so to confounding.

Substantial evidence indicates that cancer can reduce plasma cholesterol levels prior to cancer diagnosis. This phenomenon is known as the preclinical cancer effect (7). The mechanism by which cancer can lower plasma cholesterol level is unclear. However, research into this mechanism has revealed that tumor cells need cholesterol for their growth and proliferation. Therefore, there is increased uptake of cholesterol from the blood by tumor cells (22, 23). This might lead to lower plasma cholesterol levels prior to cancer diagnosis. Moreover, alterations in plasma lipids and lipoprotein fractions have been demonstrated in patients with acute leukemia and non-Hodgkin's lymphoma (24, 25). Similarly, there is ample evidence, as recently reviewed (26), for an inverse relation between the magnitude of inflammatory response and lipid levels in a variety of conditions such as sepsis, rheumatoid arthritis, and other cancers: Cholesterol levels are lowered in these illnesses but can increase dramatically and spontaneously with resolution of sepsis or with treatments which potently suppress the inflammatory response.

In 1986, one of us (M. B. K.) proposed investigating the causality of cholesterol in cancer risk by making use of data on the *ApoE* genotype (16). He reasoned that if a naturally low cholesterol level favors tumor growth, then carriers of the *ApoE2*+ genotype, who have lower levels of plasma cholesterol, should have an increased risk of cancer. Until 2004, no one had taken up his idea (27). Now, more than 20 years after this initial suggestion, we have finally addressed the causality of cholesterol in the risk of cancer.

There were some limitations to the use of the PROSPER study cohort. The PROSPER subjects were selected to have a history of vascular disease or an increased risk for such disease. Although the frequencies of the *ApoE* genotypes in our study were similar to those in the general population, when extrapolating these results to the general population,

the enrichment of cardiovascular disease in our study population should be kept in mind. Furthermore, we think that the association between plasma cholesterol and cancer risk is mostly affected by reverse causality, and less by confounding, because adjustment for potential confounders did not change the results. However, the number of confounders we adjusted for might not have been sufficient; there could be other confounders we were not aware of. Therefore, we cannot completely exclude the possibility that the association between cholesterol and cancer is due to confounding rather than disturbed by reverse causality.

Moreover, although our study had adequate statistical power to find a hazard ratio of 1.5 between cholesterol groups, it was relatively small for demonstrating equivalence between genotype groups. Given a 9% difference in cholesterol level between the most extreme *ApoE* groups, the estimated difference in cancer risk would also be small. Therefore, our study had relatively low power, which is an important drawback of Mendelian randomization studies (28). Thus, we cannot state with absolute certainty that low cholesterol levels do not cause cancer. However, given the fact that all hazard ratios were below unity, it is unlikely that low levels of cholesterol have a substantial impact on cancer risk.

One strength of our study is that we had a follow-up period of 3.2 years and were able to track more than 95% of all participants over this time. Moreover, cancer incidence and mortality were main outcomes in our study and were precisely monitored, which increases the accuracy of the findings accordingly.

In conclusion, we used Mendelian randomization to determine that the association between low plasma cholesterol levels and risk of cancer does not appear to be derived from a causal effect. Carriers of the *ApoE2+* genotype, which is associated with low plasma cholesterol levels, had no increased risk of cancer. We therefore believe that subjects with low plasma cholesterol levels are not at increased risk of cancer and that treatment with cholesterol-lowering medications does not increase cancer risk by itself.

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Conflict of interest: none declared.

REFERENCES

1. Cowan LD, O'Connell DL, Criqui MH, et al. Cancer mortality and lipid and lipoprotein levels. Lipid Research Clinics Program Mortality Follow-up Study. *Am J Epidemiol*. 1990;131(3):468–482.
2. Iribarren C, Reed DM, Chen R, et al. Low serum cholesterol and mortality. Which is the cause and which is the effect? *Circulation*. 1995;92(9):2396–2403.
3. Keys A, Aravanis C, Blackburn H, et al. Serum cholesterol and cancer mortality in the Seven Countries Study. *Am J Epidemiol*. 1985;121(6):870–883.
4. Knekt P, Reunanen A, Aromaa A, et al. Serum cholesterol and risk of cancer in a cohort of 39,000 men and women. *J Clin Epidemiol*. 1988;41(6):519–530.
5. Schuit AJ, Van Dijk CE, Dekker JM, et al. Inverse association between serum total cholesterol and cancer mortality in Dutch civil servants. *Am J Epidemiol*. 1993;137(9):966–976.
6. Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, et al. Total cholesterol and risk of mortality in the oldest old. *Lancet*. 1997;350(9085):1119–1123.
7. Kritchevsky SB, Kritchevsky D. Serum cholesterol and cancer risk: an epidemiologic perspective. *Annu Rev Nutr.* 1992;12:391–416.
8. Bonovas S, Filioussi K, Flordellis CS, et al. Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients. *J Clin Oncol*. 2007;25(23):3462–3468.
9. Browning DR, Martin RM. Statins and risk of cancer: a systematic review and metaanalysis. *Int J Cancer.* 2007;120(4):833–843.
10. Alsheikh-Ali AA, Trikalinos TA, Kent DM, et al. Statins, low-density lipoprotein cholesterol, and risk of cancer. *J Am Coll Cardiol*. 2008;52(14):1141–1147.
11. Kuoppala J, Lamminpää A, Pukkala E. Statins and cancer: a systematic review and meta-analysis. *Eur J Cancer.* 2008;44(15):2122–2132.
12. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359(21):2195–2207.
13. Shepherd J, Blauw GJ, Murphy MB, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet*. 2002;360(9346):1623–1630.

14. Peto R, Emberson J, Landray M, et al. Analyses of cancer data from three ezetimibe trials. *N Engl J Med*. 2008;359(13):1357–1366.
15. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32(1):1–22.
16. Katan MB. Apolipoprotein E isoforms, serum cholesterol, and cancer. *Lancet*. 1986;1(8479):507–508.
17. Keavney B. Commentary: Katan's remarkable foresight: genes and causality 18 years on. *Int J Epidemiol*. 2004;33(1):11–14.
18. Shepherd J, Blauw GJ, Murphy MB, et al. The design of a prospective study of pravastatin in the elderly at risk (PROSPER). PROSPER Study Group. *Am J Cardiol*. 1999;84(10):1192–1197.
19. The Lipid Research Clinics Program Epidemiology Committee. Plasma lipid distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. *Circulation*. 1979;60(2):427–439.
20. Havekes LM, de Knijff P, Beisiegel U, et al. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J Lipid Res.* 1987;28(4):455–463.
21. Packard CJ, Westendorp RG, Stott DJ, et al. Association between apolipoprotein E4 and cognitive decline in elderly adults. *J Am Geriatr Soc.* 2007;55(11):1777–1785.
22. Ueyama Y, Matsuzawa Y, Yamashita S, et al. Hypocholesterolaemic factor from gallbladder cancer cells. *Lancet*. 1990;336(8717):707–709.
23. Vitols S, Gahrton G, Björkholm M, et al. Hypocholesterolaemia in malignancy due to elevated low-density-lipoprotein-receptor activity in tumour cells: evidence from studies in patients with leukaemia. *Lancet*. 1985;2(8465):1150–1154.
24. Budd D, Ginsberg H. Hypocholesterolemia and acute myelogenous leukemia. Association between disease activity and plasma low-density lipoprotein cholesterol concentrations. *Cancer.* 1986;58(6):1361–1365.
25. Spiegel RJ, Schaefer EJ, Magrath IT, et al. Plasma lipid alterations in leukemia and lymphoma. *Am J Med*. 1982;72(5):775–782.
26. Choy E, Sattar N. Interpreting lipid levels in the context of high-grade inflammatory states with a focus on rheumatoid arthritis: a challenge to conventional cardiovascular risk actions. *Ann Rheum Dis*. 2009;68(4):460–469.
27. Katan MB. Commentary: Mendelian randomization, 18 years on. *Int J Epidemiol*. 2004;33(1):10–11.
28. Schatzkin A, Abnet CC, Cross AJ, et al. Mendelian randomization: how it can—and cannot—help confirm causal relations between nutrition and cancer. *Cancer Prev Res (Phila Pa).* 2009;2(2):104–113.