Genes, inflammation, and age-related diseases

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Chapter 9

ApoE genotype, plasma cholesterol, and cancer: A Mendelian randomization study.

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Abstract

Observational studies have shown an association between low plasma cholesterol levels and an increased risk of cancer whereas most randomized clinical trials with cholesterol lowering medications have not shown this association. The authors assessed the association between plasma cholesterol levels and cancer risk, free from confounding and reverse causality, by a Mendelian randomization study design using the ApoE genotype. ApoE genotype, plasma cholesterol levels, and cancer incidence and mortality were measured during three years in 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 an increased risk of cancer incidence (HR (95% CI) = 1.90 (1.34, 2.70)) and cancer mortality (HR (95% CI) = 2.03 (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 levels of plasma cholesterol than carriers of the ApoE4 genotype (n=635), did not have in increased risk of incident cancer incidence (HR (95% CI) = 0.86 (0.50, 1.47)) or cancer mortality (HR (95% CI) = 0.70 (0.30, 1.60)) compared to ApoE4 carriers. Our findings suggest that low levels of cholesterol are not causally related to an increased risk of cancer.

Introduction

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 treatment or lifestyle changes that lower cholesterol 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 of cancer (7). In this case, subjects with cancer have an abnormal low cholesterol level because of the cancer and 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 whether a lifelong low plasma cholesterol level increases cancer risk has remained elusive.

An alternative epidemiological method, Mendelian randomization, overcomes the problem of reverse causality and confounding since it is based on Mendel's law that inheritance of one 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 (MBK) first suggested to investigate the causality of the relation between plasma cholesterol and cancer by investigating the relationship between ApoE genotype and cancer risk (16). ApoE is involved in the clearance of lipoproteins from plasma and differences in its aminoacid 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 one 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 tumour growth, then subjects with the E2/E2 or E2/E3 phenotype should have the highest risk of cancer. Our way 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 been put to the test. We here report the association between the ApoE genotype, plasma cholesterol levels, and cancer risk in an elderly cohort.

Methods

Participants

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

PROSPER was a multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin decreases the risk of major vascular events in elderly individuals. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing 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 number of 5804 subjects were randomly assigned to pravastatin (N=2,891) or placebo (N=2,913). In our 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 our results. The primary endpoint was the combined endpoint of fatal coronary heart disease (CHD), non-fatal myocardial infarct (MI), and occurrence of clinical stroke, either fatal or non-fatal. Other study endpoints were occurrence of transient ischemic attack, disability, cognitive function, and cancer incidence and mortality. Information on all deaths were received by post-mortem 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. Mean follow-up duration 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 Center for Disease Control network. The second measurement 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.

Apolipoprotein E phenotype was determined on 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 shows very high concordance (>95%) with genotype testing by allele specific oligonucleotide assay, therefore we consider this measurement as a measurement of the ApoE genotype (21).

Statistical analysis

All statistical analyses were performed in subjects with placebo allocation so that a possible effect of pravastatin on cancer could not affect our results. For the association with ApoE genotype, participants were divided into three categories, E2+ (genotypes E2/2, E2/3), E3/3 (the most frequent genotype) and E4+ (genotypes E3/4, and E4/4). Subjects with the ApoE2/4 (n=59) were excluded from all analyses. The plasma cholesterol levels measured at baseline were divided in three equal strata representing low (<5.22 mmol/L), intermediate (5.22-6.02 mmol/L) and high plasma cholesterol levels (>6.02 mmol/L). 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 by linear regression for continuous variables. Hazard ratios (HR) with 95% confidence intervals (CI) for cancer incidence and cancer mortality were calculated using Cox-proportional hazards models. Subjects who died of other causes of death than cancer, subjects who withdrew consent, and subjects who were lost to follow-up were censored at the date of death or at the last date of follow-up. In all adjusted analyses we corrected for the potential confounders of gender, 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, Ill). *P*-values lower than 0.05 were considered statistically significant.

Results

The mean age of the participants was 75.3 years and approximately 50% were female (Table 1). Mean 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 2913 subjects allocated to placebo, apolipoprotein E phenotyping was available for 2794 (95.9%). The category E2+ consisted of 332 (12%) subjects, E3/E3 of 1768 (63%) subjects, and E4+ of 694 (25%) subjects. Translated into genotypes, the frequencies were in Hardy-Weinberg equilibrium. The genotype frequencies between the three countries were not significantly different (*P*=0.15).

Table 1: Baseline Characteristics of the Participants in the Placebo Arm of the PROSPER Study

	All participants (n=2,913)
Continuous variates (mean, SD)	
Age (years)	75.3 (3.4)
Body Mass index, (kg/m2)	26.8 (4.3)
Total cholesterol, (mmol/L)	5.7 (0.9)
LDL cholesterol, (mmol/L)	3.8 (0.8)
HDL cholesterol, (mmol/L)	1.3 (0.4)
Categorical variates (n, %)	
Female	1,505 (52)
Current smoker	805 (28)
Diabetes	320 (11)
Hypertension	1,793 (62)
ApoE genotype (n, %)*	
E2+	332 (11)
E3/E3	1,768 (61)
E4+	635 (23)

* ApoE genotype was measured in 2,735 participants

The association between the ApoE genotypes and plasma lipoprotein levels is depicted in Figure 1. As expected, ApoE2/E2 carriers had lowest plasma cholesterol levels (mean 5.26 (SE 0.25)), ApoE3/3 subjects had intermediate plasma cholesterol levels (mean 5.66 (SE 0.02)) and subjects with the ApoE4/E4 genotype had the highest plasma cholesterol levels (mean 5.97 (SE 0.12)). The p-value for trend over the five categories was statistically significant (*P*<0.01).

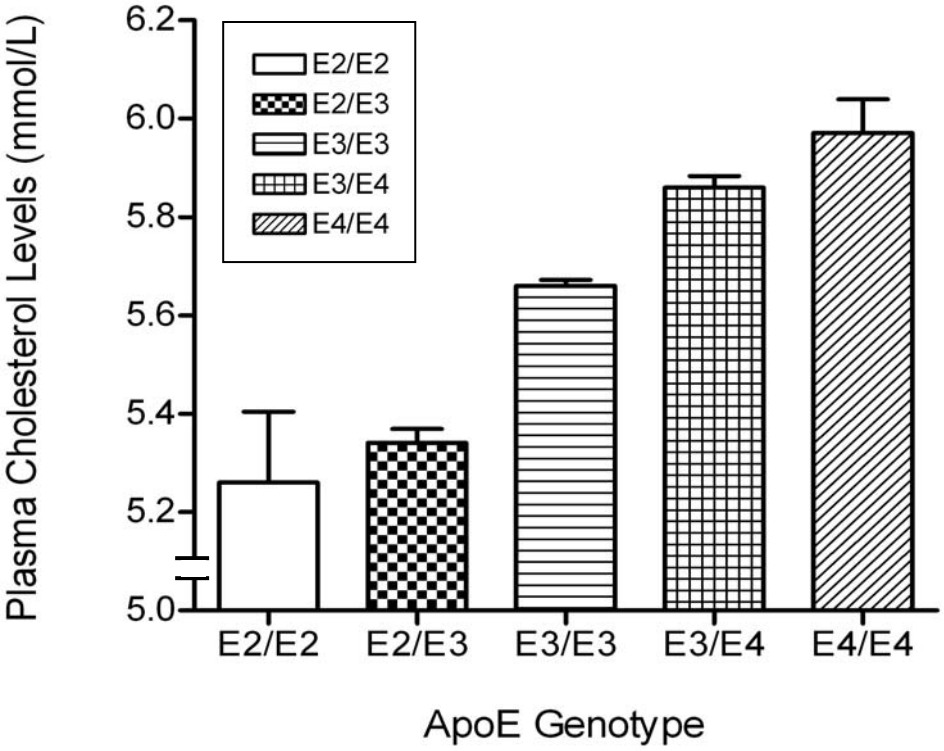

Figure 1: Association between ApoE genotype and plasma cholesterol levels.

Plasma cholesterol levels (mmol/L) are presented in mean (se).

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

During follow-up there were 199 subjects who developed cancer and 91 subjects who died of it. The results of the association between plasma cholesterol levels 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 cholesterol levels had an increased risk of cancer incidence compared to the group with intermediate cholesterol levels (HR (95%CI) = 1.45 (1.05-2.01), *P*=0.02) or high cholesterol levels (HR (95%CI) = 1.90 (1.34-2.70), *P*<0.01). After adjustment for potential confounders, subjects with low cholesterol levels still had an increased risk for cancer incidence compared to subjects with intermediate cholesterol levels (HR (95%CI) =1.35 (0.97-1.89), *P*=0.08) or compared to subjects with high cholesterol levels (HR (95%CI) = 1.70 (1.16-2.50), *P*<0.01). Results were similar for LDL cholesterol levels, subjects with low LDL cholesterol levels had an increased risk for cancer incidence compared to subjects with higher LDL cholesterol levels. Moreover, subjects with incident cancer decreased significantly more in cholesterol levels prior cancer diagnosis compared to subjects without incident cancer (mean (se) = -0.23 (0.05) vs -0.13 (0.01) respectively, *P*=0.05), this remained significant after adjustment for gender, age, and country (*P*=0.04). The association between ApoE genotype and cancer incidence presented a different picture (Table 3). E2+ carriers, with the lowest cholesterol levels, had no increased risk for cancer incidence compared to the E3/E3 subject (HR (95%CI) = 0.90 (0.56-1.45), *P*=0.67) or to E4+ carriers (HR (95%CI) = 0.91 (0.53-1.54), *P*=0.72).

A similar trend was seen for cancer mortality as with cancer incidence (table 3). Subjects with low levels of plasma cholesterol had an increased risk of cancer mortality compared to intermediate levels of cholesterol (HR (95%CI) = 2.10 (1.27-3.50), *P*=<0.01) and high levels of cholesterol (HR (95%CI) = 2.03 (0.95-3.02), *P*<0.01). 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 a similar risk for cancer mortality compared to ApoE3/E3 carriers (HR (95%CI) = 0.88 (0.55-1.41), *P*=0.59) and compared to ApoE4 carriers (HR (95%CI) = 0.86 (0.50-1.47), *P*=0.59).

Table 2: Association between ApoE, Plasma Cholesterol and Various Characteristics in Subjects Treated With Placebo[a].

	Plasma cholesterol levels[b]					ApoE genotype			
	Low (n=978)	Intermediate (n=967)	High (n=968)	P-value[c]		E2+ (n=332)	E3/E3 (n=1,768)	E4+ (n=635)	P-value[c]
Lipoprotein profile									
Total cholesterol, mmol/L (mean, SE)	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, mmol/L (mean, SE)	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, mmol/L (mean, SE)	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**
ApoE genotype									
ApoE4 carriers	151 (17)	229 (25)	255 (28)	**<0.01**		NA	NA	NA	NA
Demographics									
Age, years (mean, SE)	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 (mean, SE)	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
BMI, kg/m2 (mean, SE)	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 (mean, SE)	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	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
History of vascular disease	431 (44)	424 (44)	404 (42)	0.30		134 (40)	784 (44)	273 (43)	0.64
History of hypertension	559 (57)	594 (61)	640 (66)	**<0.01**		197 (59)	1,101 (62)	399 (63)	0.35
History of diabetes	149 (15)	101 (10)	70 (7)	**<0.01**		45 (14)	187 (11)	62 (10)	0.10
History of Stroke or TIA	103 (11)	109 (11)	109 (11)	0.61		34 (10)	193 (11)	77 (12)	0.33
History of Myocardial infarction	167 (17)	136 (14)	96 (10)	**<0.01**		54 (16)	231 (13)	89 (14)	0.54

Abbreviations: BMI, Body Mass Index; NA, Not applicable; TIA, transient ischemic attack

[a]All data are presented as number (%), unless otherwise stated.

[b]Plasma cholesterol levels are divided in three equal strata representing low, intermediate, and high plasma cholesterol levels.

[c]P-values for categorical variables are assessed with the linear-by linear association test, p-values for continuous variables are assessed with linear regression.

Table 3: Association Between ApoE, Cholesterol and Cancer Risk.

	Plasma cholesterol levels[a]						ApoE genotype					
	Low vs intermediate[c]			Low vs high[c]			E2+ vs E3/E3[c]			E2+ vs E4+[c]		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
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[b]												
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 levels are divided in three equal strata representing low, intermediate, and high plasma cholesterol levels.

[b]Adjusted model is additionally adjusted for sex, age, smokers, alcohol use, and history of hypertension, diabetes, and myocardial infarction.

[c]The last group indicates the reference category.

Discussion

In this study we assessed the association between plasma cholesterol levels and cancer risk, free of confounding and reverse causality by using the method of Mendelian randomization.

We found that subjects, when grouped by their baseline levels of cholesterol, had an increased cancer risk if the cholesterol levels were lower. This risk remained even after adjustment for potential confounders. However, when we categorized subjects according to their ApoE genotype, which also resulted in groups with significantly different cholesterol levels, no increased risk for cancer risk 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 levels and cancer risk as for the ApoE genotype and cancer risk. When we grouped subjects based on their cholesterol level, those in the low cholesterol group had an increased risk of cancer. However, when we grouped 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 two different methods gave different results. However, to our knowledge, this is only possible for continuous outcome data. Since we have dichotomous outcome data, we were unable to formally test whether the two methods actually yielded different results. When we adjusted the association between plasma cholesterol levels 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 that the association between low plasma cholesterol levels and increased risk for cancer is more likely to be due to reverse causality, and less so by 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 is unclear. However, research into this mechanism has revealed that tumor cells need cholesterol for their growth and proliferation. Therefore there is an 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 relationship 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 (MBK) proposed to investigate the causality of cholesterol in cancer risk by making use of the ApoE genotype (16). He reasoned that if naturally low cholesterol favours tumour 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 idea we finally address the causality of cholesterol in the risk of cancer.

There are some limitations to the use of the PROSPER study cohort. Subjects were selected to have a history of vascular disease or have an increased risk for such a disease. Although the frequencies of the ApoE genotypes in our study are similar to those in the general population, when extrapolating the 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 have adjusted for might not be sufficient; it could be that there are still confounders that we do not know 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 was adequately powered to find a hazard ratio of 1.5 between cholesterol groups, it was relatively small to demonstrate equivalence between genotype groups. Given a 9% difference in cholesterol level between most extreme ApoE groups, the estimated difference in cancer risk would also be small. Therefore our study has a relatively low power, which is an important drawback of Mendelian randomization studies (28). Therefore we cannot state with absolute certainty that low cholesterol does not cause cancer. But given the fact that all hazard ratios are 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 have 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 of our study and were precisely monitored, which increases the accuracy of this study accordingly.

In conclusion, we have used the Mendelian randomization concept to suggest that the association between low plasma cholesterol and risk of cancer does not derive from a cause effect. Carriers of the ApoE2+ genotype, 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 an increased risk for cancer and that treatment with cholesterol lowering medications does not increase cancer risk by itself.

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Reference List

(1) Cowan LD, O'Connell DL, Criqui MH, Barrett-Connor E, Bush TL, Wallace RB. Cancer mortality and lipid and lipoprotein levels. Lipid Research Clinics Program Mortality Follow-up Study. Am J Epidemiol 1990 Mar;131(3):468-82.

(2) Iribarren C, Reed DM, Chen R, Yano K, Dwyer JH. Low serum cholesterol and mortality. Which is the cause and which is the effect? Circulation 1995 Nov 1;92(9):2396-403.

(3) Keys A, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F, et al. Serum cholesterol and cancer mortality in the Seven Countries Study. Am J Epidemiol 1985 Jun;121(6):870-83.

(4) Knekt P, Reunanen A, Aromaa A, Heliovaara M, Hakulinen T, Hakama M. Serum cholesterol and risk of cancer in a cohort of 39,000 men and women. J Clin Epidemiol 1988;41(6):519-30.

(5) Schuit AJ, Van Dijk CE, Dekker JM, Schouten EG, Kok FJ. Inverse association between serum total cholesterol and cancer mortality in Dutch civil servants. Am J Epidemiol 1993 May 1;137(9):966-76.

(6) Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG. Total cholesterol and risk of mortality in the oldest old. Lancet 1997 Oct 18;350(9085):1119-23.

(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, Sitaras NM. Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients. J Clin Oncol 2007 Aug 10;25(23):3462-8.

(9) Browning DR, Martin RM. Statins and risk of cancer: a systematic review and metaanalysis. Int J Cancer 2007 Feb 15;120(4):833-43.

(10) Alsheikh-Ali AA, Trikalinos T.A., Kent D.M., Karas R.H. Statins, low-density lipoprotein cholesterol, and risk of cancer. J Am Coll Cardiol 2008 Aug 20.

(11) Kuoppala J, Lamminpaa A, Pukkala E. Statins and cancer: A systematic review and meta-analysis. Eur J Cancer 2008 Aug 14.

(12) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008 Nov 20;359(21):2195-207.

(13) Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet 2002 Nov 23;360(9346):1623-30.

(14) Peto R, Emberson J, Landray M, Baigent C, Collins R, Clare R, et al. Analyses of cancer data from three ezetimibe trials. N Engl J Med 2008 Sep 25;359(13):1357-66.

(15) Davey SG, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003 Feb;32(1):1-22.

(16) Katan MB. Apolipoprotein E isoforms, serum cholesterol, and cancer. Lancet 1986 Mar 1;1(8479):507-8.

(17) Keavney B. Commentary: Katan's remarkable foresight: genes and causality 18 years on. Int J Epidemiol 2004 Feb;33(1):11-4.

(18) Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, et al. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. Am J Cardiol 1999 Nov 15;84(10):1192-7.

(19) Plasma lipid distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. The Lipid Research Clinics Program Epidemiology Committee. Circulation 1979 Aug;60(2):427-39.

(20) Havekes LM, de KP, Beisiegel U, Havinga J, Smit M, Klasen E. A rapid micromethod for apolipoprotein E phenotyping directly in serum. J Lipid Res 1987 Apr;28(4):455-63.

(21) Packard CJ, Westendorp RG, Stott DJ, Caslake MJ, Murray HM, Shepherd J, et al. Association between apolipoprotein E4 and cognitive decline in elderly adults. J Am Geriatr Soc 2007 Nov;55(11):1777-85.

(22) Ueyama Y, Matsuzawa Y, Yamashita S, Funahashi T, Sakai N, Nakamura T, et al. Hypocholesterolaemic factor from gallbladder cancer cells. Lancet 1990 Sep 22;336(8717):707-9.

(23) Vitols S, Gahrton G, Bjorkholm M, Peterson C. Hypocholesterolaemia in malignancy due to elevated low-density-lipoprotein-receptor activity in tumour cells: evidence from studies in patients with leukaemia. Lancet 1985 Nov 23;2(8465):1150-4.

(24) Budd D, Ginsberg H. Hypocholesterolemia and acute myelogenous leukemia. Association between disease activity and plasma low-density lipoprotein cholesterol concentrations. Cancer 1986 Sep 15;58(6):1361-5.

(25) Spiegel RJ, Schaefer EJ, Magrath IT, Edwards BK. Plasma lipid alterations in leukemia and lymphoma. Am J Med 1982 May;72(5):775-82.

(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 Apr;68(4):460-9.

(27) Katan MB. Commentary: Mendelian Randomization, 18 years on. Int J Epidemiol 2004 Feb;33(1):10-1.

(28) Schatzkin A, Abnet CC, Cross AJ, Gunter M, Pfeiffer R, Gail M, et al. Mendelian randomization: how it can--and cannot--help confirm causal relations between nutrition and cancer. Cancer Prev Res (Phila Pa) 2009 Feb;2(2):104-13.