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Plasma total cholesterol concentration and risk of higher-grade prostate cancer: A nested case-control study and a dose-response meta-analysis

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Abstract

Our previous publication found an increased risk of higher-grade (Gleason sum ≥7) prostate cancer for men with high total cholesterol concentration (≥200 mg/dl) in the Health Professionals Follow-up Study (HPFS). With additional 568 prostate cancer cases, we are now able to investigate this association in more detail. For the nested case-control study, we included 1260 men newly diagnosed with prostate cancer between 1993 and 2004, and 1328 controls. For the meta-analyses, 23 articles studied the relationship between total cholesterol level and prostate cancer incidence were included. Logistic regression models and dose-response meta-analysis were performed. An increased risk of higher-grade (Gleason sum ≥4 + 3) prostate cancer for high vs low quartile of total cholesterol level was observed in the HPFS ($OR_{multivariable} = 1.56$; 95% CI = 1.01-2.40). This finding was compatible with the association noted in the meta-analysis of highest vs lowest group of total cholesterol level, which suggested a

Abbreviations: ATBC, alpha-tocopherol, beta-carotene cancer prevention; CI, confidence interval; CYP27A1, sterol-27-hydroxylase; EDTA, ethylenediaminetetraacetic acid; FFQ, food-frequency questionnaires; GLM, general linear model; HDL, high-density lipoprotein; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; LDL, low-density lipoprotein; OR, odds ratio; PRACTICAL, prostate cancer association group to investigate cancer associated alterations in the genome; PSA, prostate specific antigen; RR, relative risks; SQLE, squalene monooxygenase; TNM, tumor node metastasis.

moderately increased risk of higher-grade prostate cancer (Pooled RR =1.21; 95%CI: 1.11-1.32). Moreover, the dose-response meta-analysis indicated that an increased risk of higher-grade prostate cancer occurred primarily at total cholesterol levels ≥200 mg/dl, where the RR was 1.04 (95%CI: 1.01-1.08) per 20 mg/dl increase in total cholesterol level. However, total cholesterol concentration was not associated with the risk of prostate cancer overall either in the HPFS or in the meta-analysis. Our primary finding, as well as the result of the meta-analysis suggested a modest increased risk of higher-grade prostate cancer, at total cholesterol concentrations exceeding 200 mg/dl.

KEYWORDS
dose-response meta-analysis, nested case-control study, prostate cancer, total cholesterol

What's new?

Cholesterol dysregulation is potentially linked to the development and progression of prostate cancer. Relationships between total cholesterol and prostate cancer, however, remain unclear. In this study, the authors examined associations between total cholesterol, prostate cancer risk and aggressive prostate cancer using data from the Health Professionals Follow-up Study (HFPS) and meta-analysis of previous reports describing associations. Analyses indicate that risk of higher-grade prostate cancer increases in conjunction with higher total cholesterol concentrations. Increased risk of more aggressive malignancy was most pronounced at cholesterol levels above 200 mg/dl, suggesting that elevated cholesterol level is a promising biomarker for prostate cancer detection.

1 | INTRODUCTION

According to cancer statistics of 2022,[1] prostate cancer is the most common cancer among men, and the second leading cause of cancer death in the United States. Epidemiological studies suggest an association between circulating cholesterol and the burden of prostate cancer while laboratory studies implicate cholesterol dysregulation during the prostate carcinogenesis cascade.[2] In a previous publication,[3] we examined the association between plasma total cholesterol concentration and risk of prostate cancer in the Health Professionals Follow-up Study (HPFS) from 1993 through 2000, and we found that men with a higher total cholesterol level were at a risk of higher-grade disease (Gleason sum ≥7) and that this association appeared to have a threshold. We did not observe any association with prostate cancer incidence overall. Other prospective studies have also investigated this link, with some finding associations with total prostate cancer risk,[4,5] some with aggressive disease features (eg, highest grades or advanced stage),[5,6] and others finding no association.[7-9]

Since no consensus has been reached on the link between circulating total cholesterol concentrations and prostate cancer risk overall and by disease aggressiveness, we estimated the association of plasma total cholesterol level with the risk of overall prostate cancer and higher-grade disease in greater detail, and the association with metastatic disease with extended follow-up of the HPFS cohort. With follow-up through 2004, 1260 men developed prostate cancer, yielding a total of 204 men with Gleason sum ≥4 + 3 disease and 129 men with metastatic disease. Furthermore, a dose-response meta-analysis was performed to figure out the shape of the association between circulating total cholesterol concentration and overall, higher-grade and advanced prostate cancer.

2 | MATERIALS AND METHODS

2.1 | Study population

The HPFS was established in 1986, and comprised of 51 529 US male of age 40 to 75 years. As described in previous publications,[3,10] participants enrolled by responding to a baseline questionnaire on medical history and lifestyle, and mailed follow-up questionnaires every 2 years to update lifestyle and health conditions. Moreover, a semi-quantitative food-frequency questionnaire (FFQ) was mailed every 4 years to update diet information. The current study is nested in the HPFS blood cohort including 18 018 men who provided blood samples during 1993 to 1995. Excluding T1a tumors (n = 68), 1260 newly diagnosed prostate cancer cases were ascertained between 1993 and 2004. Of these, 568 were diagnosed during the extended follow-up from 2000 and 2004. We conducted a nested case-control study, where controls (n = 1328) were randomly selected in a 1:1 ratio from men who were free of prostate cancer and had at least one PSA test at the time of case ascertainment, and were matched for year of birth, PSA test prior to blood draw, and year, season and time of day of blood collection.

2.2 | Assessment of prostate cancer cases

Cases were identified from self-reports on questionnaires, then confirmed diagnosis and clinical information (including stage at diagnosis and Gleason sum) from medical and pathology records. Deaths were either reported by family members or identified by a search of the National Death Index. We defined subtypes of prostate cancer as clinically localized (stage T1b to T2b and N0 and M0), lower-grade (Gleason sum ≤3 + 4), higher-grade (Gleason sum ≥4 + 3) or metastatic disease (distant metastases or death from prostate cancer).

2.3 | Measurement of plasma total cholesterol concentration

Plasma samples were assayed adjacently in four batches for those identified by January 1996, February 1996 to January 1998, February 1998 to January 2000, and February 2000 to January 2004. As reported in previous study,[3] plasma total cholesterol concentration was measured by two enzymatic assay kits, where kit from Sigma Diagnostics (St. Louis, MO) was performed for batches 1 and 2, and kit from Equal Diagnostics (Exton, PA) for batch 3. For batch 4, plasma total cholesterol concentration was measured using an enzymatic assay kit from Roche Diagnostics (Indianapolis, IN). The intra-assay coefficient of variation for total cholesterol level were 10.9% for batches 1 and 2, 9.1% for batch 3, and 0.7% for batch 4.

2.4 | Dose-response meta-analysis

We conducted a literature search on PubMed, EMBASE and Cochrane databases for studies published up to March 2022. The search strategy was ("*Prostatic Neoplasms*" [*MeSH*] *OR prostate cancer* [*tiab*] *OR prostate tumor* [*tiab*] *OR prostate neoplas** [*tiab*] *OR prostate carcinoma* [*tiab*] *OR prostate malignanc** [*tiab*]) *AND* ("*Cholesterol*" [*MeSH*] *OR cholesterol* [*tiab*]). In addition to overall prostate cancer, higher-grade prostate cancer [defined as Gleason sum ≥7; if not available, Gleason sum ≥4 + 3 or Gleason sum ≥8 was used] and advanced prostate cancer (defined as TNM stage III/AJCC Stage 3 or above) were considered as our main outcomes. Additionally, we checked the references of selected articles and reviews for additional studies.

Studies were included according to the following inclusion criteria: (1) quantitative data on total cholesterol level at least for three categories with the estimated relative risks (RR) [risk ratio, odds ratio (OR), hazard ratio (HR)] and corresponding 95% confidence intervals (CI) were provided; (2) category-specific number of cases and noncases were available; (3) when duplicated reports were published from the same population, the most recent or complete publications were selected. We excluded studies where the only source of total cholesterol level was participants' self-report, or which defined men taking antihypertensive drugs as the control group.

Information extracted included first author, publication year, country of origin, study design (case-control or cohort), name of the cohort or study (if not a cohort), age range at baseline of the participants, sample size, number of total cases, category-specific dose of total cholesterol level (range), the most fully adjusted RRs and their 95% CIs, and adjustments.

In addition, "Risk of Bias in Non-randomized Studies of Interventions" (ROBINS-I) tool was applied to assess the method quality of observational studies.[11] Risk of bias was assessed according to the following seven domains: confounding, selection, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes and selection of the reported result. Overall risk of bias was integrated from seven domains, and risk range from low to moderate was considered acceptable.

2.5 | Statistical analyses

2.5.1 | HPFS analysis

The distribution difference of general characteristics of controls across quartiles of circulating total cholesterol level was tested using general linear model (GLM) for continuous variables and χ^2 test for categorical variables. Logistic regression (unconditional) was performed to estimate ORs and 95% CIs for the association between total cholesterol level and prostate cancer incidence, including total, lower-grade, higher-grade, clinically localized and metastatic prostate cancer. We adjusted for the matching factors age, PSA test before blood draw and year, season and time of day of blood collection and for possible confounders that are associated with prostate cancer incidence in the HPFS cohort:[3,10] family history of prostate cancer, height, vigorous physical activity, cigarette smoking in the past 10 years, intake of energy, intake of energy-adjusted lycopene, calcium and α-linolenic acid and ever use of cholesterol lowering drugs before diagnosis. Conditional logistic regression was conducted using the matched case-control pairs. The results of the conditional and unconditional logistic regression analyses were similar; thus, the results only from the unconditional models were presented, which allowed us to maximize power by including all controls in sub-group analyses.

Circulating total cholesterol levels were normally distributed in each of the four analytic batches, and the standard deviations (1996:39.7 mg/dl; 1998:41.9 mg/dl; 2000:38.2 mg/dl; 2004:34.7 mg/dl) measured in controls were similar. However, the mean concentrations of total cholesterol in controls in the 2000 batch differed from the other batches (1996:217.7 mg/dl; 1998:213.9 mg/dl; 2000:166.3 mg/dl; 2004:203.8 mg/dl). As we previously reported, the difference was likely related to differences in assay methods rather than systematic dissimilarity between the features of the controls in batch 3 vs those in other three batches.[3] In order to generate correct quartile ranking, batch-specific cholesterol ranks were used. To test for trend, an ordinal variable which ranged from 1 to 4, corresponding to the batch-specific quartile into which an individual fell was entered into the model.

Subgroup analyses were performed to assess whether the associations differed by age at prostate cancer diagnosis (<65 or ≥65 years

old), family history of prostate cancer through 1994 (yes or no), ever use of cholesterol lowering drugs at any time before diagnosis (ever or never), or time from blood draw to diagnosis (<5 or ≥5 years). For controls, the diagnosis date of their matched case was used. Analyses were conducted using SAS 9.3 software (SAS Institute, Cary, NC).

2.5.2 | Meta-analysis

The pooled RRs for the highest vs lowest categories of total cholesterol level were assessed in a highest vs lowest meta-analysis. In the dose-response meta-analysis, we initially assumed a linear relationship between total cholesterol concentration and risk of prostate cancer using the approach reported by Greenland and Longnecker[12] to calculate study-specific RRs (linear slope) and 95% CIs from the natural logarithms of extracted RRs and 95% CIs across categories of total cholesterol concentration. Then, the summary RRs and 95% CIs were calculated by pooling the study-specific RRs and variances using a random/fixed effects model depending on the results of heterogeneity tests. Next, we explored a potential non-linear relationship, using two regression models—restricted cubic spline and splines with one knot-point. The restricted cubic spline model with three knots was used for rough estimation, which produced a smooth dose-response curve. We evaluated non-linearity by testing whether the regression coefficients of the spline transformations were different from zero.[13] Then splines with one knot-point model was used for quantitative description, where, as previously described,[14] splines were modeled by changing the location of knot-point across the observed range of total cholesterol levels to test for a potential non-linear relationship. Circulating total cholesterol concentration reported in mmol/L was converted into mg/dl by multiplying 38.67.[15] For each study, the midpoint of total cholesterol level in every category was given to the corresponding RR. We set the lower boundary of total cholesterol level to 80 mg/dl if the lowest category was open-ended. Similarly, we assigned the same length of the adjacent interval to that of the highest category with the open-ended interval. When the lowest category of cholesterol was not set as the reference group in the published study,[7,16] new point estimates and 95% CIs were computed according to the method described previously.[14] Results of the dose-response meta-analyses were given as RRs per 20 mg/dl increase in plasma total cholesterol concentration.

The heterogeneity among studies was assessed by the Q test and quantified by I^2.[17] Small study effect, such as publication bias, was tested using Egger's test.[18] Meta-analysis was performed using STATA version 16 (StataCorp, College Station, TX; RRID:SCR_012763). The value of $P < .05$ was considered as a statistically significant difference.

3 | RESULTS

Among the 1260 cases (Table 1), 17.5% had higher-grade disease (Gleason sum ≥4 + 3), 85.5% had clinically localized disease (T1b, T2 and N0M0), and 10.2% had metastatic disease. Information was not available on grade for 93 (7%) cases and on stage for 75 (6%) cases. The average age at the time of prostate cancer diagnosis was 69.5 years. Mean time from blood collection to diagnosis was 5.2 years. The characteristics of 1328 controls across quartiles of plasma total cholesterol level are shown in Table 2. Men with higher total cholesterol concentration had a slightly lower calcium intake, and a higher prevalence of ever use of cholesterol-lowering drugs before the diagnosis date of their matched cases. Other lifestyle factors and dietary factors were comparable across quartiles of total cholesterol level.

We found an increased risk of higher-grade prostate cancer for men with the highest quartile of total cholesterol concentration (OR = 1.56, 95%CI: 1.01-2.40), compared with men in the lowest quartile of total cholesterol level (Table 3). This association was consistent in the direction within strata of age at diagnosis, family history of prostate cancer, ever use of cholesterol-lowering drugs before diagnosis (Tables S1-S3) and time from blood draw to diagnosis (data not shown). Moreover, the current study found a suggestive decreased risk of higher-grade prostate cancer in men who had a history of elevated cholesterol concentration before blood draw but a low cholesterol concentration at blood draw (OR = 0.52, 95%CI: 0.29-0.94), compared with individuals who had a history of elevated

TABLE 1 Characteristics of prostate cancer cases diagnosed from 1993 through 2004 in HPFS (n = 1260).

Characteristic	Prostate cancer cases
Age at blood draw (years), mean ± SD	64.4 ± 7.8
Age at diagnosis (years), mean ± SD	69.5 ± 7.5
Time to diagnosis from blood draw (years), mean ± SD	5.2 ± 2.9
Stage, no. (%)[a]	
T1, T2 (N0, M0)	1013 (85.5)
T3a (N0, M0)	95 (8.0)
T3b (N0, M0)	41 (3.4)
T4 (N0, M0)	2 (0.2)
N1	15 (1.3)
M1	19 (1.6)
Grade Group, no. (%)[b]	
Gleason sum 2 to 6	703 (60.2)
Gleason sum 3 + 4 or no major score defined	260 (22.3)
Gleason sum 4 + 3	81 (6.9)
Gleason sum 8 to 10	123 (10.6)
Metastatic prostate cancer, No. (%)	129 (10.2)
Metastases at diagnosis	19 (14.7)
Metastases to bone or other organs on follow-up	38 (29.5)
Prostate cancer deaths without recorded date of metastases	72 (55.8)

[a]Stage was not available for 75 cases.
[b]Gleason sum was not available for 93 cases.

TABLE 2 Characteristics of HPFS by quartile of plasma cholesterol concentration among controls (n = 1328).

Characteristic	Quartile of plasma cholesterol				P
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Batch-adjusted cholesterol level, mg/dl (median)	160.3	186.0	207.4	240.6	
Age at blood draw, years	64.0	64.8	64.2	63.8	.36
Height, inches	70.3	70.1	70.0	70.1	.40
Vigorous physical activity, MET-h/week	15.4	12.7	16.7	15.7	.27
Energy intake, kcal/day	2007	2046	2033	2033	.87
Energy-adjusted calcium, mg/day	970	977	939	894	**.04**
Energy-adjusted lycopene, μg/day	7316	7231	7784	7558	.44
Energy-adjusted α-linolenic acid, g/day	1.1	1.1	1.1	1.1	.55
Cigarette smoking in the past 10 years from blood draw, %					.82
Yes	15.7	16.6	16.6	18.4	
No	84.3	83.4	83.4	81.6	
Family history of prostate cancer through 1992, %					.61
Yes	11.5	9.6	10.2	12.7	
No	88.5	90.4	89.8	87.3	
Prostate specific antigen (PSA) test before blood draw, %					.49
Yes	71.1	74.7	75.3	71.4	
No	28.9	25.3	24.7	28.6	
Ever use of cholesterol-lowering drugs, %					**<.001**
Yes	18.1	26.2	33.1	43.7	
No	81.9	73.8	66.9	56.3	

Note: (1) Mean value were given, otherwise specified. (2) Ever use of cholesterol-lowering drugs was defined before diagnosis, using the diagnosis date of their matched case. Unless otherwise indicated, values were from the 1994 follow-up questionnaire. (3) Two-sided *P* value for association across quartiles (Chi-square test for categorical variables, *F*-test for continuous variables).

cholesterol concentration before blood draw and a current high cholesterol concentration. There was no appreciable association between quartiles of total cholesterol level and risk of overall prostate cancer, or with lower-grade, clinically localized or metastatic disease either before or after multivariable adjustment or within strata of age at diagnosis, family history or cholesterol-lowering drug use (Tables S1-S3).

The flowchart of study selection for the dose-response meta-analysis is presented in Figure S1. 2754 articles were identified among three databases, 2421 studies remained after excluding duplicates. After title and abstract screening, full-text reviewing was performed for 35 studies. Finally, 23 studies were eligible for meta-analysis, including two case-control studies,[19,20] two nested case-control studies (including our current study)[8] and 19 cohort studies[4-7,9,16,21-33] (Table S4). Except for one study that only reported on higher-grade cancer,[33] 22 studies reported data on the association between circulating total cholesterol level and overall prostate cancer. Of the 23 studies, 11 studies (including our current study) presented associations for higher-grade disease,[5-8,16,20,21,26,29,33] and five studies (including our current study) presented associations for advanced disease.[5,7,8,24] As the traffic light plot showed (Figure S2), the risk of bias measured by the ROBINS-I tool was low or moderate for each study.

Figure 1 provides the RRs from the highest vs lowest meta-analysis of the association between total cholesterol level with the risk of overall, higher-grade and advanced prostate cancer. The risk of higher-grade disease was 21% higher in men in the highest cholesterol category (Pooled RR = 1.21, 95%CI: 1.11-1.32, $P < .001$, $I^2 = 44.7\%$) than in those in the lowest cholesterol category (Figure 1B). Moreover, this association was not altered even excluding our HPFS cohort (Pooled RR = 1.20, 95%CI: 1.10-1.31, $P < .001$, $I^2 = 49.4\%$). The pooled RRs were close to the null for overall prostate cancer (Pooled RR of 1.04, 95% CI: 0.97-1.12, $P = .27$, $I^2 = 55.3\%$; Figure 1A) and advanced disease (Pooled RR = 1.05, 95%CI: 0.85-1.30, $P = .66$, $I^2 = 41.4\%$; Figure 1C). Pooled RRs for prostate cancer overall were null within subgroups (Table S5); subgroup analyses could not be performed for advanced disease due to the limited number of studies reporting on this outcome. Pooled RRs for higher-grade disease were statistically significant in the subgroups of studies that were prospective design (including either cohort study or nested case-control study), had <1000 cases, in European populations, with a follow-up time >10 years, and with adjustment for BMI (Table S6).

In the dose-response meta-analysis, we assessed the non-linear relationship between total cholesterol level and prostate cancer risk, integrating evidence of non-linear associations between total cholesterol concentration and higher-grade disease reported in some of the original publications, and the non-significant *P* value of the slope in

TABLE 3 Odds ratio (OR) and 95% confidence interval (CI) of prostate cancer in HPFS, by quartile of plasma cholesterol concentration.

	Quartile of plasma cholesterol[b]				
Prostate cancer[a]	Quartile 1 (reference)	Quartile 2	Quartile 3	Quartile 4	*P* trend[c]
Total					
No. cases/controls	324/332	319/332	297/332	320/332	
OR[d] (95%CI)	1	0.98 (0.79-1.22)	0.92 (0.74-1.14)	0.99 (0.80-1.23)	.81
OR[e] (95%CI)	1	1.01 (0.81-1.26)	0.95 (0.76-1.18)	1.02 (0.81-1.27)	.99
Lower-grade (Gleason sum ≤ 3 + 4)					
No. cases/controls	253/332	240/332	231/332	239/332	
OR[d] (95%CI)	1	0.95 (0.75-1.20)	0.91 (0.72-1.15)	0.94 (0.75-1.19)	.60
OR[e] (95%CI)	1	0.97 (0.77-1.23)	0.95 (0.74-1.20)	0.96 (0.76-1.23)	.74
Higher-grade (Gleason sum ≥ 4 + 3)					
No. cases/controls	43/332	54/332	43/332	64/332	
OR[d] (95%CI)	1	1.26 (0.82-1.93)	1.00 (0.64-1.57)	1.49 (0.98-2.25)	.11
OR[e] (95%CI)	1	1.29 (0.84-2.00)	1.04 (0.66-1.65)	**1.56 (1.01–2.40)**	.08
Clinically localized					
No. cases/controls	252/332	259/332	245/332	257/332	
OR[d] (95%CI)	1	1.03 (0.82-1.29)	0.97 (0.77–1.23)	1.02 (0.81-1.28)	.97
OR[e] (95%CI)	1	1.05 (0.83-1.32)	1.00 (0.79-1.27)	1.05 (0.83-1.33)	.78
Metastatic					
No. cases/controls	31/332	36/332	32/332	30/332	
OR[d] (95%CI)	1	1.16 (0.70-1.92)	1.03 (0.62-1.73)	0.97 (0.57-1.64)	.78
OR[e] (95%CI)	1	1.26 (0.75-2.12)	1.21 (0.71-2.07)	1.16 (0.67-2.01)	.65

Note: Bold indicates higher-grade prostate cancer.

[a]Adjusted ORs of advanced prostate cancer were Quartile 2: 0.88 (0.56-1.40), Quartile 3: 0.83 (0.51-1.33), and Quartile 4: 1.08 (0.69-1.68); and adjusted ORs of high grade prostate cancer (Gleason sum ≥7) were Quartile 2: 1.23 (0.91-1.68), Quartile 3: 1.08 (0.79-1.48) and Quartile 4: 1.31 (0.96-1.79). These data were used in the meta-analysis.

[b]The median batch-adjusted cholesterol level is 160.3 mg/dl for Quartile 1, 186.0 mg/dl for Quartile 2, 207.4 mg/dl for Quartile 3 and 240.6 mg/dl for Quartile 4.

[c]*P* value for trend was calculated based on the median of each plasma cholesterol concentration quantile as a continuous variable.

[d]Estimated from an unconditional logistic regression model.

[e]Estimated from an unconditional logistic regression model and adjusted for age at diagnosis, PSA test before blood draw, family history of prostate cancer, height, vigorous physical activity, cigarette smoking in the past 10 years, intake of energy, intake of energy-adjusted lycopene, calcium and α-linolenic acid, and ever use of cholesterol-lowering drugs before diagnosis.

the linear model (Figure 2). A significant non-linear relationship between total cholesterol concentration and higher-grade prostate cancer was observed in the restricted cubic spline model (P_{model} < .001, Figure 2B). Noting the sharp change of the curve based on the restricted cubic spline model and given the clinical cut-off point of abnormal total cholesterol level, we selected a total cholesterol level of 200 mg/dl as the break point in one knot-point model. In that analysis, we found that the RR of higher-grade prostate cancer remained stable (null) until 200 mg/dl (Pooled RR = 1.01, 95%CI: 1.00-1.02, *P* = .27), but increased by 4% for each 20 mg/dl increase of total cholesterol level >200 mg/dl (Pooled RR = 1.04, 95%CI: 1.01-1.08, *P* = .008). In the restricted cubic spline model, total cholesterol level was not associated with the risk of overall prostate cancer (P_{model} = .31, Figure 2A) or advanced prostate cancer (P_{model} = .43, Figure 2C). Funnel plots (Figure S3) and the Egger's test (*P* > .05) indicated that there was no evidence of publication bias in our meta-analysis.

4 | DISCUSSION

In this nested case-control study, which is an extension of our prior publication,[3] a significant positive relationship between circulating total cholesterol concentrations and incidence of higher-grade prostate cancer was observed. In the meta-analysis, which included the current results, a high total cholesterol level was associated with a moderately elevated risk of higher-grade prostate cancer, and a complementary dose-response meta-analysis indicated the association was non-linear and that the risk increased mostly at levels of cholesterol above approximately 200 mg/dl. However, circulating total cholesterol concentration was not associated with total prostate cancer risk either in our HFPS cohort or in the meta-analysis. Together, these findings support an association between higher cholesterol as a biomarker for detection of clinically significant prostate cancer.

Our current finding for higher-grade prostate cancer, defined as Gleason sum ≥4 + 3 in the HPFS cohort is consistent with our prior

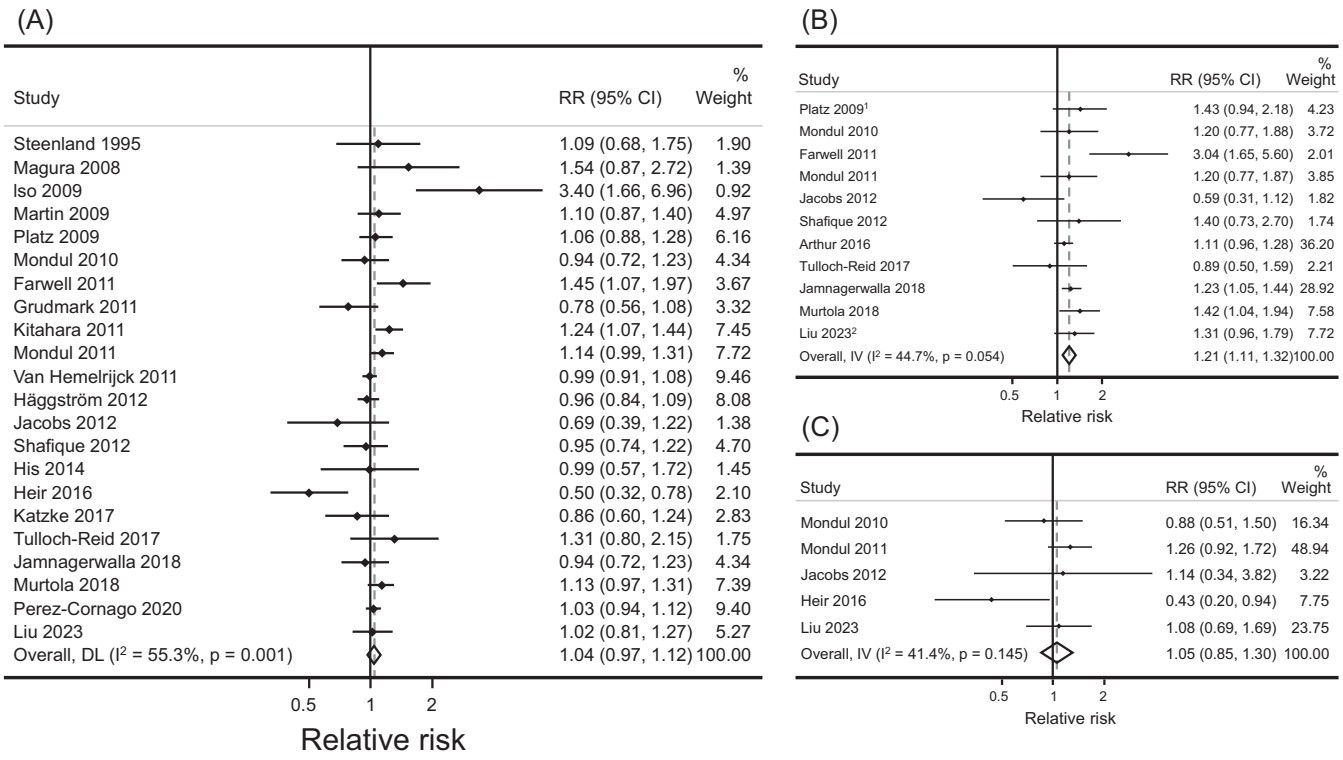

FIGURE 1 Highest vs lowest meta-analysis on the association of total cholesterol level and risk of prostate cancer. (A) Overall prostate cancer; (B) Higher-grade prostate cancer; (C) Advanced prostate cancer. [1]For PCPT study (Platz 2009), RR of Gleason sum ≥7 was used rather than that of Gleason sum ≥8 (RR = 1.43, 95%CI: 0.94-2.18). [2]For HPFS study (Liu 2023, current study), OR of Gleason sum ≥7 was used rather than that of Gleason sum ≥7 (4 + 3).

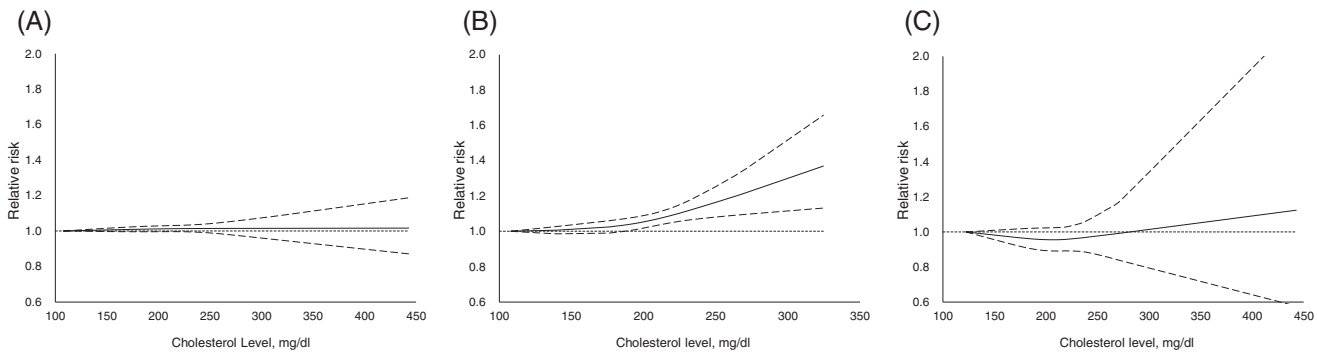

FIGURE 2 Dose-response meta-analysis on the association of total cholesterol level and risk of prostate cancer using 3-knot restricted cubic spline model. (A) Overall prostate cancer; (B) Higher-grade prostate cancer and (C) Advanced prostate cancer.

finding for higher-grade prostate cancer defined as Gleason ≥7.[3] Our finding is also in line with that from Shafique et al,[6] who found higher plasma total cholesterol concentrations (≥240 mg/dl) positively associated with an increased risk of prostate cancer with Gleason sum 8 to 10 (HR = 1.75, 95% CI: 1.03-2.97) in a UK prospective cohort study. Moreover, Platz et al[16] reported a positive association between total cholesterol concentration (≥200 mg/dl) and risk of Gleason 8 to 10 prostate cancer in the placebo arm of the Prostate Cancer Prevention Trial. Further, our dose-response meta-analysis indicated that the risk of higher-grade prostate cancer (Gleason sum ≥7; if not available, Gleason sum ≥4 + 3 or Gleason sum ≥8 was used) appeared above a circulating cholesterol level of 200 mg/dl, which is the clinical cut-off point for borderline high total cholesterol concentration in the context of cardiovascular disease risk.

Our findings of HPFS cohort and the meta-analysis both support no association between total cholesterol level and overall prostate cancer risk. However, some prospective studies found an increased risk of total prostate cancer for men with higher cholesterol concentrations;[4,5] while other studies did not find any significant association,[7,9,31] and one inverse association.[24] The disparity of

results between studies may be contributed either by underestimation of the association due to the young age of participants at baseline or the short length of follow-up, or reverse causation due to the short period between total cholesterol test and case identification. Mendelian randomization method which could infer a putative causal link between exposure and outcome was applied in the PRACTICAL consortium, and the finding indicated that cholesterol-related metabolites such as total cholesterol and ratios or cholesterol esters and ratios were not associated with the risk of total prostate cancer.[34] Yet this study did not investigate higher-grade cancer or account for the possibility of a non-linear association.

We did not observe a significant association between total cholesterol concentration and metastatic prostate cancer. A positive association might have been expected given the association for higher-grade prostate cancer at diagnosis. While the number of metastatic or lethal events was small leading to wide confidence intervals (Table 3 and Figure 2C), nevertheless, the direction of the ORs were compatible with the higher-grade results, albeit attenuated in the size. Of note, progression of higher-grade prostate cancer to distant metastasis and death occurs over may years, even a decade or more after the initial development of the cancer, and survival with metastatic disease is dependent upon sensitivity of the cancer to many treatment interventions over time while a large number of men succumb to competing mortality in this age group. Thus, a sufficiently long follow-up in large numbers of men is needed to be able to test the significant association between total cholesterol level and metastatic or lethal disease.

As an important component of the cellular membrane, cholesterol has critical regulating effects on membrane fluidity, signal transduction and cellular trafficking.[35] Cholesterol might influence the progression of prostate cancer through altered membrane organization, cell proliferation and differentiation, as well as steroidogenesis.[2] Of note, use of statins in cholesterol-lowering therapies may reduce the risk of advanced prostate cancer.[36] Our finding of an inverse association comparing men who previously reported elevated cholesterol concentration but had a low cholesterol level at blood draw with men who previously reported elevated cholesterol concentration and had a high cholesterol level at blood draw also hints that intervening on cholesterol dysregulation by reducing circulating cholesterol level may reduce higher-grade prostate cancer risk.

The strengths of our study included not only the prospective study design, but also its large sample size and numerous covariates to adjust for confounding. The HPFS blood cohort was followed for at least 10 years, which allowed for the ascertainment of cases detected early by PSA-based screening but that later progressed to distant metastases or caused death (ie, lethal prostate cancer). We required all controls to have had a PSA test after the collection of the blood sample in which we measured total cholesterol level; we made this restriction to reduce detection bias that could have occurred if the likelihood of a PSA test was dependent on cholesterol level.[3] Blood samples for the test of plasma total cholesterol concentration were taken months to years ahead of the diagnosis date of prostate cancer, which ameliorates the concern for reverse causation. Furthermore, findings in the current HPFS cohort were not altered after excluding individuals in the first 5 years of follow-up. Reverse causation cannot be entirely excluded but may be less likely in this highly screened population because most of the cases were diagnosed at a relatively early stage.

However, some limitations should be discussed. First, we measured total cholesterol concentration using three methods. Although the three assay approaches provided obviously different means, the distributions were comparable. Thus, though the ranking was intact, our estimation of absolute cholesterol level was limited. Additionally, total cholesterol concentrations were detected at a single time point, herein, did not present the variation of total cholesterol levels over time. Second, because of the high prevalence of PSA screening in this cohort, our total number of cases was high but the number of metastatic or lethal cases was lower, and we may have been underpowered to detect associations with metastatic prostate cancer. Third, we could not estimate the association for cholesterol carried by different lipoproteins such as low-density lipoproteins (LDL) and high-density lipoproteins (HDL) among the cases and controls from 1994 through 2000, yet the full profile of lipoproteins may provide a detailed picture of the association between lipids and prostate cancer risk.

Our meta-analysis may also have some limitations. For example, the divergent classification of prostate cancer grade over time and by different investigators might contribute to the heterogeneity (Table S4). In the meta-analysis, we defined higher-grade prostate cancer as Gleason sum ≥7 because this was the most common definition for high-grade disease among the published studies. Five studies,[5,7,16,20,26] plus our study, reported associations for cholesterol and Gleason sum ≥7; however, Jacobs et al[8] and Farwell et al[21] only reported associations for Gleason sum ≥4 + 3, while Shafique et al,[6] Murtola et al,[29] and Arthur et al[33] only mentioned associations for Gleason sum ≥8. The result was unchanged when the analysis was restricted using studies that defined cases as Gleason sum ≥7. The heterogeneity may also be due partly to differences in the sample size, country, duration of follow-up, inconsistent definition of low total cholesterol levels, variation in total cholesterol concentration measure, discrepancy of adjustment, the prevalence of PSA screening, or the prevalence of the prescription of statin drugs. Due to small numbers and limited information in the identified publications, especially the use of statins, we could not further explore these sources of the heterogeneity.

In conclusion, our findings integrating results from the HPFS nested case-control analysis and dose-response meta-analysis suggest an increased risk of higher-grade prostate cancer, at higher total cholesterol levels, particularly when they exceeded 200 mg/dl. By robustly confirming an association between high total cholesterol concentration and increased risk of aggressive prostate cancer, our results support further research elucidating the differences in the association of cholesterol with the risk of higher-grade vs lower-grade prostate cancer, and the underlying mechanisms of cholesterol that could serve as a potential target for prevention of clinically important prostate cancer.

AUTHOR CONTRIBUTIONS

The work reported in the paper has been performed by the authors, unless clearly specified in the text. Conceptualisation: Edward

L. Giovannucci, Elizabeth A. Platz; formal analysis: Hui Liu, Irene M. Shui, NaNa Keum, Xudan Shen; validation: Irene M. Shui, Xudan Shen; writing-original draft: Hui Liu; writing-review & editing: all authors.

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CONFLICT OF INTEREST STATEMENT

All authors declared that we have no conflicts of interest. Dr. Kana Wu is currently an employee and stockholder of Vertex Pharmaceuticals. This work was not funded by this commercial entity.

DATA AVAILABILITY STATEMENT

Research data are available from the corresponding author upon request.

ETHICS STATEMENT

Our study was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. Completion of the questionnaire was considered to imply informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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