Gallstones are crystalline deposits in the gallbladder, primarily formed by abnormal bile constituents (eg, cholesterol, phospholipids, and bile salts). Gallstone disease is one of the most common and costly gastrointestinal disorders requiring hospitalization in the United States, affecting 5% to 25% adults in the Western world. Gallstone disease has been linked to diverse cardiovascular risk factors, such as obesity, diabetes, and metabolic syndrome. In addition, patients with gallstones are characterized by distorted secretion of bile acids, which play a key role in regulating gut microbiota composition and activity. Gut microbiota may influence a range of host functions related to cardiovascular risk, and metabolites produced by gut microbiota such as trimethylamine N-oxide and L-carnitine have been directly linked with cardiovascular risk.

Several previous studies have related a history of gallstone disease to cardiovascular risk. However, some studies had short-term follow-up, did not confirm gallstone cases, or did not account for important covariates such as lifestyle and dietary habits. Notably, most of the previous studies were not conducted in the US population; and data from the US cohorts are lacking.

In this study, we analyzed the relationship between a history of gallstone disease and risk of incident coronary heart disease (CHD) in 3 large prospective US cohorts, followed up for ≤30 years and accounting for a wide spectrum of traditional risk factors. We performed several sensitive analyses to minimize the influence from potential reverse causation and confounding. We also conducted a systematic review of previously published studies and meta-analyzed our results with the published data from other prospective cohorts.

Objective—Gallstone disease has been related to cardiovascular risk factors; however, whether presence of gallstones predicts coronary heart disease (CHD) is not well established.

Approach and Results—We followed up 269,142 participants who were free of cancer and cardiovascular disease at baseline from 3 US cohorts: the Nurses’ Health Study (112,520 women; 1980–2010), Nurses’ Health Study II (112,919 women; 1989–2011), and the Health Professionals Follow-up Study (43,703 men; 1986–2010) and documented 21,265 incident CHD cases. After adjustment for potential confounders, the hazard ratio for the participants with a history of gallstone disease compared with those without was 1.15 (95% confidence interval, 1.10–1.21) in Nurses’ Health Study, 1.33 (95% confidence interval, 1.17–1.51) in Nurses’ Health Study II, and 1.11 (95% confidence interval, 1.04–1.20) in Health Professionals Follow-up Study. The associations seemed to be stronger in individuals who were not obese, not diabetic, or were normotensive, compared with their counterparts. We identified 4 published prospective studies by searching PUBMED and EMBASE up to October 2015, coupled with our 3 cohorts, involving 842,553 participants and 51,123 incident CHD cases. The results from meta-analysis revealed that a history of gallstone disease was associated with a 23% (15%–33%) increased CHD risk.

Conclusion—Our findings support that a history of gallstone disease is associated with increased CHD risk, independently of traditional risk factors.

Key Words: adult □ bile □ cohort study □ coronary disease □ gallstones
Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

At cohort-specific baselines, 14,023 participants out of 225,439 (6.2%) women (Nurses’ Health Study [NHS] and NHS II) and 1449 out of 43,703 (3.3%) men (HPFS [Health Professionals Follow-Up Study]) reported a history of gallstone disease. Table 1 shows the characteristics of participants by cohort and gallstone status at cohort-specific baselines. Consistently across the 3 cohorts, participants who reported a history of gallstone disease were more likely to be older, current smokers, regular aspirin users, less physically active, to consume less alcohol, to have a higher body mass index, and to have a history of hypertension, diabetes, or hypercholesterolemia (Table 1).

Across ≤30 years of follow-up, we confirmed 21,265 incident CHD cases. In general, the crude incidence rates of total CHD were nearly double among participants who had reported gallstone disease compared with those who had never reported gallstone disease (Table 2). There was a significant age-adjusted association between a history of gallstone disease and CHD risk in each cohort. After multivariable adjustment, the associations between history of gallstones and CHD were largely attenuated but remained significant in each cohort: NHS (hazard ratio [HR] [95% confidence interval (CI)]: 1.15 [1.10–1.21]), NHS II (1.33 [1.17–1.51]), and HPFS (1.11 [1.04–1.20]). An interim meta-analysis of these 3 cohorts revealed that the results in women (NHS and NHS II pooled HR [95% CI] 1.22 [1.06–1.41]) were not different from those in men (HPFS alone; P for heterogeneity=0.23).

When we limited the exposure to a history of cholecystectomy only, its association with total CHD risk was similar to when our definition of gallstone disease included both history of gallstones and cholecystectomy (data not shown). In secondary analyses of the total CHD subgroup definitions (fatal or nonfatal myocardial infarction and revascularization), multivariable-adjusted analyses of the individual outcomes revealed significant associations between the history of

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
</tr>
<tr>
<td>CI</td>
</tr>
<tr>
<td>HPFS</td>
</tr>
<tr>
<td>HR</td>
</tr>
<tr>
<td>NHS</td>
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<tr>
<td>NHS II</td>
</tr>
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</table>

Table 1. Age-Adjusted Baseline Characteristics of Participants from the Nurses’ Health Study (NHS; 112,520 Women; 1980–2010), NHS II (112,919 Women; 1989–2011) and the Health Professionals Follow-Up Study (HPFS; 43,703 Men; 1986–2010)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>103,724</td>
<td>87,96</td>
<td>42,254</td>
</tr>
<tr>
<td>Age, y*</td>
<td>46.4 (7.2)</td>
<td>48.6 (6.9)</td>
<td>25.0 (6.9)</td>
</tr>
<tr>
<td>White, %</td>
<td>96.7</td>
<td>98.2</td>
<td>73.7</td>
</tr>
<tr>
<td>Married, %</td>
<td>74.0</td>
<td>78.2</td>
<td>77.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.2 (5.6)</td>
<td>25.0 (6.9)</td>
<td>23.9 (4.8)</td>
</tr>
<tr>
<td>Physical activity (METs/week)</td>
<td>14.2 (21.0)</td>
<td>12.4 (19.0)</td>
<td>25.0 (36.9)</td>
</tr>
<tr>
<td>Premenopausal, %</td>
<td>57.5</td>
<td>49.7</td>
<td>93.0</td>
</tr>
<tr>
<td>Current use of postmenopausal hormones, %</td>
<td>9.2</td>
<td>12.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>26.7</td>
<td>30.9</td>
<td>13.2</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>14.9</td>
<td>24.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.0</td>
<td>4.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>4.8</td>
<td>7.4</td>
<td>10.1</td>
</tr>
<tr>
<td>Family history of MI ≤60 y, %</td>
<td>18.3</td>
<td>21.3</td>
<td>14.5</td>
</tr>
<tr>
<td>Regular aspirin use, %</td>
<td>32.1</td>
<td>37.3</td>
<td>11.0</td>
</tr>
<tr>
<td>Daily energy intake, kcal/d</td>
<td>1566 (500)</td>
<td>1563 (516)</td>
<td>1789 (547)</td>
</tr>
<tr>
<td>Daily cholesterol intake, mg/d</td>
<td>336 (122)</td>
<td>336 (127)</td>
<td>242 (68)</td>
</tr>
<tr>
<td>Alternative Health Eating Index Score</td>
<td>47.8 (10.8)</td>
<td>46.8 (10.6)</td>
<td>58.7 (7.3)</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>4.9 (9.7)</td>
<td>4.0 (8.6)</td>
<td>2.6 (5.7)</td>
</tr>
</tbody>
</table>

Values are means (SD) or percentages and are standardized to the age distribution of the study population. BMI indicates body mass index; HPFS, Health Professionals Follow-up Study; METs, metabolic equivalent task; MI, myocardial infarction; and NHS, Nurses’ Health Study.

*Value is not age adjusted.
gallstone disease and myocardial infarction in older women from NHS, but not in younger women from NHS II or men from HPFS (HR [95% CI] in NHS, 1.14 [1.05–1.22]; in NHS II, 1.15 [0.95–1.40]; and in HPFS, 1.08 [0.98–1.19]; Table 2). Further exploratory analysis showed an attenuated and insignificant association (maybe because of a smaller sample size of the cases) between gallstone disease and fatal myocardial infarction only (pooled HR [95% CI], 1.03 [0.93–1.15]). There were significant associations between a history of gallstone disease and revascularization in both women and men (HR [95% CI] in NHS, 1.20 [1.13–1.26]; in NHS II, 1.35 [1.18–1.56]; and in HPFS, 1.14 [1.05–1.24]; Table 2).

The direct associations between gallstone disease and risk of CHD remained (HR [95% CI] for lagged analyses and baseline gallstone disease only, respectively, in NHS, 1.17 [1.12–1.23] and 1.17 [1.10–1.24]; in NHS II, 1.28 [1.11–1.47] and 1.24 [1.05–1.47]; and in HPFS, 1.15 [1.06–1.24] and 1.16 [1.07–1.25]).

The results did not change materially when we evaluated the associations separately among participants not taking lipid-lowering medication (interim 3-cohort meta-analyzed HR [95% CI], 1.18 [1.10–1.27]), in those who were not obese (1.19 [1.13–1.24]), in those who were nonsmokers (1.15 [1.10–1.20]), in those without diabetes mellitus (1.16 [1.11–1.21]), or in those with normal blood cholesterol levels (1.15 [1.08–1.22]). However, obesity status and disease status of diabetes mellitus and hypertension significantly modified the association between gallstone disease and CHD risk (all P for interaction <0.05, in the interim meta-analyses of our 3 cohorts; Figure 1). Participants with a history of gallstone disease who were not obese, or did not have diabetes or hypertension, seemed to have a greater increased risk of CHD compared with those participants who were obese, did have diabetes mellitus, or hypertension. Age, smoking, dietary quality, physical activity, or hyperlipidemia did not modify the association between history of gallstone disease and CHD. Although the pooled modification effect from physical activity was statistically significant, the direction differed across 3 cohorts. Therefore, we did not conclude physical activity modified the association between gallstone disease and CHD risk.
Results of Systematic Review and Meta-Analysis

We also conducted a systematic review and meta-analysis to incorporate the results of our 3 cohorts with previously published reports from prospective cohort studies. Our search of English-language articles resulted in 1243 citations from PUBMED and 73 from EMBASE, with 16 duplicates. We screened the titles and abstracts using general inclusion criteria as described in the Materials and Methods section in the online-only Data Supplement. We identified 52 abstracts for further full-text review and eventually included 4 prospective cohorts for final meta-analysis. Together with the current study, a total of 7 cohort studies were included in our meta-analysis. Characteristics of the 7 prospective cohorts included in the meta-analysis are shown in Table I in the online-only Data Supplement. We detected significant heterogeneity among these study results ($F=72\%$, $P$ for homogeneity <0.01), which could be from a wide variation in study characteristics, such as study population, follow-up period, and measurements of gallstone disease and CHD. However, we were unable to detect the sources of heterogeneity because of a relatively small number of studies. Nevertheless, most studies showed directionally consistent associations between gallstone disease and CHD risk. On the basis of data from all cohort studies combined, including 842553 participants and 51 123 incident cases of CHD, the pooled risk ratio from the random-effects model was 1.23 (95% CI, 1.15–1.33) for those with gallstone disease compared with those without gallstone disease (Figure 2). In meta-analysis, participants from our 3 internal cohorts with a history of gallstone disease had a 17% increased risk of CHD (HR, 1.17 [95% CI, 1.09–1.26]), whereas participants from external cohorts with a history of gallstone disease had a 30% increased risk of CHD (HR, 1.30 [95% CI, 1.15–1.47]). We also conducted sensitivity analyses excluding the only study that presented an odds ratio estimate, and the pooled risk ratio was 1.23 (95% CI, 1.15–1.33), indicating that the overall results were not influenced by this study. Neither visual inspection of the funnel plot (Figure I in the online-only Data Supplement) nor the Egger test ($P=0.52$) suggested publication bias.

Discussion

In our 3 prospective studies of 269 142 women and men, we found that a history of gallstone disease was associated with a 17% increased risk of CHD, independent of traditional risk factors. The associations were consistent in both men and women and modified by obesity and hypertension and diabetes status. Results from the meta-analysis incorporating previously published data from 4 additional prospective cohort studies further confirmed our findings.

One of the first reports on the relationship between gallstone disease and incident cardiovascular disease was by Bortnick et al, who found that male gallstone patients were at an increased risk of incident CHD, whereas there was no such association in women. Significant associations between gallstone disease and CHD risk were also observed in cross-sectional studies and other prospective cohort studies in Asian and German populations. The most recent and largest prospective study to date emerged from the China Kadoorie Biobank study, which involved 199 292 men and 288 081 women, followed up for a median 7.2 years. Authors reported 23% higher risk of ischemic heart disease for participants with gallstone disease at baseline, as compared with those without...
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The disease, which is an estimated risk consistent with our present results in the US cohorts. As to other cardiovascular outcomes, both ultrasound-diagnosed gallstones and cholecystectomy were related to a 30% higher risk in overall mortality and 49% higher risk in cardiovascular mortality in a large US population, and participants who had gallstone disease had a 28% higher risk in developing ischemic stroke and 33% higher risk in hemorrhagic stroke in a nationwide study in Taiwan.

Gallstone disease and CHD share many common risk factors, and it has been challenging to disentangle these shared risk factors from potentially causally linked pathogenesis. Both diseases share multiple common traditional risk factors, including obesity, diabetes mellitus, metabolic syndrome, hypercholesterolemia, hypertension, and an unhealthy diet. In our analyses, the increased risk of incident CHD among gallstone patients was attenuated to a large degree but remained significant after we adjusted for these risk factors, and when we repeated main analyses among populations free of obesity, diabetes mellitus, and hypertension and among those with healthier lifestyle and dietary habits after stratifying by these factors. Our results were similar when we limited the disease to cholecystectomy, and it might suggest that cholecystectomy might not ameliorate the deleterious influence of gallstone diseases on CHD risk. Our observed associations were modified by obesity, diabetes mellitus, and hypertension status, with perhaps counterintuitively stronger associations observed among the presumably healthier subsets of the population, ie, those without obesity, diabetes mellitus, and hypertension. In addition, because cholesterol is the main component of most gallstones and atherosclerotic plaques, and the use of statins seems to prevent gallstone formation, we included hypercholesterolemia as a covariate in our statistical models and also conducted sensitivity analyses among the participants who had normal blood cholesterol levels, as well as among those not taking lipid-lowering medications, and yet the association of gallstone disease and CHD risk remained significant. Taken together, these observations may point to pathways independent of these important risk factors that link gallstone disease to CHD. Further research is warranted to explore potential mechanisms.

The potential mechanisms for the association of gallstone diseases with CHD may, at least, include the primary metabolic pathway and the bacterial pathway. Take an example in the metabolic pathway, among patients with gallstones, especially those with cholesterol gallstones, their bile acid and lecithin secretion rates tend to be depressed and cholesterol secretion rates elevated, which could indicate enhanced cholesterol synthesis and therefore increase cardiovascular disease risk. In the bacterial pathway, gut microbiota dysbiosis may directly link gallstone disease to CHD risk. The presence of gallstones is related to microbiota dysbiosis in gut and biliary tract (maybe via biotransformation of secondary bile acids), for example, an overgrowth of the bacterial phylum Proteobacteria. At the same time, gut microbiota dysbiosis is found to be linked to a wide range of metabolic disturbances, including an increased risk of obesity and cardiovascular disease. Of note, the bacterial link may be a targeted pathway through which diets influence both diseases. For example, circulating trimethylamine N-oxide, which is a gut flora–generated metabolite of red meat intakes, inhibits bile acid transporters in mouse liver and at the same time promotes atherosclerosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>HR (95% CI)</th>
<th>%Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrnochak, 1984 (16) males from FHS</td>
<td>1.60 (1.13, 2.28)</td>
<td>3.45</td>
</tr>
<tr>
<td>Botrnochak, 1984 (16) females from FHS</td>
<td>0.72 (0.42, 1.22)</td>
<td>1.63</td>
</tr>
<tr>
<td>Wirth, 2013 (17) EPIC-Potsdam</td>
<td>1.19 (0.83, 1.70)</td>
<td>3.33</td>
</tr>
<tr>
<td>Wirth, 2013 (17) EPIC-Heidelberg</td>
<td>1.49 (1.01, 2.20)</td>
<td>2.89</td>
</tr>
<tr>
<td>Olaiya, 2013 (18) Taiwan NHIRD</td>
<td>1.42 (1.28, 1.58)</td>
<td>15.21</td>
</tr>
<tr>
<td>Lv, 2015 (37) China Kadoorie Biobank Study</td>
<td>1.23 (1.17, 1.28)</td>
<td>21.00</td>
</tr>
<tr>
<td>Zheng et al, 2015 (Present) NHS</td>
<td>1.15 (1.10, 1.21)</td>
<td>20.78</td>
</tr>
<tr>
<td>Zheng et al, 2015 (Present) NHS II</td>
<td>1.33 (1.17, 1.51)</td>
<td>13.14</td>
</tr>
<tr>
<td>Zheng et al, 2015 (Present) HPFS</td>
<td>1.11 (1.04, 1.20)</td>
<td>18.58</td>
</tr>
<tr>
<td>Overall (I-squared = 71.8%, p = 0.000)</td>
<td>1.23 (1.15, 1.33)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 2. Forest plot of the multivariate-adjusted hazard ratio of coronary heart disease for history of gallstone disease in individual cohort studies and all studies combined in inverse weighted random-effects meta-analysis. Relative risks approximate hazard ratios in these studies. Diamonds indicate point estimates; bars indicate 95% confidence intervals (CIs); the size of the gray squares corresponds to the weight of the study in the meta-analysis. EPIC indicates European Prospective Investigation into Cancer and Nutrition; FHS, Framingham Heart Study; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; NHS, Nurses’ Health Study; and NHIRD, National Health Insurance Research Database.
Our results indicate that the gallstone patients who were not affected by obesity, diabetes mellitus, or hypertension had a greater increased CHD risk compared with those who were affected by these diseases. The underlying mechanisms remained to be further explored. Patients with obesity, diabetes mellitus, or hypertension had a higher absolute risk of CHD; therefore, the increased risk attributable to gallstone disease might be relatively less than those without these diseases. In addition, patients with obesity, diabetes mellitus, and hypertension might modify their lifestyle to be healthier after disease diagnosis, and such modifications might also attenuate the relation between gallstone and CHD.

Although this study may not be able to prove a causal relationship between gallstone disease and CHD, the long-term implications of gallstone disease on heart health are important in clinical practice.28 Patients with gallstone disease should be recommended a multidisciplinary program based on a careful assessment of coexisting cardiometabolic risk factors. Statins have been shown to dissolve cholesterol gallstones, suggesting their potential for a pharmacological therapy for gallstones beyond their well-known benefits on CHD prevention.25 However, randomized clinical trials are warrant to test the effect of statins on gallstone disease to more clearly address this issue.28

Strengths and Limitations

The major strengths of this study included the large sample size and well-characterized study populations. The sensitivity analyses, including the 4-year lag analysis, minimized the possibility of reverse causality. We also incorporated previously published prospective cohort studies in a meta-analysis to explore the repeatability and generalizability of our results. Nevertheless, our results need to be interpreted in the context of several potential limitations. First, the assessment of gallstone disease in our cohorts was self-reported. However, validation studies in our populations have confirmed that gallstones and history of cholecystectomy were reported with high accuracy (99%) according to validation of medical records.29,30 Second, we did not measure in our cohorts insulin resistance or nonalcoholic fatty liver disease, which could be important mediators of the reported associations. This limited our ability to explore underlying molecular mechanisms or pathways. However, our results were similar among participants reporting normal blood lipid levels.

Conclusions

In summary, we observed that gallstone disease is significantly and consistently associated with an increased risk of incident CHD independent of traditional risk factors in both women and men in the United States. The combined, metaanalyzed observations from our cohorts and previous prospective cohorts support our results. Our findings highlight the potential implication of gastrointestinal disorders on prevention of cardiovascular disease.

Acknowledgments

We thank all the participants for generously helping us in this research.

Sources of Funding

The cohorts were supported by grants of UM1 CA186107, R01 HL034594, UM1 CA176726, UM1 CA167552, and R01 HL35464 from the National Institutes of Health. The current study was supported by grants from the National Heart, Lung, and Blood Institute (HL071981, HL034594, HL126024), the National Institute of Diabetes and Digestive and Kidney Diseases (DK091718, DK100383, and DK078616), the Boston Obesity Nutrition Research Center (DK46200), and United States-Israel Binational Science Foundation Grant 2011036. The National Heart, Lung, and Blood Institute had no role in the design, conduct, analysis, or reporting of this study.

Disclosures

Dr Qi is a recipient of the American Heart Association Scientist Development Award (0730094 N) and Shanghai Thousand Talents Program for Distinguished Scholars. The other authors report no conflicts.

References

Gallstones and Risk of Coronary Heart Disease


**Highlights**

- Gallstone disease and cardiovascular disease share a few risk factors; however, prospective data in the United States regarding the association of gallstone disease and cardiovascular risk are limited.

- In our 3 prospective studies of 270,067 women and men, we found that a history of gallstone disease was associated with an 18% increased risk of CHD, independent of traditional risk factors.

- Results from the meta-analysis incorporating previously published data from 4 additional prospective cohort studies further confirmed our findings.

- Our findings highlight the potentially implication of gastrointestinal disorders on prevention of cardiovascular disease.
Gallstones and Risk of Coronary Heart Disease: Prospective Analysis of 270,000 Men and Women From 3 US Cohorts and Meta-Analysis
Yan Zheng, Min Xu, Yanping Li, Adela Hruby, Eric B. Rimm, Frank B. Hu, Janine Wirth, Christine M. Albert, Kathryn M. Rexrode, JoAnn E. Manson and Lu Qi

Arterioscler Thromb Vasc Biol. 2016;36:1997-2003; originally published online August 18, 2016;
doi: 10.1161/ATVBAHA.116.307507
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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MATERIALS AND METHODS

Study Populations

The Nurses’ Health Study (NHS) began in 1976 with the enrollment of 121,700 female nurses aged 30 to 55 years who completed an initial questionnaire on medications, lifestyle, and medical history. The Nurses’ Health Study II (NHS II) began in 1989, enrolling 116,430 female nurses aged 25 to 42 years. The Health Professionals Follow-up Study (HPFS) began in 1986 with the enrollment of 51,529 male health professionals (including dentists, optometrists, osteopaths, pharmacists, podiatrists, and veterinarians) aged 40 to 75 years. For the current study, the baseline year was the first year for which detailed information was available on gallstone disease, lifestyle, dietary habits, and physical activity: 1980 for NHS, 1989 for NHS II, and 1986 for HPFS. History of gallstone disease, CHD, and other medical conditions has been updated every two years until the end of follow-up: June 2010 for NHS, June 2011 for NHS II, and January 2010 for HPFS. Participants with a baseline self-reported history of myocardial infarction (MI), coronary revascularization, or cancer were excluded from the analysis.

The study protocol was approved by the institutional review boards of Brigham and Women’s Hospital and the Harvard T.H. Chan School of Public Health, with informed consent indicated by return of the baseline questionnaire.

Assessment of Gallstone Disease

The exposure of interest was a history of gallstone disease, including unremoved gallstones and cholecystectomy. In NHS, biennial questionnaires were used to assess the occurrence and date of cholecystectomy. Previously, we randomly selected 50 nurses who self-reported cholecystectomy, and 43 who agreed to requests for additional information reiterated their earlier report. Cholecystectomy was confirmed in all 36 nurses for whom medical records were available.¹

For NHS II and HPFS, at baseline and in each biennial follow-up questionnaire, participants reported whether they had cholecystectomy or had received a diagnosis of gallstones from a physician. The participants were also asked whether their gallstone disease was symptomatic and whether the diagnosis had been confirmed by radiography or ultrasonography. To verify self-reports of gallstone disease including symptomatic unremoved gallstones and cholecystectomy, we previously reviewed a random sample of 441 medical records of men who reported having gallstone disease or cholecystectomy; of these, 99% (all but 5) confirmed the diagnosis.²

Assessment of CHD

The primary outcome was total CHD, defined as a composite of nonfatal or fatal MI, or coronary revascularization procedure (coronary artery bypass grafting surgery or percutaneous transluminal coronary angioplasty).³ Secondary outcomes were nonfatal
or fatal MI, and coronary revascularization, examined separately. We defined fatal MI as documented fatal MI or fatal CHD determined by deaths identified from state death certificates or the National Death Index or reported by the participant’s next of kin or the postal system. Fatal and nonfatal MI events were confirmed through medical record review and required characteristic symptoms with either diagnostic electrocardiographic changes or positive myocardial enzymes; revascularization was self-reported but has been found to be virtually 100% specific in the HPFS.4

Assessment of Covariates

The covariates considered in the current study were traditional CHD risk factors: age (months, continuous); race (white/nonwhite); family history of MI (yes/no); marital status (married/not married); smoking status (never smoked, past smoker, current smoker); body mass index (BMI, calculated as weight in kilograms divided by height in meters squared; continuous); physical activity (metabolic equivalent task hours/d in quintiles); diabetes (yes/no); hypertension (yes/no); hypercholesterolemia (yes/no); regular use of aspirin (yes/no); daily intake of alcohol (0, 0.1–5.0, 5.0–9.9, 10.0–14.9, ≥15.0 g/d), daily intake of the energy-adjusted dietary cholesterol (g/d in quintiles), 2010 Alternate Healthy Eating Index (AHEI score in quintiles), and daily energy intake (kcal/d in quintiles). In women from NHS and NHS II, postmenopausal status (yes/no), postmenopausal hormone uses (yes/no), and uses of oral contraceptive pills (yes/no) were also considered as covariates. Detailed information on cigarette smoking, physical activity, and several lifestyle factors and health outcomes were updated every 2 years. Marital status and status with respect to a family history of MI were assessed periodically. Dietary information was collected from validated food-frequency questionnaires approximately every 4 years in each cohort.5,6 Diet quality was assessed using the 2010 AHEI (higher scores indicating a healthier diet),7 which predicts cardiovascular disease risk well in our cohorts.8

Statistical Analysis
We used Cox proportional hazards models to calculate the crude, age- and multivariable-adjusted hazard ratios (HRs) and 95% CIs for the risk of incident CHD in participants with a history of gallstone disease compared with those without. We used updated information on unremoved gallstones or cholecystectomy history, and updated information on covariates for each 2-year follow-up period in multivariable-adjusted models. Person-time was calculated from the date of return of the baseline questionnaire (1980 for NHS, 1989 for NHS II, and 1986 for HPFS) to the date of incident CHD, death, or end of follow-up (June 1, 2010 for NHS, June 1, 2011 for NHS II, and January 31, 2010 for HPFS), whichever came first. The proportional-hazards assumption was evaluated with a likelihood-ratio test comparing the model with and without an interaction term between time period and the status of gallstone disease; there was no evidence suggesting that the proportional-hazards assumption was violated (NHS: P=0.53; NHS II: P=0.62; and HPFS: P=0.49 in the multivariate model).

To minimize possible reverse causation, we also conducted several sensitivity analyses: 1) we conducted 4-year-lagged analyses by starting the follow-up period four years after the assessment of gallstone disease; and 2) we analyzed the association
between gallstone disease reported at baseline and incident CHD, without updating information for exposures or covariates across the follow-up period.

To minimize possible residual confounding, we conducted the following analyses: 1) we repeated the above analysis among (a) participants with normal blood lipid levels, (b) those not taking lipid-lowering medication, and (c) those who were not obese. The baseline years for analyses among participants not taking lipid-lowering medication were 1990 for NHS, 1998 for NHS II, and 1986 for HPFS, because information on lipid medications was collected biennially beginning with these questionnaires. 2) We included history of kidney stones as a covariate in the fully adjusted model, because previous publications have shown that kidney stones are related to both gallstone disease and CHD.3,9

In addition, we repeated our analyses after stratifying by age, BMI, physical activity, current smoking status, moderate alcohol intake, AHEI, and disease status of diabetes, hypertension, and hyperlipidemia. Effect modification by the above factors was assessed using the multiplicative interaction term between gallstone disease and the effect modifier, added to the multivariable model which included both main effect variables.

Finally, we repeated the above analyses in secondary analyses that either limited the exposure to history of cholecystectomy alone, or assessed the secondary outcomes when defined separately as nonfatal or fatal MI, or as coronary revascularization.

Analyses were carried out with SAS software, version 9.3 (SAS Institute), at a two-tailed alpha level of 0.05.

We conducted a meta-analysis to combine our new results from the 3 cohorts with previously published reports from other prospective cohort studies. We conducted a systematic review in accordance with PRISMA guidelines.10 We systematically searched PUBMED (up to October 26, 2015) and EMBASE (up to October 26, 2015) for published studies that examined gallstone disease in relation to risk of coronary heart disease, using the following search protocol and related extensions to filter information: (((“heart disease”[Title/Abstract]) OR cardiovascular diseases[Title/Abstract])) AND (((cholecystectomy[Title/Abstract]) OR gallstone[Title/Abstract]) OR cholelithiasis[Title/Abstract]). The keywords were combined using the Boolean operators “AND” and “OR”. No restrictions in the search strategy were inserted. Additionally, we hand-searched the reference lists of all identified publications. Articles were considered for inclusion in the systematic review if: the investigators reported data from an original, peer-reviewed study (i.e., not review articles or conference abstracts); the report was of a prospective study done in adults without CHD at baseline; and the investigators reported risk estimates of CHD by gallstone disease status. We assessed eligible articles first by screening titles or abstracts, followed by full-text review. One author (Y.Z.) assessed study eligibility and extracted the data, and another (M.X.) independently double-checked the data.

We extracted the following information using a predesigned collection form: study characteristics (study name, authors, publication year, journal, study location, follow-up...
length, number of participants, and number of incident cases of CHD), participant characteristics (mean age or age range, sex, and ethnic origin), CHD outcome assessment methods (self-report, medical record, or clinical examination), and analysis strategy (statistical models and covariates included in the models).

For one prospective study that used odds ratio to present results,\textsuperscript{11} we converted the reported adjusted odds ratio to approximate relative risk,\textsuperscript{12,13} and for the other studies, fully-adjusted relative risks presented were extracted and analyzed as the common measure of association across studies. We pooled relative risks using inverse-variance-weighted random-effects models that incorporated both a within-study and an additive between-studies component of variance,\textsuperscript{14} and produced forest plots. We calculated heterogeneity of study results using the $I^2$ statistic.\textsuperscript{15} Publication bias was assessed by use of the Egger’s test and visual inspection of funnel plots.\textsuperscript{16} Analyses were carried out with Stata 12.0 (StataCorp, College Station, TX) at a two-tailed alpha level of 0.05.
Reference:


Supplemental Figure I. Funnel Plots Assessing for Publication Bias in Publications on Gallstone Disease and Risk of Coronary Heart Disease.
**Supplemental Table I.** Cohort studies of gallstone disease and risk of coronary heart disease from previous studies and our 3 cohorts

<table>
<thead>
<tr>
<th>Citation (y)</th>
<th>Study population</th>
<th>Sample size, n</th>
<th>Baseline age (y)</th>
<th>Follow-up (y)</th>
<th>Participants with gallstone disease</th>
<th>CHD cases</th>
<th>Adjustment for potential confounders</th>
<th>Relative risk (95% Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortnichak *, 1984&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Framingham Heart Study original cohort</td>
<td>4,647 (males: 2171, females: 2476)</td>
<td>28–62</td>
<td>Up to 26</td>
<td>421 (diagnosed cholesterol gallstone disease)</td>
<td>699 (myocardial infarction, sudden death, coronary insufficiency, and angina pectoris)</td>
<td>Age, gender, overweight, left ventricular hypertrophy, smoking, systolic blood pressure, diabetes, serum cholesterol, and length of follow-up</td>
<td>Males: 1.60 (1.13–2.28) Females: 0.72 (0.42–1.22)</td>
</tr>
<tr>
<td>Olaiya, 2013&lt;sup&gt;2&lt;/sup&gt;</td>
<td>The Taiwan National Health Insurance Research Database</td>
<td>34,905</td>
<td>18–80</td>
<td>6</td>
<td>6,981 (diagnosed)</td>
<td>Not known (3,693 including stroke, CHD and heart failure cases)</td>
<td>Age, gender, peripheral vascular disease, chronic obstructive pulmonary disease, hypertension, diabetes, hyperlipidemia, alcoholism, chronic liver disease, and anemia</td>
<td>1.42 (1.28–1.58) (this estimate was for CHD only)</td>
</tr>
<tr>
<td>Wirth, 2013&lt;sup&gt;3&lt;/sup&gt;</td>
<td>EPIC–Potsdam</td>
<td>24,422</td>
<td>35–65</td>
<td>On average 8.2</td>
<td>3,178 (self–reported)</td>
<td>243 (myocardial infarction)</td>
<td>Age, gender, education, physical activity, smoking, alcohol intake, BMI, waist circumference, hypertension and hyperlipidemia</td>
<td>1.19 (0.83–1.70)</td>
</tr>
<tr>
<td></td>
<td>EPIC–Heidelberg</td>
<td>22,064</td>
<td></td>
<td></td>
<td>1,650 (self–reported)</td>
<td>264 (myocardial infarction)</td>
<td></td>
<td>1.49 (1.01–2.20)</td>
</tr>
<tr>
<td>Lv, 2015&lt;sup&gt;4&lt;/sup&gt;</td>
<td>China Kadoorie Biobank study</td>
<td>487,373</td>
<td>30–79</td>
<td>7.2 (median)</td>
<td>28,345 (self–reported)</td>
<td>24,959 (ischemic heart disease)</td>
<td>Age, gender, education, level of education; marital status; alcohol consumption; smoking; physical activity; intake of red meat, fresh fruits, and vegetables; prevalent hypertension and</td>
<td>1.23 (1.17–1.28)</td>
</tr>
<tr>
<td><strong>Zheng, (present study)</strong></td>
<td><strong>Nurses’ Health Study</strong></td>
<td>112,520</td>
<td>30–55</td>
<td>Up to 30</td>
<td><strong>8,796 (self–reported with high validity)(^5)</strong></td>
<td><strong>10,923 (myocardial infarction, and revascularization)</strong></td>
<td><strong>Age, BMI, myocardial infarction family history, smoking, alcohol intake, daily cholesterol intake, daily energy intake, physical activity, race, marital status, post–menopausal hormone replacement, Alternative Health Eating Index Score, hypercholesterolemia, hypertension, diabetes, and regular aspirin use</strong></td>
<td><strong>1.15 (1.10–1.21)</strong></td>
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<tr>
<td><strong>Zheng, (present study)</strong></td>
<td><strong>Nurses’ Health Study II</strong></td>
<td>112,919</td>
<td>25–42</td>
<td>Up to 22</td>
<td><strong>5,227 (self–reported with high validity)(^5)</strong></td>
<td><strong>1,397 (myocardial infarction, and revascularization)</strong></td>
<td><strong>Age, BMI, myocardial infarction family history, smoking, alcohol intake, daily cholesterol intake, daily energy intake, physical activity, race, marital status, post–menopausal hormone replacement, Alternative Health Eating Index Score, hypercholesterolemia, hypertension, diabetes, and regular aspirin use</strong></td>
<td><strong>1.33 (1.17–1.51)</strong></td>
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<tr>
<td><strong>Zheng, (present study)</strong></td>
<td><strong>Health Professionals Follow–up Study</strong></td>
<td>43,703</td>
<td>40–75</td>
<td>Up to 24</td>
<td><strong>1,449 (self–reported with high validity)(^5)</strong></td>
<td><strong>8,945 (myocardial infarction, and revascularization)</strong></td>
<td><strong>Age, BMI, myocardial infarction family history, smoking, alcohol intake, daily cholesterol intake, daily energy intake, physical activity, race, marital status, post–menopausal hormone replacement, Alternative Health Eating Index Score, hypercholesterolemia, hypertension, diabetes, and regular aspirin use</strong></td>
<td><strong>1.11 (1.04–1.20)</strong></td>
</tr>
</tbody>
</table>

*: this study excluded the participants with baseline prevalent gallstone disease, this study presented odds ratio as the final result, and we used a statistical method to convert odds ratio to relative risk approximately.\(^6\),\(^7\)
Supplemental References:


History of gallstone disease and risk of coronary heart disease

Hazard Ratios

<table>
<thead>
<tr>
<th></th>
<th>NHS</th>
<th>NHSII</th>
<th>HPFS</th>
<th>OVERALL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.15</td>
<td>1.33</td>
<td>1.11</td>
<td>1.23</td>
</tr>
</tbody>
</table>