Research Square

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A Restricted Biliary and Cholesterol-Related Biomarker Pattern Associated with Cholecystectomy: Population-Level and Mouse Transcriptomic Evidence

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Abstract

Background

Cholecystectomy may influence hepatobiliary physiology and lipid metabolism, but population-level biomarker patterns and their distinction from associations with underlying gallstone disease remain unclear. We examined liver and lipid biomarkers in relation to self-reported gallbladder surgery and explored potentially relevant hepatic molecular pathways.

Methods

We conducted a cross-sectional analysis of adults aged ≥ 20 years in the nationally representative NHANES 2017–March 2020 pre-pandemic cycle. Survey-weighted linear regression models evaluated associations of gallbladder surgery with seven liver and four lipid biomarkers after adjustment for demographic, socioeconomic, metabolic, lifestyle, and medication-related factors. Secondary analyses examined joint gallstone–surgery categories, time since surgery, effect modification, and multiple sensitivity analyses. Public mouse liver transcriptomic data were re-analyzed using Hallmark gene-set enrichment analysis.

Results

Among 9,232 adults, 1,025 reported previous gallbladder surgery. In fully adjusted models, surgery history was associated with 12.4% higher total bilirubin (95% CI, 6.6%–18.6%), 2.7% higher alkaline phosphatase (95% CI, 0.6%–4.8%), and 5.87 mg/dL lower total cholesterol (95% CI, − 10.04 to − 1.69) after false-discovery-rate correction. Bilirubin elevation was concentrated among participants reporting both gallstones and surgery, whereas lower total cholesterol was observed in both surgery groups irrespective of gallstone history. Cholesterol-homeostasis and bile-acid-metabolism pathways were negatively enriched 30 days after cholecystectomy.

Conclusion

Gallbladder surgery history was associated with a restricted biliary and cholesterol-related biochemical pattern rather than generalized hepatic dysfunction. Longitudinal studies are needed to distinguish effects of surgery from those of underlying gallstone disease.

1. Introduction

Cholecystectomy is one of the most common surgical procedures worldwide, although its prevalence varies across regions (1). It has traditionally been the standard treatment for gallstone disease, which itself is a common gastrointestinal disorder (2, 3). The prevalence and risk of gallbladder disease appear

to vary across racial and ethnic populations, with certain groups showing particularly high susceptibility; for example, American Indian populations have been reported to be at increased risk, although systematic evaluations across diverse populations remain limited (4).

Beyond its therapeutic role, cholecystectomy is increasingly recognized as having broader gastrointestinal and metabolic implications. A history of cholecystectomy has been associated with a greater burden of gastrointestinal symptoms, which may reflect either new conditions related to the procedure or persistence of disorders of gut–brain interaction that were initially attributed to biliary disease, potentially resulting in unnecessary surgery (1). Importantly, evidence suggests that cholecystectomy itself may be associated with metabolic and liver-related disorders. For example, cholecystectomy—but not gallstones—has been associated with nonalcoholic fatty liver disease (NAFLD) (5). Similarly, cholecystectomy has been reported as a predictor of cirrhosis and has been associated with elevated serum liver enzymes, whereas cholelithiasis alone did not show the same associations (6). A large-scale meta-analysis involving millions of individuals further demonstrated an association between cholecystectomy and an increased risk of liver disease (7). In addition, the prevalence of metabolic syndrome and NAFLD has been reported to be higher following cholecystectomy (8).

The relationship between cholecystectomy and liver function has also been examined in the perioperative setting. Transient elevations in aminotransferases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] are commonly observed following laparoscopic cholecystectomy, although values typically return to normal within 7 days and are generally clinically inconsequential in patients with otherwise normal liver function (9). Other postoperative alterations in liver function indices, such as increases in bilirubin or alkaline phosphatase, have also been reported but are generally considered to have limited clinical consequences. These changes may occur more frequently in female patients but appear to be unaffected by age, duration of surgery, or severity of inflammation; routine assessment of direct bilirubin and alkaline phosphatase has generally been considered sufficient (10). Preoperative liver function tests, however, do not appear to predict conversion from laparoscopic to open cholecystectomy (11, 12).

In addition to liver function, cholecystectomy may influence lipid metabolism. Patients with a history of the procedure have been reported to show differences in lipid metabolism compared with individuals with other gallbladder conditions (13). Similarly, cholecystectomy has been shown to affect serum lipid profiles rather than glucose-related indices (14). However, the direction and consistency of these lipid changes remain uncertain (15). Some studies have reported potentially favorable changes, including reductions in total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG), together with increased high-density lipoprotein (HDL) levels following cholecystectomy in patients with gallstones (16). Conversely, other evidence suggests that alterations in lipid and metabolic homeostasis after cholecystectomy may contribute to susceptibility to metabolic syndrome and NAFLD (8).

Previous studies have linked cholecystectomy with hepatic and metabolic abnormalities, but it remains unclear whether a consistent biomarker pattern is detectable at the population level, whether observed associations can be distinguished from those related to underlying gallstone disease, and whether corresponding alterations in hepatic molecular pathways may provide mechanistic context for these associations. Therefore, we investigated the associations of self-reported gallbladder surgery with liver and lipid biomarkers in a nationally representative sample of U.S. adults. We further examined the joint patterns of gallstone and surgery history, variation according to time since surgery, potential effect modification, and the robustness of the findings across sensitivity analyses. Finally, we performed a secondary re-analysis of published gene-level hepatic transcriptomic results from a mouse cholecystectomy model to explore molecular pathways potentially relevant to the population-level findings.

2. Materials and Methods

2.1. Study Design and Population

We conducted a cross-sectional analysis of the National Health and Nutrition Examination Survey (NHANES) 2017–March 2020 pre-pandemic cycle. NHANES uses a complex multistage probability sampling design to obtain a nationally representative sample of the civilian, noninstitutionalized U.S. population. The present study included adults aged ≥ 20 years. Among 15,560 NHANES participants, 9,232 adults constituted the source population; 8,203 reported no history of gallbladder surgery, 1,025 reported previous gallbladder surgery, and four had indeterminate exposure status.

NHANES protocols were approved by the National Center for Health Statistics Research Ethics Review Board, and all participants provided informed consent. The present analysis used publicly available, deidentified data.

2.2. Gallbladder Surgery and Gallstone History

History of gallbladder surgery was determined from the NHANES Medical Conditions Questionnaire based on participants' responses to whether they had ever undergone gallbladder surgery. Participants with affirmative and negative responses were classified as the surgery and no-surgery groups, respectively.

Gallstone history was assessed separately and was used in a secondary analysis combining gallstone and surgery status into four mutually exclusive categories: no gallstones/no surgery, gallstones without surgery, gallstones with surgery, and surgery without reported gallstones. The no gallstones/no surgery group served as the reference. Among participants reporting surgery, approximate time since first reported gallbladder surgery was calculated as age at screening minus reported age at first surgery; participants with top-coded age, top-coded surgery age, special or missing surgery-age responses, or surgery age greater than current age were excluded from this analysis.

2.3. Liver and Lipid Biomarkers

Liver biomarkers included ALT, AST, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, total bilirubin, and total protein. Lipid biomarkers included TC, HDL cholesterol, LDL cholesterol calculated using the NIH Eq. 2 variable (LBDLDLN), and triglycerides. Non-HDL cholesterol and the total cholesterol/HDL (TC/HDL) ratio were additionally examined as secondary lipid outcomes.

2.4. Covariates

Covariates were selected to account for demographic, socioeconomic, metabolic, and lifestyle factors potentially associated with gallbladder surgery and the evaluated biomarkers. These included age, sex, race/ethnicity, educational attainment, body mass index (BMI), diabetes, smoking status, alcohol consumption, and physical activity. Models of lipid biomarkers additionally adjusted for lipid-lowering medication use. Statin use was separately considered in sensitivity and effect-modification analyses. Lipid-lowering medication and statin use were derived from the NHANES prescription medication inventory for the preceding 30 days.

BMI was calculated from measured height and weight. Obesity was defined as BMI ≥ 30 kg/m². Diabetes was based on self-reported physician diagnosis, with borderline diabetes grouped with no diabetes. Smoking was categorized as never, former, or current smoking; alcohol consumption as none, within sex-specific limits (≤ 7 drinks/week for women and ≤ 14 drinks/week for men), or above these limits; and recreational physical activity as meeting recommendations (≥ 150 moderate-equivalent minutes/week), insufficiently active (> 0 to < 150 minutes/week), or inactive (0 minutes/week).

2.5. Survey-weighted Regression Models

Associations between gallbladder surgery and liver and lipid biomarkers were evaluated using survey-weighted linear regression models. Model 0 was unadjusted. Model 1 was adjusted for age, sex, and race/ethnicity. Model 2, the fully adjusted model, additionally included education, BMI, diabetes, smoking, alcohol consumption, and physical activity; models of lipid biomarkers further included lipid-lowering medication use. Age and BMI were modeled using restricted cubic splines with four survey-weighted knots at the 5th, 35th, 65th, and 95th percentiles (age: 22, 39, 57, and 79 years; BMI: 20.1, 26.3, 31.3, and 43.4 kg/m²).

ALT, AST, ALP, GGT, total bilirubin, and triglycerides were modeled on the natural-log scale because of their right-skewed distributions, with estimates back-transformed and expressed as percentage differences. Other biomarkers were analyzed on their original scales and expressed as mean differences.

Analyses were performed using outcome-specific complete cases with valid survey weights; consequently, analytic sample sizes varied across biomarkers. No missing values were imputed. Mobile Examination Center (MEC) examination weights were used for most biomarkers, whereas fasting subsample weights were used for LDL cholesterol and triglycerides.

2.6. Secondary, Sensitivity, and Effect-Modification Analyses

Secondary analyses examined the four-category gallstone/surgery phenotype and biomarker patterns according to time since surgery. In the four-category analysis, adjusted contrasts compared each non-reference group with participants reporting neither gallstones nor surgery, and overall tests evaluated differences across phenotype categories. Time-since-surgery associations were modeled using restricted cubic splines with knots at 1, 8, 16, and 40 years. Model-based differences were calculated relative to 1 year after surgery; for visualization, adjusted estimates and pointwise 95% CIs were displayed at 5, 10, 20, and 40 years.

Sensitivity analyses of the principal findings included exclusion of participants with known liver disease, original-scale analyses of biomarkers modeled primarily on the log scale, replacement of the broader lipid-lowering medication covariate with statin use, exclusion of statin users, evaluation of the alternative official Friedewald and Martin-Hopkins LDL equations, and exclusion of pregnant participants.

Effect modification was evaluated by sex, obesity, and diabetes, with statin use additionally examined for lipid biomarkers. Effect modification was assessed using interaction terms between gallbladder surgery and each potential modifier.

2.7. Mouse Liver Transcriptomic Re-analysis

To complement the NHANES findings and gain mechanistic insight into the hepatic response to cholecystectomy, we performed a secondary re-analysis of gene-level differential-expression results from the mouse cholecystectomy study by Liang *et al.* (17). The original study used male C57BL/6 mice subjected to surgical cholecystectomy or sham operation, with liver transcriptomic profiling performed 7 and 30 days after cholecystectomy and at day 7 after sham surgery. Differentially expressed genes (DEGs) reported from the RNA-sequencing analysis were examined for the day-7 versus sham and day-30 versus day-7 comparisons and visualized using volcano plots.

Gene Set Enrichment Analysis was performed in preranked mode using GSEA (version 4.4.0) with the mouse Hallmark gene sets from the Mouse Molecular Signatures Database (MSigDB). All genes were ranked by log2 fold change from the differential-expression results of Liang et al., and the resulting ranked list was used as the input file. Pathways with a nominal $P < 0.05$, an FDR $q < 0.25$, and an absolute normalized enrichment score ($|NES|$) > 1.5 were considered significantly enriched; enrichment direction was determined by the sign of the NES. Enrichment plots were generated for pathways of particular relevance to the human findings, including Cholesterol Homeostasis and Bile Acid Metabolism.

2.8. Statistical Analysis

All NHANES analyses accounted for the complex survey design, including sampling weights, strata, and primary sampling units. Continuous participant characteristics were summarized as survey-weighted means with standard errors (SEs), and categorical characteristics as survey-weighted percentages with SEs. Between-group differences were evaluated using survey-weighted t tests for continuous variables and design-adjusted Rao-Scott tests for categorical variables. Regression confidence intervals and coefficient tests used the survey design degrees of freedom (primary sampling units minus strata).

Effect estimates are reported with 95% confidence intervals (CIs), and all statistical tests were two-sided.

To account for multiple comparisons, Benjamini–Hochberg false discovery rate (BH-FDR) correction was applied separately within the liver and lipid biomarker families for each regression model. Model 2 q values were used for primary inference, and the two secondary lipid outcomes were corrected as a separate family. FDR-adjusted $q < 0.05$ was considered statistically significant. Secondary phenotype and sensitivity analyses were evaluated using nominal P values, whereas interaction P values were BH-FDR corrected across the 37 evaluated interaction tests.

Statistical analyses were performed using R version 4.5.0, primarily with the survey package (version 4.4-2) for complex survey analyses. Figures were generated using ggplot2 (version 4.0.1).

3. Results

3.1. Study population and participant characteristics

An overview of the study design, integrating the population-based NHANES analysis with a complementary re-analysis of mouse liver transcriptomic data following cholecystectomy, is presented in Fig. 1. Among 15,560 participants in the NHANES 2017–March 2020 pre-pandemic cycle, 9,232 adults aged ≥ 20 years constituted the source population for the present analysis. Of these, 8,203 reported no previous gallbladder surgery, 1,025 reported a history of gallbladder surgery, and four had indeterminate exposure status.

The characteristics of the study population according to gallbladder surgery status are presented in Table 1. Compared with participants without a history of gallbladder surgery, those reporting surgery were older (weighted mean age, 58.42 vs. 47.09 years), had a higher BMI (33.11 vs. 29.37 kg/m²), and were more frequently female (77.0% vs. 48.6%; all $P < 0.001$). The surgery group also had a higher prevalence of diabetes (22.5% vs. 10.2%), obesity (57.5% vs. 39.9%), lipid-lowering medication use (33.5% vs. 18.9%), and statin use (31.4% vs. 17.6%; all $P < 0.001$). As expected, a history of gallstones was substantially more common among participants reporting gallbladder surgery (74.5% vs. 2.7%; $P < 0.001$). Differences were also observed in race/ethnicity, education, smoking status, alcohol consumption, and physical activity (Table 1).

Table 1
Characteristics of the NHANES study population according to self-reported gallbladder surgery status

Characteristic	Overall	No surgery	Surgery	P value
Participants, unweighted n	9,232	8,203	1,025	—
Age, years	48.41 (0.52)	47.09 (0.52)	58.42 (0.70)	< 0.001
BMI, kg/m²	29.80 (0.18)	29.37 (0.16)	33.11 (0.44)	< 0.001
Sex				< 0.001
Male	48.1 (0.8)	51.4 (0.8)	23.0 (1.6)	
Female	51.9 (0.8)	48.6 (0.8)	77.0 (1.6)	
Race/ethnicity				< 0.001
Mexican American	8.3 (1.2)	8.5 (1.2)	6.8 (1.0)	
Other Hispanic	7.7 (0.8)	7.9 (0.8)	6.2 (1.0)	
Non-Hispanic White	62.6 (2.4)	61.3 (2.5)	72.6 (3.1)	
Non-Hispanic Black	11.5 (1.4)	12.1 (1.5)	6.8 (1.0)	
Non-Hispanic Asian	6.0 (0.9)	6.4 (1.0)	2.5 (0.5)	
Other/Multiracial	4.0 (0.4)	3.9 (0.3)	5.2 (1.5)	
Education				0.001
Less than 9th grade	3.8 (0.4)	3.9 (0.4)	2.9 (0.6)	
Grades 9–11	7.3 (0.4)	7.1 (0.4)	8.9 (1.3)	
High school/GED	27.0 (1.3)	26.1 (1.4)	33.3 (1.8)	
Some college/AA	30.5 (1.0)	30.4 (1.0)	30.9 (2.1)	
College graduate+	31.5 (2.0)	32.5 (2.0)	24.0 (2.9)	
Diabetes				< 0.001
No/Borderline	88.4 (0.4)	89.8 (0.4)	77.5 (1.9)	
Yes	11.6 (0.4)	10.2 (0.4)	22.5 (1.9)	
Smoking status				0.007
Never	57.4 (1.1)	58.0 (1.0)	52.9 (3.1)	

Values are presented as survey-weighted mean (SE) for continuous variables and survey-weighted percentage (SE) for categorical variables; participant counts are unweighted. Four participants with indeterminate gallbladder surgery status were included in the overall population but not in either exposure group.

Characteristic	Overall	No surgery	Surgery	P value
Former	25.8 (0.8)	25.0 (0.8)	32.1 (2.2)	
Current	16.7 (1.0)	17.0 (1.1)	15.1 (1.3)	
Alcohol consumption				< 0.001
None	23.1 (0.6)	21.3 (0.7)	36.3 (2.0)	
Within limit	69.5 (0.8)	70.9 (0.8)	58.7 (2.2)	
Above limit	7.5 (0.5)	7.8 (0.5)	5.0 (1.3)	
Physical activity				0.002
Meets guideline	38.9 (1.4)	40.2 (1.3)	28.9 (2.4)	
Inactive	45.4 (1.3)	44.3 (1.3)	53.4 (2.7)	
Insufficient	15.7 (0.7)	15.4 (0.8)	17.7 (2.3)	
Obesity (BMI ≥ 30 kg/m²)				< 0.001
No	58.0 (1.2)	60.1 (1.2)	42.5 (2.3)	
Yes	42.0 (1.2)	39.9 (1.2)	57.5 (2.3)	
Lipid-lowering medication use	20.6 (1.1)	18.9 (0.9)	33.5 (2.6)	< 0.001
Statin use	19.2 (1.1)	17.6 (1.0)	31.4 (2.5)	< 0.001
History of gallstones	11.0 (0.8)	2.7 (0.3)	74.5 (2.0)	< 0.001

Values are presented as survey-weighted mean (SE) for continuous variables and survey-weighted percentage (SE) for categorical variables; participant counts are unweighted. Four participants with indeterminate gallbladder surgery status were included in the overall population but not in either exposure group.

3.2. Associations of gallbladder surgery with liver and lipid biomarkers

The observed distributions of liver and lipid biomarkers according to gallbladder surgery history are shown in Fig. 2A. Associations across the unadjusted, minimally adjusted, and fully adjusted survey-weighted models are presented in Table 2, with the fully adjusted estimates summarized graphically in Fig. 2B. Several associations observed in the unadjusted or minimally adjusted models were attenuated after multivariable adjustment, whereas associations with alkaline phosphatase, total bilirubin, and total cholesterol remained significant after false discovery rate correction in the fully adjusted model.

Table 2
Survey-weighted associations between history of gallbladder surgery and liver and lipid biomarkers in NHANES 2017–March 2020

Biomarker	Analytic N	Surgery, n	Difference (95% CI)	P value	q value
Model 0 — Unadjusted					
Liver biomarkers					
ALT[#]	7,876	870	−7.2% (− 12.2 to − 1.8)	0.0113	0.0159
AST[#]	7,845	866	−6.5% (− 9.4 to − 3.5)	0.0002	0.0003
ALP[#]	7,876	870	+ 9.2% (7.3 to 11.1)	< 0.0001	< 0.0001
GGT[#]	7,876	870	−3.0% (− 9.8 to 4.3)	0.3951	0.4610
Albumin[‡]	7,879	870	−0.13 (− 0.16 to − 0.09)	< 0.0001	< 0.0001
Total bilirubin[#]	7,878	870	+ 0.2% (− 4.7 to 5.5)	0.9275	0.9275
Total protein[‡]	7,876	870	−0.12 (− 0.15 to − 0.08)	< 0.0001	< 0.0001
Lipid biomarkers					
Total cholesterol[‡]	7,917	878	−2.63 (− 6.02 to 0.75)	0.1222	0.2445
HDL cholesterol[‡]	7,917	878	−0.371 (− 2.10 to 1.36)	0.6634	0.6634
LDL cholesterol[‡]	3,726	417	−3.450 (− 9.93 to 3.03)	0.2833	0.3777
Triglycerides[#]	3,734	419	+ 15.7% (8.3 to 23.7)	0.0001	0.0005
Model 1 — Minimally adjusted					
Liver biomarkers					
ALT[#]	7,876	870	+ 4.2% (− 0.8 to 9.5)	0.0979	0.1371
AST[#]	7,845	866	−2.8% (− 5.8 to 0.2)	0.0683	0.1195
ALP[#]	7,876	870	+ 6.3% (4.2 to 8.4)	< 0.0001	< 0.0001

Biomarker	Analytic N	Surgery, n	Difference (95% CI)	P value	q value
GGT#	7,876	870	+ 6.4% (− 2.6 to 16.2)	0.1625	0.1653
Albumin‡	7,879	870	−0.04 (− 0.08 to − 0.01)	0.0026	0.0091
Total bilirubin#	7,878	870	+ 7.4% (2.1 to 13.0)	0.0079	0.0185
Total protein‡	7,876	870	−0.02 (− 0.07 to 0.01)	0.1653	0.1653
Lipid biomarkers					
Total cholesterol‡	7,917	878	−8.01 (− 11.62 to − 4.40)	0.0001	0.0002
HDL cholesterol‡	7,917	878	−4.53 (− 6.19 to − 2.87)	< 0.0001	< 0.0001
LDL cholesterol‡	3,726	417	−5.50 (− 12.06 to 1.05)	0.0961	0.0961
Triglycerides#	3,734	419	+ 12.7% (6.8 to 19.0)	0.0001	0.0002
Model 2 — Fully adjusted					
Liver biomarkers					
ALT#	7,285	796	−1.1% (− 6.7 to 4.7)	0.6883	0.9340
AST#	7,256	793	−3.1% (− 6.4 to 0.4)	0.0809	0.1889
ALP#	7,285	796	+ 2.7% (0.6 to 4.8)	0.0134	0.0469
GGT#	7,285	796	−0.4% (− 8.9 to 8.9)	0.9340	0.9340
Albumin‡	7,288	796	+ 0.003 (− 0.02 to 0.02)	0.8288	0.9340
Total bilirubin#	7,287	796	+ 12.4% (6.6 to 18.6)	0.0001	0.0010
Total protein‡	7,285	796	−0.02 (− 0.07 to 0.01)	0.2325	0.4069
Lipid biomarkers					
Total cholesterol‡	7,220	791	−5.87 (− 10.04 to − 1.69)	0.0077	0.0309

Biomarker	Analytic N	Surgery, n	Difference (95% CI)	P value	q value
HDL cholesterol‡	7,220	791	−1.107 (− 2.77 to 0.55)	0.1829	0.2439
LDL cholesterol‡	3,441	378	−5.09 (− 11.54 to 1.36)	0.1166	0.2332
Triglycerides#	3,448	379	+ 0.6% (− 4.2 to 5.6)	0.8177	0.8177
secondary lipid outcomes§					
Non-HDL cholesterol	7,220	791	−4.76 (− 9.07 to − 0.45)	0.0316	0.0632
Total cholesterol/HDL ratio	7,220	791	−0.036 (− 0.16 to 0.09)	0.5777	0.5777
# ALT, AST, ALP, GGT, total bilirubin, and triglycerides were analyzed on the natural-log scale and are presented as percentage differences					
‡ Albumin, total protein, total cholesterol, HDL cholesterol, LDL cholesterol, and non-HDL cholesterol are presented as mean differences in their original units.					
§ Non-HDL cholesterol and the total cholesterol/HDL ratio were prespecified secondary lipid outcomes and are presented for the fully adjusted model only.					
Measurement units: ALT, U/L; AST, U/L; ALP, IU/L; GGT, IU/L; Albumin, g/dL; total bilirubin, mg/dL; total protein, g/dL; total cholesterol, mg/dL; HDL cholesterol, mg/dL; LDL cholesterol, mg/dL; Non-HDL cholesterol, mg/dL. **Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CI, confidence interval.					

Among liver biomarkers, total bilirubin was 12.4% higher in participants with a history of gallbladder surgery (95% CI, 6.6% to 18.6%; P = 0.0001; q = 0.0010), while alkaline phosphatase was 2.7% higher (95% CI, 0.6% to 4.8%; P = 0.0134; q = 0.0469). No FDR-significant associations were observed for ALT, AST, GGT, albumin, or total protein in the fully adjusted model (Table 2; Fig. 2B).

Among lipid biomarkers, total cholesterol was lower in participants with a history of gallbladder surgery, with an adjusted mean difference of − 5.87 mg/dL (95% CI, − 10.04 to − 1.69; P = 0.0077; q = 0.0309). Associations with HDL cholesterol (− 1.11 mg/dL; 95% CI, − 2.77 to 0.55; q = 0.2439), LDL cholesterol (− 5.09 mg/dL; 95% CI, − 11.54 to 1.36; q = 0.2332), and triglycerides (+ 0.6%; 95% CI, − 4.2% to 5.6%; q = 0.8177) did not remain significant after FDR correction. Overall, three of the 11 prespecified biomarkers—total bilirubin, alkaline phosphatase, and total cholesterol—met the FDR threshold in the fully adjusted analysis (Table 2; Fig. 2B).

Among the secondary lipid outcomes, non-HDL cholesterol was modestly lower in participants with a history of gallbladder surgery (mean difference, − 4.76 mg/dL; 95% CI, − 9.07 to − 0.45; P = 0.0316), although the association did not remain significant after FDR correction (q = 0.063); the total cholesterol/HDL ratio was not significantly associated with gallbladder surgery (P = 0.577; q = 0.5777) (Table 2).

3.3. Sensitivity analyses

The principal findings were generally robust across sensitivity analyses (Table 3). The association with total bilirubin persisted after excluding participants with known liver disease (+ 10.5%; 95% CI, 4.4% to 17.0%; P = 0.0014) and after excluding pregnant participants (+ 12.1%; 95% CI, 6.2% to 18.3%; P = 0.0002), and remained evident when bilirubin was analyzed on its original scale. The association with alkaline phosphatase was also maintained after exclusion of pregnant participants and in the original-scale analysis, although it was attenuated and no longer statistically significant after excluding participants with known liver disease (+ 2.1%; 95% CI, − 0.2% to 4.4%; P = 0.0726).

Table 3
Sensitivity analyses of the principal NHANES findings

Sensitivity analysis	Biomarker	Analytic N	Surgery, n	Difference (95% CI)	P value
Excluding participants with known liver disease	Total bilirubin	6,896	726	+ 10.5% (4.4 to 17.0)	0.0014
	ALP	6,894	726	+ 2.1% (− 0.2 to 4.4)	0.0726
Original-scale analysis	Total bilirubin	7,287	796	+ 0.06 (0.03 to 0.10)	0.0004
	ALP	7,285	796	+ 2.45 (0.78 to 4.11)	0.0056
Statin use replacing any lipid-lowering medication*	Total cholesterol	7,219	791	−5.72 (− 9.83 to -1.61)	0.0084
Excluding statin users	Total cholesterol	5,590	518	−6.20 (− 10.07 to -2.32)	0.0030
Excluding pregnant participants	Total bilirubin	7,220	791	+ 12.1% (6.2 to 18.3)	0.0002
	ALP	7,218	791	+ 2.8% (0.7 to 4.9)	0.0115
	Total cholesterol	7,154	786	−5.51 (− 9.74 to − 1.28)	0.0128

Values are survey-weighted effect estimates with 95% CIs. Percentage differences are reported for log-transformed outcomes; estimates from original-scale analyses are reported in the corresponding original units. P values are nominal and were not additionally adjusted for multiple testing.

*In the statin-specific analysis, statin use replaced the broader lipid-lowering medication covariate used in the fully adjusted lipid model.

Measurement units: Total bilirubin, mg/dL; ALP, IU/L; total cholesterol, mg/dL

Abbreviations: ALP, alkaline phosphatase; CI, confidence interval

The inverse association with total cholesterol was similarly robust to alternative approaches to lipid-lowering medication adjustment. The association remained significant when statin use replaced the broader lipid-lowering medication covariate (− 5.72 mg/dL; 95% CI, − 9.83 to − 1.61; P = 0.0084), after excluding statin users (− 6.20 mg/dL; 95% CI, − 10.07 to − 2.32; P = 0.0030), and after excluding pregnant participants (− 5.51 mg/dL; 95% CI, − 9.74 to − 1.28; P = 0.0128) (Table 3). Overall, the associations with total bilirubin and total cholesterol were particularly consistent across sensitivity analyses, whereas the smaller association with alkaline phosphatase was more sensitive to the exclusion of participants with known liver disease.

3.4. Joint analysis of gallstone history and gallbladder surgery

To further distinguish associations related to underlying gallstone disease from those associated with surgery history, participants were classified into four mutually exclusive groups according to self-reported gallstone and gallbladder surgery history. Participants reporting neither gallstones nor surgery served as the reference group, and the three remaining groups are therefore displayed as contrasts against this reference in Fig. 2C.

For total bilirubin, the strongest association was observed among participants reporting both gallstones and gallbladder surgery, with concentrations 15.9% higher than in the reference group (95% CI, 8.6% to 23.7%; P < 0.0001). In comparison, bilirubin was 7.2% higher among participants with gallstones without surgery (95% CI, − 4.0% to 19.7%; P = 0.2069) and 3.9% higher among those reporting surgery without gallstones (95% CI, − 6.3% to 15.3%; P = 0.4514), although neither association was statistically significant. The overall difference across groups was significant (P = 0.0005).

A similar, although weaker, pattern was observed for alkaline phosphatase. Participants reporting both gallstones and surgery had 3.8% higher alkaline phosphatase than the reference group (95% CI, 0.4% to 7.2%; P = 0.0276), whereas no significant differences were observed among participants with gallstones without surgery or surgery without reported gallstones; however, the overall group test was not statistically significant (P = 0.0802).

The pattern for total cholesterol differed. Lower total cholesterol was observed among participants reporting both gallstones and surgery (− 5.15 mg/dL; 95% CI, − 10.05 to − 0.26; P = 0.0400) and among those reporting surgery without gallstones (− 7.16 mg/dL; 95% CI, − 12.87 to − 1.45; P = 0.0161), whereas participants with gallstones without surgery showed no evidence of lower total cholesterol (+ 2.80 mg/dL; 95% CI, − 8.19 to 13.80; P = 0.6039). The overall difference across groups was significant (P = 0.0290).

Other biomarker associations in this secondary analysis were generally less consistent across exposure groups (Fig. 2C). Overall, elevations in total bilirubin were concentrated among participants reporting both gallstones and surgery, whereas lower total cholesterol was observed in both groups reporting a history of surgery but not among those reporting gallstones without surgery. The alkaline phosphatase pattern was directionally similar to that for bilirubin, but the overall group test did not reach statistical significance.

3.5. Associations according to time since gallbladder surgery

Among participants reporting a history of gallbladder surgery, we further examined whether liver and lipid biomarker levels varied according to time since surgery (Fig. 2D). No significant overall association with time since surgery was observed for any of the evaluated liver or lipid biomarkers (all overall P >

0.05). Total cholesterol showed the strongest evidence of variation over time, although the overall association did not reach statistical significance (P = 0.079). There was also no evidence of significant nonlinearity for any biomarker (all P for nonlinearity > 0.05). Overall, these analyses did not identify a clear time-dependent pattern in biomarker levels according to years since reported gallbladder surgery.

3.6. Effect modification analyses

Potential effect modification by sex, obesity, diabetes, and, for lipid biomarkers, statin use was examined in exploratory interaction analyses (Fig. 2E). After BH-FDR correction across 37 interaction tests, evidence of effect modification was observed only for the association between gallbladder surgery and albumin by sex (P for interaction = 0.0007; q = 0.0245). Several additional interactions were nominally significant, including those of sex with total protein (P = 0.0049), total cholesterol (P = 0.0062), and LDL cholesterol (P = 0.0195), and of obesity with LDL cholesterol (P = 0.0188), total cholesterol (P = 0.0332), and albumin (P = 0.0457); however, none remained significant after FDR correction (all q > 0.05).

Notably, there was no evidence of effect modification of the total bilirubin or alkaline phosphatase associations by sex, obesity, or diabetes. Overall, these analyses provided limited evidence of heterogeneity in the associations according to the examined participant characteristics, with the sex interaction for albumin being the only interaction that remained significant after correction for multiple testing (Fig. 2E).

Overall, the NHANES analyses identified a distinct biochemical pattern associated with gallbladder surgery history, characterized primarily by higher total bilirubin and alkaline phosphatase and lower total cholesterol, with the bilirubin and cholesterol associations showing the greatest robustness across secondary and sensitivity analyses.

3.7. Hepatic transcriptomic remodeling following cholecystectomy

To complement the population-level findings with experimental evidence and gain mechanistic insight into the hepatic response to cholecystectomy, we re-analyzed liver transcriptomic data from the mouse model reported by Liang *et al.* (17), which involved direct surgical cholecystectomy and longitudinal assessment of hepatic transcriptomic responses at days 7 and 30 after surgery. The experimental design is schematically summarized in Fig. 3A. Differential expression analysis showed a relatively limited transcriptional response at day 7 compared with sham-operated mice, whereas substantially broader transcriptional remodeling was evident at day 30 relative to day 7, with numerous genes differentially expressed in both directions (Fig. 3B).

Hallmark pathway enrichment analysis further revealed distinct early and late transcriptional responses (Fig. 3C). At day 7, E2F Targets, G2M Checkpoint, and Unfolded Protein Response were positively enriched relative to sham, consistent with an early response involving cell-cycle activity and proteostatic stress, whereas Apical Surface was enriched in sham-operated mice. In contrast, the day-30 versus day-7 comparison was characterized predominantly by negative enrichment of metabolic pathways, with no

Hallmark pathway significantly enriched in the day-30 direction. Notably, Cholesterol Homeostasis and Bile Acid Metabolism were negatively enriched at day 30 relative to day 7, accompanied by negative enrichment of other metabolic programs including Oxidative Phosphorylation and Xenobiotic Metabolism.

GSEA enrichment profiles further demonstrated marked negative enrichment of Cholesterol Homeostasis (NES = − 2.46, P < 0.001) and Bile Acid Metabolism (NES = − 1.52, P < 0.001) at day 30 relative to day 7, with coordinated contributions from multiple genes within each pathway (Fig. 3D). Together, these findings indicate that the later hepatic response following cholecystectomy is characterized by broad transcriptional remodeling involving pathways central to cholesterol and bile acid metabolism.

4. Discussion

In the present study, a history of gallbladder surgery was associated with a relatively specific biochemical pattern rather than broad abnormalities across liver and lipid biomarkers. After comprehensive adjustment and correction for multiple testing, participants with prior gallbladder surgery had higher total bilirubin, modestly higher ALP, and lower total cholesterol. In contrast, ALT, AST, GGT, albumin, total protein, HDL cholesterol, LDL cholesterol, and triglycerides were not independently associated with surgery history after FDR correction. The bilirubin and total cholesterol findings were particularly consistent across sensitivity analyses, whereas the ALP association was smaller and less robust. Complementary re-analysis of hepatic transcriptomic data from the mouse cholecystectomy model of Liang *et al.* (17) further identified later alterations in pathways related to cholesterol homeostasis and bile acid metabolism. Taken together, these findings suggest a modest biliary/cholesterol-related biochemical pattern associated with gallbladder surgery history rather than generalized hepatocellular dysfunction.

The higher total bilirubin and ALP levels observed in participants with previous gallbladder surgery may reflect persistent alterations in biliary physiology after loss of the gallbladder reservoir. Cholecystectomy changes the storage and delivery of bile acids to the intestine and may consequently alter enterohepatic cycling and hepatic handling of bile constituents (18, 19). Importantly, however, the present biochemical pattern does not support diffuse hepatocellular injury. Neither ALT nor AST was independently elevated, and GGT was also unrelated to surgery history after full adjustment. The magnitude of the ALP association was modest and was attenuated after excluding participants with known liver disease, whereas the bilirubin association remained evident across sensitivity analyses. Thus, these findings should not be interpreted as evidence that cholecystectomy produces chronic liver dysfunction. Rather, they are more consistent with a limited biliary biochemical signature that may reflect long-term differences in bile handling or the clinical conditions leading to surgery.

The joint analysis of gallstone and surgery history provides an important qualification to this interpretation. The largest bilirubin difference was observed among participants reporting both

gallstones and gallbladder surgery, whereas bilirubin was not significantly elevated among those reporting surgery without a history of gallstones. ALP showed a broadly similar but weaker pattern. This distinction suggests that the bilirubin and, to a lesser extent, ALP findings cannot be attributed confidently to gallbladder removal itself. Gallstone disease is metabolically and hepatobiliarily heterogeneous, and individuals who ultimately undergo surgery may differ from those without surgery in previous disease severity, biliary obstruction, inflammation, or other unmeasured characteristics. Previous population studies have similarly raised the difficulty of separating gallbladder disease from the consequences of its treatment; for example, cholecystectomy has been associated with NAFLD and metabolic syndrome in observational studies (20–22). Our four-category analysis extends this literature by explicitly separating reported gallstone history from surgery history within the same population. The results emphasize the potential for confounding by indication and argue against a simple causal interpretation of the biliary biomarker findings.

The cholesterol finding showed a different pattern. Lower total cholesterol remained evident after adjustment for lipid-lowering medication, when statin use specifically replaced the broader medication variable, and after exclusion of statin users. Moreover, in the joint gallstone–surgery analysis, lower total cholesterol was observed in both groups with a history of surgery but not in participants reporting gallstones without surgery. Although this pattern does not establish causality, it is more compatible with a surgery-associated metabolic difference than was the bilirubin finding. This result is broadly concordant with the NHANES and Mendelian-randomization study by Wang *et al.* (14), in which propensity-score-matched participants with cholecystectomy had lower TC, LDL-C, and HDL-C and higher triglycerides, while the MR analyses supported lower TC and particularly LDL-C. In our study, however, only total cholesterol remained significant after FDR correction; LDL and HDL estimates were also negative but their confidence intervals included the null, and triglycerides were not associated with surgery history. An important advantage of the present analysis is that it used the longer NHANES 2017–March 2020 pre-pandemic cycle and therefore included a substantially larger observational sample than the 1,721 participants included in the 2017–2018 NHANES component of Wang *et al.* (14). In addition, our survey-weighted regression framework incorporated a broader set of demographic, metabolic, and lifestyle covariates, outcome-specific analytic samples, and explicit control of multiple testing. These methodological differences, together with their use of propensity-score matching, may partly explain why our evidence was concentrated on total cholesterol rather than multiple lipid fractions.

The broader literature on lipid changes after cholecystectomy has also been inconsistent. Malik *et al.* reported reductions in TC and LDL-C following cholecystectomy (23), while Walmsley *et al.* described substantial postoperative reductions in serum cholesterol (18). Conversely, Juvonen *et al.* found that early reductions in TC and LDL-C after gallbladder removal returned rapidly toward preoperative values (24), and Gill *et al.* reported increased HDL-C with relatively stable LDL-C (25). A pilot study by Cortes *et al.* found no major changes in conventional serum lipid concentrations but reported increased apolipoprotein B after cholecystectomy (26). These heterogeneous observations may reflect differences in baseline lipid status, postoperative diet, duration of follow-up, underlying gallstone disease, and analytic design. Against this heterogeneous background, the reproducibility of the lower total cholesterol

association across our primary and sensitivity analyses, together with its absence in the gallstone-without-surgery group, strengthens the rationale for investigating cholesterol metabolism specifically in relation to gallbladder removal.

Several biological mechanisms could potentially connect gallbladder removal with altered cholesterol metabolism. The gallbladder normally concentrates bile and regulates its meal-related delivery, whereas after cholecystectomy bile enters the intestine more continuously, potentially modifying enterohepatic circulation and the composition of the bile acid pool. Such changes may influence hepatic cholesterol utilization, as conversion of cholesterol to bile acids represents a major route of cholesterol catabolism. Cholecystectomy-associated alterations in the gut microbiota and secondary bile acids may further contribute to changes in lipid metabolism (13, 27). Consistent with these possibilities, experimental studies have reported alteed basal metabolic rate and increased hepatic triglyceride content after cholecystectomy (28, 29), while Cortes *et al.* reported increased hepatic fat, serum insulin, and apolipoprotein B two years after surgery (26).

Our transcriptomic re-analysis provided complementary biological context for the human findings. The results of our Hallmark GSEA demonstrated marked enrichment changes in both Cholesterol Homeostasis and Bile Acid Metabolism. The cholesterol-homeostasis signal involved several key regulators of sterol synthesis and feedback control, including Hmgcs1, Hmgcr, Srebf2, Sqle, and Dhcr7, whereas the bile-acid pathway involved genes central to hepatic bile-acid synthesis and handling, including Cyp7a1, Cyp7b1, Cyp27a1, Cyp8b1, Nr0b2, and Slc27a5. These coordinated transcriptional changes are biologically consistent with remodeling of the hepatic cholesterol–bile acid axis after gallbladder removal. In particular, Cyp7a1 encodes the rate-limiting enzyme of the classical bile-acid synthesis pathway (30), and its regulation is closely linked to bile-acid feedback signaling through the FXR–FGF19–FGFR4 axis (31–33). Together with changes in sterol-synthetic genes such as Hmgcr and Hmgcs1, this provides a plausible molecular link between continuous post-cholecystectomy bile delivery, altered enterohepatic bile-acid signaling, and the lower circulating total cholesterol observed in our NHANES analysis. Thus, our re-analysis extends the original observations of Liang *et al.* (17) by identifying coordinated pathway-level changes that closely parallel the biliary and cholesterol-related biochemical pattern observed in the human population.

We found little evidence that the biomarker pattern varied systematically with elapsed time since surgery. Neither the overall nor nonlinear time-since-surgery tests were significant for the evaluated biomarkers, providing no clear evidence for progressive increase, decline, or normalization of these biochemical differences over time. This finding may indicate that the observed pattern is relatively stable, but such an interpretation remains uncertain because both age at surgery and surgery history were self-reported and the analysis was cross-sectional rather than longitudinal. Similarly, there was little evidence that the principal bilirubin, ALP, or cholesterol findings differed materially by sex, obesity, or diabetes status. The albumin-by-sex interaction was the only interaction surviving FDR correction; because albumin itself showed no overall association with surgery history, this isolated interaction should be considered exploratory rather than evidence of a clinically distinct subgroup.

This study has several strengths. NHANES provides a large sample with standardized laboratory measurements and detailed demographic, metabolic, behavioral, and medication information. The use of complex-survey methods, including appropriate MEC and fasting-subsample weights, allowed estimates to account for the sampling design, while outcome-specific complete-case analyses avoided unnecessarily restricting all outcomes to a single smaller analytic sample. Skewed biomarkers were modeled on the logarithmic scale, nonlinear effects of age and BMI were accommodated, and multiplicity across the primary biomarker families was addressed using FDR correction. The robustness of the principal findings was further examined through analyses excluding participants with known liver disease or pregnancy, alternative outcome scales, and several approaches to lipid-lowering medication and statin use. Importantly, the joint classification of gallstone and surgery history provided information about potential confounding by indication that is not available from a simple surgery-versus-no-surgery comparison. The analysis additionally examined time since surgery and effect modification and incorporated complementary experimental transcriptomic evidence.

Several limitations should also be considered. First, the NHANES analysis is cross-sectional; although gallbladder surgery generally preceded the examination, preoperative biomarker levels were unavailable, preventing assessment of within-person change and precluding causal attribution. Second, gallbladder surgery, gallstone history, and age at surgery were self-reported and may be affected by recall or classification error. Third, residual confounding and confounding by indication remain possible despite extensive covariate adjustment, particularly because information on the precise indication for surgery, previous gallstone severity or biliary complications, surgical technique, and detailed postoperative dietary changes was unavailable. Fourth, biomarkers were measured at a single examination. The absence of a detectable association with time since surgery must also be interpreted in light of uncertainty in the self-reported timing of surgery. Next, the NHANES questionnaire assessed self-reported gallbladder surgery without confirming the specific procedure; however, cholecystectomy constitutes the overwhelming majority of gallbladder surgeries. Finally, in the original transcriptomic data, the day-30 contrast was made against day 7 rather than a concurrent sham group.

In conclusion, a history of gallbladder surgery was associated with a restricted biochemical pattern characterized by higher total bilirubin, modestly higher ALP, and lower total cholesterol rather than generalized abnormalities in hepatic biomarkers or lipid fractions. The joint gallstone–surgery analysis suggests that the bilirubin and ALP findings may partly reflect underlying gallstone disease or factors related to the indication for surgery, whereas the cholesterol association was more consistently observed among participants with a history of surgery and remained robust to analyses addressing lipid-lowering therapy. Complementary mouse transcriptomic findings implicating cholesterol homeostasis and bile acid metabolism provide biological context for this metabolic signal. Longitudinal human studies with pre- and post-cholecystectomy measurements, detailed characterization of the surgical indication, and parallel assessment of bile acids and cholesterol-related pathways will be needed to distinguish effects of gallbladder removal from those of the underlying biliary disease and to clarify the long-term metabolic consequences of cholecystectomy.

Declarations

Author contributions

Conceptualization: Lingli Xia,Yanzhong Wang

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Writing – original draft: Yanzhong Wang

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Conflict of interest statement

There is no conflict of interest.

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Institutional Review Board Statement

Not applicable.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Figures

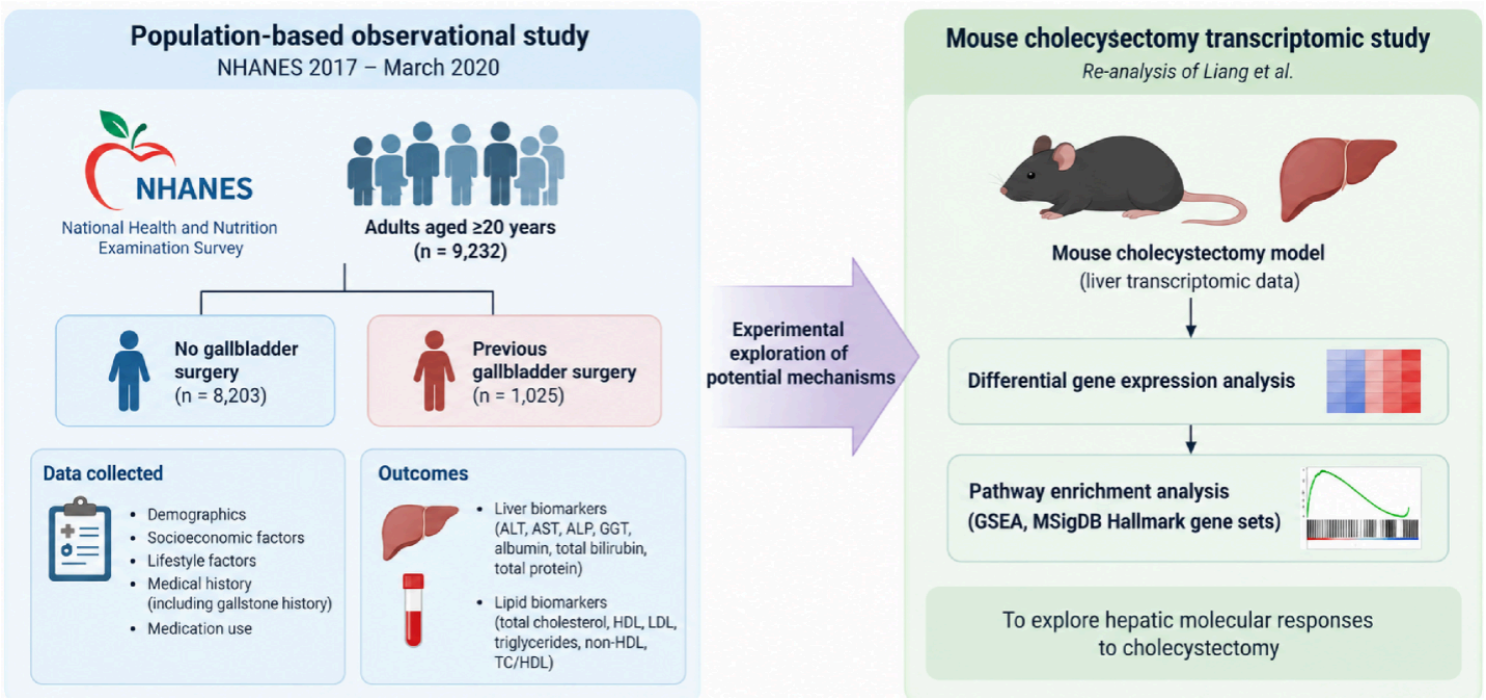


Figure 1

Overview of the study design. The study included a population-based analysis of NHANES 2017–March 2020 examining associations of gallbladder surgery history with liver and lipid biomarkers, followed by complementary re-analysis of mouse liver transcriptomic data to explore potential mechanisms through differential gene expression and pathway enrichment analyses. In NHANES, four participants with indeterminate gallbladder surgery status were excluded from group-specific analyses.

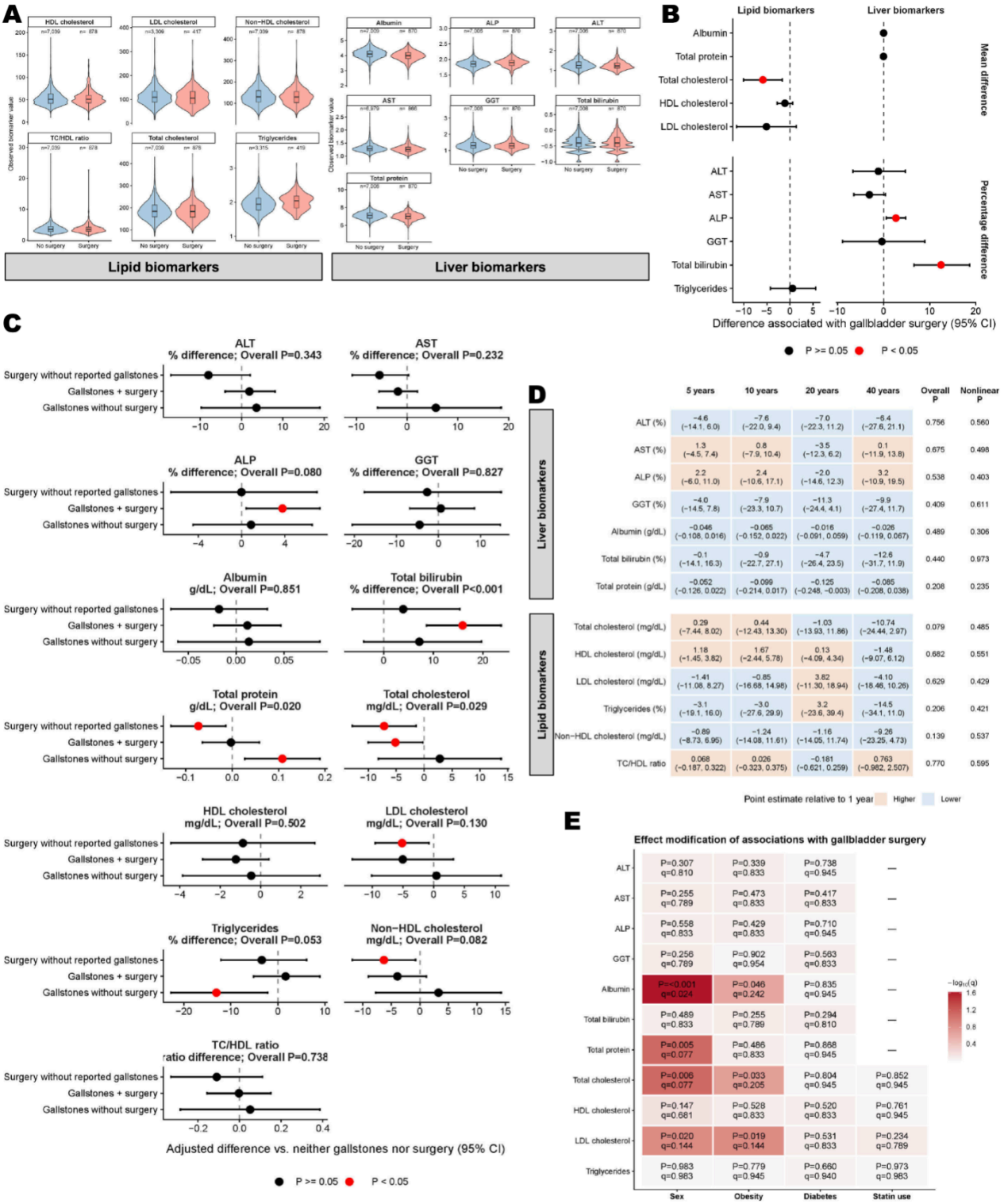


Figure 2

NHANES analyses of gallbladder surgery and liver and lipid biomarkers.

(A) Distributions of observed liver and lipid biomarker values according to self-reported gallbladder surgery history; violin densities incorporate the corresponding NHANES examination weights, and embedded boxplots indicate the median and interquartile range. ALT, AST, ALP, GGT, total bilirubin, and

triglycerides are displayed on a log10 scale because of their right-skewed distributions. LDL cholesterol and triglycerides use fasting subsample weights, whereas the remaining biomarkers use MEC examination weights.

(B) Forest plot showing associations between gallbladder surgery and biomarkers in the fully adjusted model. ALT, AST, ALP, GGT, total bilirubin, and triglycerides were modeled on the natural-log scale and are presented as percentage differences; albumin, total protein, total cholesterol, HDL cholesterol, and LDL cholesterol are presented as mean differences in their original units. Points and horizontal lines represent estimates and 95% confidence intervals, respectively. Red points indicate associations significant after BH-FDR correction ($q<0.05$), whereas black points indicate $q\geq0.05$.

(C) Associations according to the four-category gallstone/surgery phenotype: no gallstones/no surgery, gallstones without surgery, gallstones with surgery, and surgery without reported gallstones. No gallstones/no surgery served as the reference category; therefore, three estimates are displayed for each biomarker, each representing comparison with this common reference. For biomarkers modeled on the natural-log scale, estimates are expressed as percentage differences; other outcomes are expressed as mean differences in their original units. Points and horizontal lines represent adjusted estimates and 95% confidence intervals, respectively. Red points indicate nominal $P<0.05$, whereas black points indicate $P\geq0.05$. The overall P value shown in each panel corresponds to the global test across the four-category phenotype.

(D) Heatmap of adjusted biomarker differences at 5, 10, 20, and 40 years since surgery relative to 1 year after surgery. Cells show point estimates with pointwise 95% CIs; log-transformed outcomes are expressed as percentage differences and other outcomes in their original units. Colors indicate direction only. Overall and nonlinearity P values test the full fitted time association.

(E) Heatmap of effect modification by sex, obesity, diabetes, and, for lipid biomarkers, statin use. Cell color represents −log10 of the BH-FDR–adjusted q value, with greater intensity indicating stronger evidence of interaction; interaction P and q values are shown within cells. White cells with an em dash (—) indicate interactions that were not evaluated.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BH-FDR, Benjamini–Hochberg false discovery rate; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MEC, Mobile Examination Center; NHANES, National Health and Nutrition Examination Survey; TC, total cholesterol.

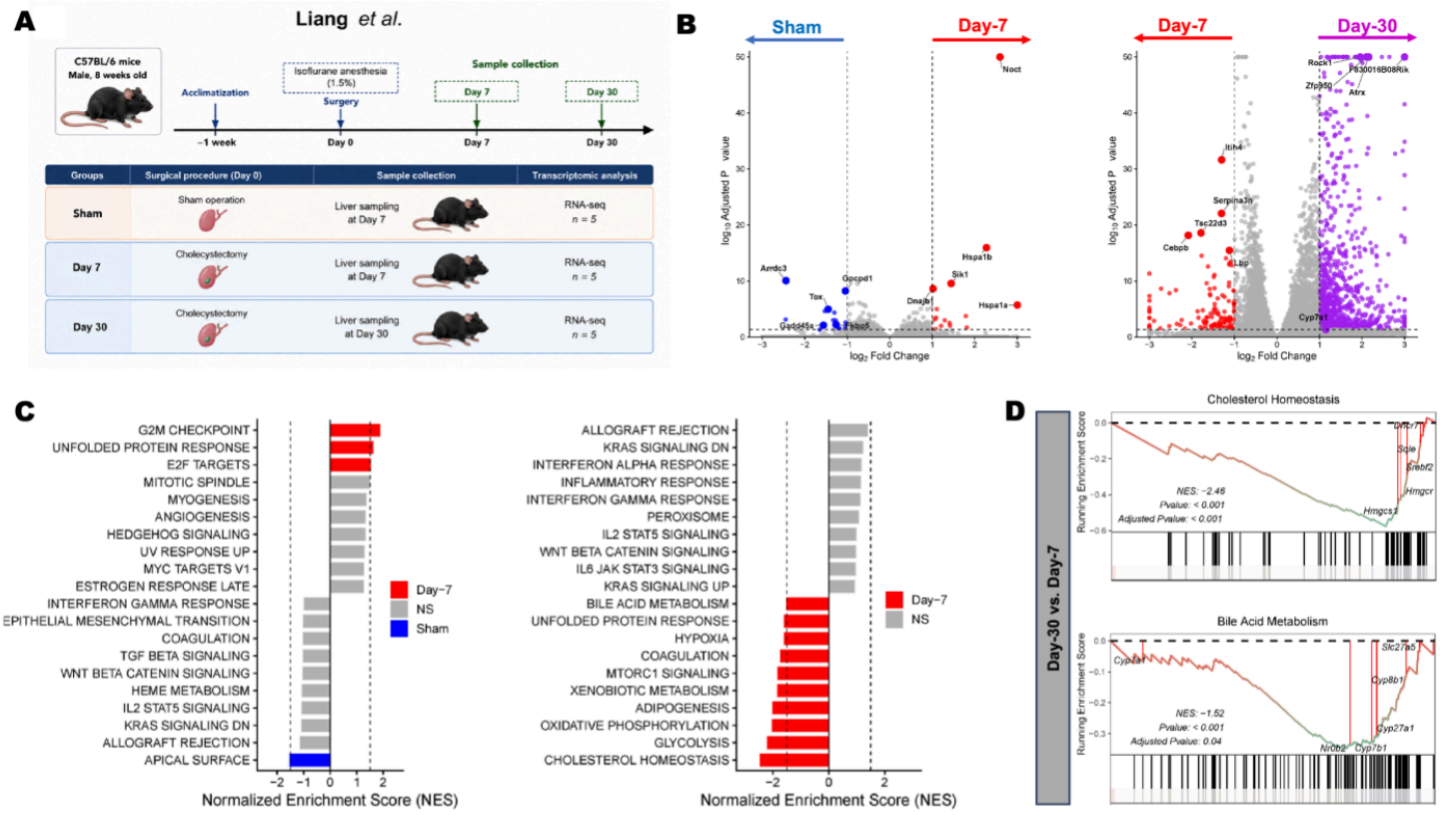


Figure 3

Hepatic transcriptomic remodeling following cholecystectomy in mice.

(A) Schematic overview of the experimental mouse cholecystectomy model reported by Liang *et al.*, including sham-operated controls and liver sampling at 7 and 30 days after surgery.

(B) Volcano plots showing differential gene expression at day 7 versus sham and at day 30 versus day 7.

(C) Hallmark gene-set enrichment analysis showing early pathway enrichment at day 7 versus sham and predominantly negative enrichment of metabolic pathways at day 30 versus day 7.

(D) GSEA enrichment plots highlighting negative enrichment of Cholesterol Homeostasis and Bile Acid Metabolism at day 30 relative to day 7.

Abbreviations: NES, normalized enrichment score; GSEA, gene set enrichment analysis.