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Serum Butyrylcholinesterase and Hepatic Steatosis in U.S. Adults: A Nationally Representative NHANES Study

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Abstract

Background

Serum butyrylcholinesterase (BChE), a hepatocyte-derived hepatic synthetic-function marker, has also been linked to adiposity, insulin resistance, and hepatic fat. Whether BChE is independently associated with steatosis and fibrosis in a nationally representative population is unknown.

Methods

This cross-sectional analysis included adults ≥ 18 years in NHANES (August 2021–August 2023). The primary cohort comprised 5,123 adults with valid BChE activity and complete transient elastography examinations. Steatosis was defined as controlled attenuation parameter (CAP) ≥ 274 dB/m; significant fibrosis as liver stiffness ≥ 8.2 kPa. MASLD was defined per 2023 consensus criteria. Survey-weighted regression models sequentially adjusted for demographic, lifestyle, socioeconomic, and metabolic factors; secondary and sensitivity analyses examined BChE concentration, alternative thresholds, an expanded cohort, hepatitis exclusion, and fasting-subsample classification.

Results

Weighted mean CAP rose from 232.7 dB/m (lowest BChE activity quartile) to 285.8 dB/m (highest), with MASLD prevalence rising from 19.4% to 52.5%. After full adjustment, each 1-SD increase in BChE activity was associated with higher CAP (β = 7.85, 95% CI 5.59–10.12), steatosis (OR = 1.42, 95% CI 1.29–1.57), and MASLD (OR = 1.38, 95% CI 1.26–1.51; all $p < 0.001$); BChE concentration showed concordant associations (CAP β = 6.51; steatosis OR = 1.36; MASLD OR = 1.35). Neither measure was associated with significant fibrosis, and findings were unchanged across all secondary/sensitivity analyses.

Conclusions

Higher serum BChE activity and concentration remained associated with greater hepatic steatosis and MASLD in U.S. adults after multivariable adjustment, but not with significant fibrosis. These findings identify BChE as a potential adjunct biomarker of the steatotic phenotype, supporting prospective study of its temporal and clinical significance.

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) affected an estimated 33.7% of U.S. adults (86.3 million people) in 2020, projected to reach 41.4% (121.9 million) by 2050, with parallel rises in hepatocellular carcinoma, liver transplantation, and liver-related mortality.[1] Despite this scale, MASLD is substantially underrecognized: fewer than 6% of NHANES-identified adults with MASLD reported

awareness of their condition.[2] Established approaches for identifying steatosis and staging fibrosis — MRI-based imaging, vibration-controlled transient elastography (VCTE), and liver biopsy — are accurate but require specialized equipment, trained personnel, or an invasive procedure, and none is universally available where much of this burden will occur. An inexpensive, widely measurable blood-based adjunct signal could meaningfully extend screening capacity.

Serum butyrylcholinesterase (BChE) is a hepatocyte-synthesized serine hydrolase long used as a marker of hepatic synthetic function, with characteristically low levels in decompensated cirrhosis.[3] Independently of this role, a smaller literature spanning nearly two decades has linked higher circulating BChE to adiposity, insulin resistance, and hepatic fat. In Japanese adults, BChE activity correlated with visceral fat, a CT-based hepatic fat marker, and HOMA-IR;[4] in Thai adults with obesity, BChE tracked with insulin resistance and lower HDL.[5] A case-control study of NAFLD patients found BChE elevated overall but reduced among those with more advanced fibrosis,[6] and a recent German cohort found cholinesterase independently associated with hepatic fat among metabolic syndrome components.[7] This human correlational picture runs opposite to causal data from mice, in which BChE-knockout animals accumulate more hepatic triglyceride on a high-fat diet than wild-type controls.[8,9] Population-level data cannot resolve this discrepancy, but can help characterize it. To our knowledge, prior human studies have not combined a nationally representative sample, a standardized steatosis-and-fibrosis phenotype, and a within-cohort comparison of BChE activity against its independently measured protein concentration. Prior samples have ranged from dozens to several hundred participants from single clinics or countries.

The NHANES August 2021–2023 cycle closes this gap. For the first time, it pairs a newly introduced BChE component (BCHE_L, measuring both enzymatic activity and protein concentration) with the established VCTE examination (LUX_L, providing CAP for steatosis and LSM for fibrosis; fielded in NHANES since 2017–2018), allowing BChE to be evaluated against standardized elastography phenotypes in the same nationally representative participants. We used these linked data to test whether serum BChE activity and concentration are independently associated with hepatic steatosis, MASLD, and clinically significant fibrosis across a prespecified adjustment hierarchy. We hypothesized that higher BChE would be associated with greater steatosis and MASLD prevalence, attenuated but not eliminated by metabolic adjustment. Because BChE's established role is as a marker of hepatic synthetic capacity rather than fibrotic injury, we did not expect this association to extend to significant fibrosis.

Materials and Methods

Study design and data source

We conducted a cross-sectional analysis of the NHANES August 2021–August 2023 cycle, a nationally representative, complex multistage probability sample of the noninstitutionalized U.S. civilian population conducted by NCHS. Eighteen public-use files were linked by SEQN: Demographics (DEMO_L); Butyrylcholinesterase Activity & Concentration (BCHE_L); Liver Ultrasound Transient Elastography

(LUX_L); Body Measures (BMX_L); Oscillometric Blood Pressure (BPXO_L); Blood Pressure & Cholesterol questionnaire (BPQ_L); Diabetes questionnaire (DIQ_L); Glycohemoglobin (GHB_L); Fasting Glucose (GLU_L); LDL & Triglycerides (TRIGLY_L); HDL (HDL_L); Total Cholesterol (TCHOL_L); Standard Biochemistry Profile (BIOPRO_L); Alcohol Use (ALQ_L); Cigarette Use (SMQ_L); Physical Activity (PAQ_L); Hepatitis questionnaire (HEQ_L); Hepatitis B core/surface antigen/D antibody (HEPBD_L); and Hepatitis C RNA/antibody (HEPC_L).

Participants

The survey design was constructed on the complete, unfiltered demographic file (N = 11,933) before eligibility restriction, with the analytic cohort then defined via the survey package's subset() function rather than by physically filtering rows first (see Survey weighting, below; Fig. 1). Eligible participants were adults ≥ 18 years (n = 8,153) who completed the MEC exam (RIDSTATR = 2; n = 6,337), were not pregnant (n = 6,296), had valid serum BChE activity (LBXBA non-missing; n = 5,797), and a valid FibroScan CAP reading (LUXCAPM non-missing; n = 5,441).

The cohort was further restricted to a complete VCTE examination (LUAXSTAT = 1: fasting ≥ 3 h, ≥ 10 valid liver stiffness measurements, interquartile-range-to-median ratio [LUXSIQRM] < 30% — a reliability threshold applying to liver stiffness specifically, not CAP), yielding the primary cohort (N = 5,123). Participants with valid CAP but a partial VCTE examination (LUAXSTAT = 2; n = 318) were retained for a sensitivity analysis using the expanded cohort (N = 5,441; Supplementary Table 1).

BChE measurement

Serum BChE activity (LBXBA, U/mL) was measured by absorption spectroscopy (Ellman method). Serum BChE protein concentration (LBXCH, ng/mL) was measured independently by immunomagnetic separation LC-MS/MS. Both analytes were newly introduced in this NHANES cycle (first released August 2026); all primary-cohort values were at or above the assay's lower reportable limit.

VCTE outcomes and thresholds

Hepatic steatosis was defined a priori as median CAP ≥ 274 dB/m, the cutoff providing 90% sensitivity for any steatosis in a large biopsy-based cohort[10] and used in prior NHANES VCTE studies; CAP ≥ 248 dB/m, an independently derived Youden-optimal cutoff,[11] was prespecified as an alternative definition, with CAP also analyzed continuously. Liver fibrosis was assessed by median liver stiffness (LSM, kPa); the prespecified primary outcome used LSM ≥ 8.2 kPa, an elastography cutoff for biopsy-confirmed stage ≥F2 fibrosis in the same cohort[10] rather than a direct histological measurement, with continuous LSM analyzed as a secondary, exploratory outcome.

MASLD definition

Metabolic dysfunction-associated steatotic liver disease (MASLD) was defined per the 2023 multisociety consensus nomenclature[12] as hepatic steatosis (CAP ≥ 274 dB/m) plus ≥ 1 of five cardiometabolic criteria: (1) adiposity — BMI ≥ 25 kg/m², or waist circumference > 94 cm (men)/>80 cm (women); (2) dysglycemia — diagnosed diabetes or insulin/oral hypoglycemic use, HbA1c ≥ 5.7%, or fasting glucose ≥ 100 mg/dL; (3) elevated blood pressure — oscillometric systolic ≥ 130 or diastolic ≥ 85 mmHg, or antihypertensive medication; (4) hypertriglyceridemia — triglycerides ≥ 150 mg/dL or lipid-lowering medication; (5) low HDL — ≤40 mg/dL (men)/≤50 mg/dL (women), or lipid-lowering medication. MASLD-positive participants were further classified by alcohol intake — ALQ121 categorical drinking-frequency converted to weekly frequency via NHANES category midpoints, multiplied by ALQ130 (drinks/occasion) and 14 g ethanol/standard drink — as MASLD (< 140 g/week women, < 210 g/week men), MetALD (140–350/210–420 g/week), or ALD (above those thresholds), per the 2023 nomenclature.

Because triglycerides are measured only in the NHANES fasting morning subsample (54.7% of the primary cohort), a missing triglyceride value in a participant not on lipid-lowering medication was treated as indeterminate rather than imputed as normal: participants were classified as meeting the cardiometabolic criterion if any component was positive regardless of others' missingness, as not meeting it only if all five were definitively negative, and as indeterminate otherwise. This affected 242/5,123 participants (4.7%); combined with 627 (12.2%) missing alcohol-frequency data, primary MASLD classification was indeterminate for 841 participants (16.4%), yielding an analytic n of 4,282 (unadjusted/demographic models) and 3,719 (fully adjusted model). A sensitivity analysis restricted to the fasting subsample, where all five criteria could be fully ascertained without indeterminacy, is reported in Supplementary Table 4.

Covariates

Models were adjusted for age (RIDAGEYR), sex (RIAGENDR), race/ethnicity (RIDRETH3, collapsed to four categories — non-Hispanic White [reference], non-Hispanic Black, Hispanic, other/non-Hispanic Asian — to conserve design degrees of freedom), current smoking (binary current-vs-not-current), alcohol intake (continuous g/week, as derived above), family income-to-poverty ratio (INDFMPIR), body mass index (BMXBMI), glycated hemoglobin (LBXGH) and mean oscillometric systolic blood pressure, as parameter-efficient glycemic/hypertension proxies.

Survey weighting

All analyses accounted for the complex NHANES sample design using Taylor series linearization (R survey package, version 4.2)[13]: full-sample two-year MEC weight (WTMEC2YR), primary sampling unit (SDMVPSU) nested within masked stratum (SDMVSTRA), lonely-PSU adjustment enabled. Per NHANES analytic guidance,[14] the svydesign object was constructed once on the complete, unfiltered demographic file, with the primary cohort and all reported subgroups obtained via subset() rather than by pre-filtering

the data frame — a choice that made no numerical difference here but was followed throughout as recommended. Analyses involving fasting-subsample variables (triglycerides, fasting glucose) used a separate design specified with the fasting subsample weight (WTSAF2YR), built the same way.

Regression models

Associations with continuous outcomes (CAP, LSM) were assessed by survey-weighted linear regression (svyglm, Gaussian family); binary outcomes (steatosis, significant fibrosis, MASLD) by survey-weighted logistic regression (svyglm, quasibinomial family). BChE activity was modeled continuously (per 1 U/mL) and by quartile (Q1 referent); concentration was modeled continuously. A four-model hierarchy was prespecified: Model 1, unadjusted; Model 2, + age, sex, race/ethnicity; Model 3, + current smoking, alcohol intake, income-to-poverty ratio; Model 4, + BMI, HbA1c, systolic blood pressure. Metabolic covariates were deliberately withheld until Model 4 because BChE is biologically intertwined with metabolic phenotype and these variables may lie on the causal pathway between BChE and hepatic fat rather than acting purely as confounders; Model 4 is therefore an extended metabolic-adjustment model, not the single "correct" model.

The NHANES August 2021–2023 masked public-use design has 15 pseudo-strata (30 primary sampling units), fixing the Taylor-series design degrees of freedom at 15 regardless of sample size. Because the survey package's default coefficient test subtracts each estimated parameter from this design df (Korn & Graubard) — producing non-estimable tests for richly parameterized models — we used a parsimonious, collapsed covariate parameterization throughout and report coefficient-level inference using df.resid = degf(design) (= 15 for every model) rather than the parameter-subtracted default, per package documentation,[13] which notes the default can be overly conservative here. All reported models retained ≥ 8 residual/design degrees of freedom, per NCHS reliability guidance.[14] Two-sided $p < 0.05$ was considered statistically significant.

Restricted cubic spline

The shape of the BChE activity–CAP association was examined using a natural cubic spline (4 degrees of freedom, quantile-placed knots) in an age-, sex-, and race/ethnicity-adjusted survey-weighted linear model, restricted to the primary cohort. Nonlinearity was tested against a linear-term model by design-based Wald test (survey::anova); its denominator df is not adjustable in the current implementation (conservative default, 6 residual df), so its p-value is interpreted with caution.

Secondary and sensitivity analyses

- • BChE concentration (LBXCH) substituted for activity (LBXBA) as the primary exposure across all five outcomes, to test whether circulating enzyme quantity reproduces the activity signal (effects per 1 SD of each exposure).

- • Expanded cohort (N = 5,441) additionally including the 318 partial-examination participants (LUAXSTAT = 2), to test dependence on the VCTE completeness restriction.
- • Alternative steatosis threshold, CAP ≥ 248 dB/m, substituted for the primary CAP ≥ 274 dB/m definition.
- • Exclusion of active/current viral hepatitis — current HBV (HBsAg positive [LBDHBG = 1] with anti-HBc positivity [LBXHBC = 1]) or active HCV (RNA positive [LBXHCR = 1]; n = 49) — since a single timepoint cannot establish the ≥ 6-month persistence a chronic HBV diagnosis requires. Indeterminate/untested hepatitis status (n = 35) was retained.
- • MASLD reclassification restricted to the fasting subsample (WTSAF2YR design), where all five cardiometabolic criteria could be fully ascertained without indeterminacy.

Statistical software

All analyses were conducted in R using the haven, foreign, dplyr, survey, and splines packages.

Results

From the full NHANES August 2021–2023 public-use sample (N = 11,933), sequential eligibility restrictions yielded a primary analytic cohort of N = 5,123 (complete VCTE examination, LUAXSTAT = 1) and an expanded sensitivity cohort of N = 5,441 (including 318 participants with a partial VCTE examination, LUAXSTAT = 2).

Table 1
Weighted Participant Characteristics by Serum BChE Activity Quartile

Characteristic	Q1 (lowest)	Q2	Q3	Q4 (highest)
N (unweighted)	1,287	1,284	1,272	1,280
Age, years	46.7 (1.0)	47.9 (0.8)	48.4 (0.7)	47.3 (0.6)
Female, %	62.5%	52.2%	46.1%	41.9%
BMI, kg/m²	26.5 (0.3)	29.0 (0.3)	29.9 (0.3)	31.1 (0.2)
Waist circumference, cm	91.8 (0.9)	98.2 (0.8)	101.1 (0.6)	104.7 (0.7)
BChE activity, U/mL	2.84 (0.01)	3.57 (0.00)	4.10 (0.01)	5.04 (0.05)
BChE concentration, ng/mL	44.7 (0.4)	55.4 (0.4)	63.5 (0.6)	76.3 (0.6)
Diagnosed diabetes, %	7.3%	9.7%	10.9%	12.5%
HbA1c, %	5.44 (0.03)	5.60 (0.04)	5.72 (0.03)	5.91 (0.05)
Fasting glucose, mg/dL*	99.1 (0.8)	105.5 (1.9)	108.6 (1.2)	117.4 (2.9)
Systolic BP, mmHg	118.3 (0.6)	120.1 (0.8)	121.4 (0.5)	122.0 (0.5)
Diastolic BP, mmHg	71.7 (0.5)	73.8 (0.5)	75.3 (0.4)	76.9 (0.3)
Triglycerides, mg/dL*	82.7 (2.2)	99.3 (1.4)	124.4 (6.3)	158.1 (6.0)
HDL cholesterol, mg/dL	58.6 (0.6)	55.2 (0.7)	52.5 (0.6)	49.3 (0.4)
ALT, U/L	18.6 (0.6)	20.2 (0.5)	23.9 (0.6)	28.0 (0.7)
AST, U/L	22.3 (0.8)	21.6 (0.3)	22.9 (0.4)	23.9 (0.5)
GGT, U/L	24.4 (1.3)	25.5 (1.4)	28.8 (1.2)	38.7 (2.2)
Current smoker, %	13.4%	14.2%	12.6%	14.2%
Physical activity, MET-min/wk	1,541 (86)	1,598 (119)	1,597 (148)	1,482 (92)
CAP, dB/m	232.7 (2.5)	251.3 (1.8)	270.3 (1.9)	285.8 (2.6)
Steatosis (CAP ≥ 274), %	20.4%	32.9%	45.7%	57.1%
Liver stiffness, kPa	5.80 (0.21)	5.96 (0.25)	6.02 (0.22)	6.42 (0.26)
Significant fibrosis (LSM ≥ 8.2), %	8.1%	10.1%	12.0%	12.9%
MASLD, %	19.4%	33.4%	40.9%	52.5%
Fatty Liver Index*	30.4 (2.2)	45.9 (2.6)	55.1 (1.7)	65.0 (1.5)

Primary analytic cohort, N = 5,123. Values are survey-weighted (WTMEC2YR) means (SE) or percentages unless noted. Quartile boundaries defined on unweighted BChE activity (LBXBA) within the primary cohort.

**Fasting-subsample variable (WTSAF2YR); n restricted accordingly. BMI = body mass index; BP = blood pressure; CAP = controlled attenuation parameter; LSM = liver stiffness measurement; MASLD = metabolic dysfunction-associated steatotic liver disease; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase.*

Table 2
Association Between Serum BChE Activity and Hepatic Steatosis Measures Across Progressive Adjustment Models

Model	n	CAP, β (95% CI), dB/m per U/mL	Steatosis (CAP ≥ 274), OR (95% CI)	MASLD, OR (95% CI)
Model 1 (unadjusted)	5123	22.60 (19.57, 25.63)***	2.00 (1.79, 2.25)***	1.81 (1.64, 1.99)***
Model 2 (+ age, sex, race/ethnicity)	5123	21.91 (18.89, 24.92)***	1.99 (1.78, 2.23)***	1.82 (1.64, 2.03)***
Model 3 (+ smoking, alcohol, SES)	3958	21.30 (17.47, 25.13)***	1.99 (1.72, 2.30)***	1.88 (1.65, 2.14)***
Model 4 (+ BMI, HbA1c, SBP)	3903	9.08 (6.47, 11.69)***	1.50 (1.34, 1.68)***	1.45 (1.30, 1.61)***

*Primary analytic cohort (adults ≥ 18y, complete VCTE examination, valid BChE activity), N = 5,123. Model 1: unadjusted. Model 2: + age, sex, race/ethnicity (4 categories). Model 3: + current smoking, alcohol intake (g/week), family income-to-poverty ratio. Model 4: + BMI, HbA1c, systolic blood pressure. All models survey-weighted (WTMEC2YR) accounting for the NHANES masked design (15 strata, 30 PSU); coefficient inference uses design df = 15[13] rather than the conservative parameter-subtracted default. ***p < 0.001. CAP = controlled attenuation parameter; MASLD = metabolic dysfunction-associated steatotic liver disease.*

Table 3
BChE Activity and Concentration in Relation to Hepatic Steatosis and Fibrosis Outcomes, Fully Adjusted (Model 4)

Outcome	Exposure	n	Estimate (95% CI)
CAP, β (dB/m per SD)	BChE activity (SD = 0.87 U/mL)	3903	7.85 (5.59, 10.12)***
	BChE concentration (SD = 14.9 ng/mL)	3902	6.51 (4.54, 8.48)***
Steatosis (CAP ≥ 274), OR	BChE activity	3903	1.42 (1.29, 1.57)***
	BChE concentration	3902	1.36 (1.20, 1.54)***
MASLD, OR	BChE activity	3719	1.38 (1.26, 1.51)***
	BChE concentration	3718	1.35 (1.20, 1.50)***
Liver stiffness, β (kPa per SD)	BChE activity	3903	−0.47 (− 1.01, 0.06), p=.077
	BChE concentration	3902	−0.52 (− 1.03, − 0.01), p=.046
Significant fibrosis (LSM ≥ 8.2), OR	BChE activity	3903	0.96 (0.82, 1.13), p=.609
	BChE concentration	3902	0.95 (0.83, 1.08), p=.401

Primary analytic cohort (N = 5,123). Estimates standardized per 1 SD of each exposure (activity SD = 0.87 U/mL; concentration SD = 14.9 ng/mL) to allow direct comparison across the two assays, which are measured on different scales. Model 4 covariates as in Table 2. ****p < 0.001; exact p-values shown for non-asterisked estimates. LSM = liver stiffness measurement.*

Among the 5,123 adults in the primary cohort (weighted mean age 47.5 years [SE 0.56]; 49.7% female; Table 1), serum BChE activity was positively and monotonically associated with steatosis measures across quartiles, with weighted mean CAP rising from 232.7 to 285.8 dB/m and MASLD prevalence rising from 19.4% to 52.5% (Table 1).

In progressively adjusted regression (Table 2), each 1 U/mL increase in BChE activity was associated with 22.60 dB/m higher CAP unadjusted (95% CI 19.57–25.63, p < 0.001), attenuating modestly through demographic (β = 21.91) and lifestyle/socioeconomic adjustment (β = 21.30), then substantially — but remaining robust — after metabolic adjustment (Model 4: β = 9.08, 95% CI 6.47–11.69, p < 0.001). The pattern held for steatosis (OR 2.00→1.50) and MASLD (OR 1.81→1.45, both p < 0.001), indicating metabolic factors explain part, not all, of the association.

BChE concentration reproduced this pattern (Table 3). In fully adjusted, SD-standardized models, both exposures were independently associated with CAP (activity β = 7.85/SD, 95% CI 5.59–10.12;

concentration β = 6.51, 95% CI 4.54–8.48), steatosis (OR = 1.42 vs 1.36), and MASLD (OR = 1.38 vs 1.35; all p < 0.001).

Neither activity nor concentration was independently associated with significant fibrosis (LSM ≥ 8.2 kPa) after full adjustment (activity OR = 0.96, p = 0.609; concentration OR = 0.95, p = 0.401). For continuous liver stiffness, concentration showed a modest inverse association (β=−0.52/SD, p = 0.046) and activity a similar non-significant trend (β=−0.47, p = 0.077); this strengthened in the expanded cohort but lost significance after excluding active/current viral hepatitis (Supplementary Table 3) — an inconsistent, exploratory signal.

Steatosis and MASLD associations were robust across every prespecified secondary and sensitivity analysis: materially unchanged in the expanded N = 5,441 cohort (steatosis OR: activity 1.39, concentration 1.34; Supplementary Table 1); with the alternative CAP ≥ 248 dB/m threshold (activity 1.43, concentration 1.33; Supplementary Table 2); after excluding 49 participants with active/current viral hepatitis (MASLD OR: activity 1.38, concentration 1.35; Supplementary Table 3); and in the fasting-subsample MASLD analysis (Supplementary Table 4).

A restricted cubic spline of CAP on BChE activity (Fig. 2) showed a generally monotonic increase in predicted CAP, from 236.4 dB/m at the 1st percentile to 309.4 dB/m at the 99th, crossing the steatosis threshold near the median. A test against a linear term suggested some deviation from linearity (p = 0.017), interpreted cautiously given the limited denominator df for nonlinearity testing in this single cycle.

Primary analytic cohort (N = 5,123). Natural cubic spline (4 df, quantile-placed knots) for BChE activity, adjusted for age, sex, and race/ethnicity; survey-weighted. Shaded band is the 95% confidence band. Dashed line marks the steatosis threshold (CAP = 274 dB/m).

Discussion

Principal findings

In this nationally representative sample, higher serum BChE activity — and, independently, higher BChE concentration by an orthogonal LC-MS/MS assay — was positively associated with hepatic steatosis (CAP) and MASLD across the full adjustment hierarchy and all secondary and sensitivity analyses. The association attenuated after adjustment for BMI, HbA1c, and blood pressure but persisted, indicating BChE carries steatosis information beyond these variables. Neither BChE measure was independently associated with significant fibrosis after full adjustment; a nominally significant inverse signal for continuous liver stiffness did not survive hepatitis exclusion or replicate consistently. Concordant per-SD effects between activity and concentration support a real biological association rather than an assay-specific artifact.

Comparison with prior literature

This positive relationship between circulating BChE and hepatic/metabolic fat burden is consistent with nearly two decades of literature. Iwasaki et al. found BChE activity correlated with visceral fat, hepatic fat, triglycerides, HDL, and HOMA-IR in 259 Japanese adults, with one-year changes in BChE tracking hepatic fat.[4] Heni et al. asked which metabolic-syndrome component — fatty liver, insulin resistance, or dysglycemia — best explains elevated cholinesterase activity, the question our Model 1→4 hierarchy also addresses;[7] our attenuated, persistent BChE–steatosis association after full adjustment supports a fatty-liver-specific component. This study adds objective VCTE phenotyping and replication across an orthogonal enzymatic/mass-spectrometry assay, and — since prior BChE–NAFLD studies rarely examined fibrosis in the same cohort — a direct, population-based test of whether the steatosis signal extends to fibrosis; it does not.

Biological plausibility

Two threads of evidence bear on mechanism, pointing in different directions. Human observational data — the present study and Iwasaki et al. — associate higher BChE with more hepatic fat and worse insulin resistance, consistent with BChE being co-regulated with hepatic lipogenic and lipoprotein-secretory pathways: hepatocytes synthesize BChE alongside albumin and VLDL apolipoproteins, and the hyperinsulinemic, lipogenic state driving triglyceride accumulation in MASLD may also upregulate BChE. Under this model, BChE would mark the steatotic phenotype rather than cause it. BChE is also a negative acute-phase reactant, falling only once inflammation becomes moderate-to-severe;[15] milder inflammation in early metabolic dysfunction is unlikely to suppress BChE at our steatosis threshold's disease stage.

Causal animal data point the opposite way. BChE-knockout mice on a high-fat diet accumulate more hepatic triglyceride and inflammatory markers than wild-type mice, an effect reversed by AAV-mediated BChE restoration and attributed partly to elevated ghrelin and reduced energy expenditure;[8] BChE gene transfer separately suppresses acyl-ghrelin and improves glucose tolerance in mice.[9] Taken at face value, this implies BChE is protective against steatosis, opposite our human association. The apparent discrepancy may reflect differences between experimental loss-of-function models and cross-sectional measurements in humans with established metabolic disease: the mouse experiments isolate a causal, whole-body effect before disease develops, while our survey captures established, chronic status. Any protective baseline effect, if real, could be outweighed later by a hypothesized compensatory response to insulin resistance and fat accumulation; our design can neither test this directly nor adjudicate between BChE reflecting versus contributing to the phenotype, and we do not read our findings as evidence for the latter.

Interpreting the fibrosis null

BChE was not independently associated with significant fibrosis after multivariable adjustment. This finding may reflect the different biological relationships of BChE with hepatic fat accumulation and advanced loss of synthetic function. BChE is a marker of hepatic synthetic function and classically falls with decompensated cirrhosis, reflecting loss of functioning hepatocyte mass rather than fibrosis per

se.[3] A cohort of confirmed NAFLD patients showed a comparable pattern — BChE elevated overall but reduced with elevated hyaluronic acid, a marker of more advanced fibrosis[6] — and hepatocyte BCHE expression reportedly declines with fibrosis progression in HCV-infected drug users.[16] Our LSM ≥ 8.2 kPa threshold captures clinically significant (≥ F2) fibrosis short of the decompensated range where BChE's classical decline occurs, consistent with a two-phase pattern — an early rise tracking hepatic fat, followed much later by a decline in synthetic function — that our cross-sectional data cannot confirm.

Strengths and limitations

This study benefits from several strengths: a nationally representative sample; objective elastography-based ascertainment of both steatosis (CAP) and fibrosis (LSM); to our knowledge, the first population-based comparison of BChE activity against an orthogonal concentration assay in the same participants; survey-appropriate statistical inference, including degrees-of-freedom handling; adherence to 2023 consensus MASLD nomenclature with transparent handling of missing cardiometabolic data; and prespecified secondary and sensitivity analyses reported regardless of outcome.

Several limitations warrant emphasis. First, this cross-sectional design cannot establish temporality or distinguish elevated BChE as consequence, cause, or a marker of shared unmeasured confounding. Second, the masked design provides only 15 design degrees of freedom regardless of sample size, limiting subgroup precision; single-cycle data cannot be pooled since BCHE_L and LUX_L have not been co-fielded before. Third, VCTE-based CAP and LSM are validated surrogates, not biopsy, and threshold misclassification is possible. Fourth, MASLD phenotyping was indeterminate for 16.4% of the cohort from nonresponse, reducing MASLD-model samples and risking nonresponse bias. Fifth, BCHE_L is newly released with no established reference ranges, and we lacked data on BCHE genetic variants or medications/exposures that could confound the activity measure. Finally, NHANES likely underrepresents advanced fibrosis, limiting our ability to test rather than refute a BChE–fibrosis relationship at more advanced stages; our fibrosis null is specific to early-to-moderate (≥ F2) fibrosis.

Implications for clinical practice and future research

Clinically, serum BChE — already in some biochemistry panels, and now separately quantifiable by concentration — may hold promise as an accessible adjunct biomarker for steatosis and MASLD risk stratification, if validated prospectively against established noninvasive strategies. We are not proposing BChE as a replacement for VCTE, imaging, or biopsy, nor as a fibrosis-staging tool; our data argue against the latter. Three research directions follow: Mendelian-randomization studies using BCHE genetic variants to test whether the steatosis association is causal, compensatory, or reflects shared upstream biology; extension of the fibrosis analysis to cohorts enriched for advanced disease, where synthetic-function decline would be expected; and mechanistic work on hepatocyte co-regulation of BChE with lipogenic and VLDL-secretory pathways, clarifying whether concentration and activity capture the same processes.

Declarations

Competing Interests

The authors declare no competing interests.

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Author Contributions

Maie Hassan Al-Za’areer: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing, Project administration. Abdulhaq Al Jafari: Methodology (supporting), Writing – review & editing. Celeen Hassan Al-Za’areer: Methodology (supporting), Writing – review & editing. All authors approved the final manuscript.

Data Availability Statement

All data analyzed are publicly available from the National Center for Health Statistics (NCHS) at https://wwwn.cdc.gov/nchs/nhanes/; component files are listed under Study design and data source (Materials and Methods). Analysis code is available from the corresponding author upon reasonable request.

References

1. Le P, Tatar M, Dasarathy S et al (2025) Estimated burden of metabolic dysfunction-associated steatotic liver disease in US adults, 2020 to 2050. JAMA Netw Open 8(1):e2454707
2. Alp J, Sripongpun P, Roldan GA, Fletcher JA, Davie T, Udompap P (2025) Low awareness of MASLD among U.S. adults: trends from NHANES 2017–2023. Hepatol Commun 9(11):e0829
3. Biochemistry P In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. NCBI Bookshelf ID: NBK545284
4. Iwasaki T, Yoneda M, Nakajima A, Terauchi Y (2007) Serum butyrylcholinesterase is strongly associated with adiposity, the serum lipid profile, and insulin resistance. Intern Med 46(19):1633–1639
5. Tangvarasittichai S, Pongthaisong S, Meemark S, Tangvarasittichai O (2015) Abdominal obesity associated with elevated serum butyrylcholinesterase activity, insulin resistance and reduced high-density lipoprotein-cholesterol levels. Indian J Clin Biochem 30(3):275–280
6. Turecký L, Kupčová V, Durfinová M, Uhlíková E (2021) Serum butyrylcholinesterase activities in patients with non-alcoholic fatty liver disease. Comparison with liver proteosynthetic function and

liver fibrosis. Bratisl Lek Listy 122(10):689–694

7. Heni M, Hummel J, Fritsche L, Wagner R, Relker L, Machann J et al (2025) Elevated cholinesterase activity and the metabolic syndrome—dissecting fatty liver, insulin resistance and dysglycaemia. Liver Int 45(5):e70046
8. Chen VP, Gao Y, Geng L, Stout MB, Jensen MD, Brimijoin S (2016) Butyrylcholinesterase deficiency promotes adipose tissue growth and hepatic lipid accumulation in male mice on high-fat diet. Endocrinology 157(8):3086–3096
9. Chen VP, Gao Y, Geng L, Brimijoin S (2017) Butyrylcholinesterase regulates central ghrelin signaling and has an impact on food intake and glucose homeostasis. Int J Obes (Lond) 41(9):1413–1419
10. Eddowes PJ, Sasso M, Allison M et al (2019) Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 156(6):1717–1730
11. Karlas T, Petroff D, Sasso M et al (2017) Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 66(5):1022–1030
12. Rinella ME, Lazarus JV, Ratziu V et al (2023) A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 78(6):1966–1986
13. Lumley T (2004) Analysis of complex survey samples. J Stat Softw 9(1):1–19
14. National Center for Health Statistics (2023) Analytic guidelines for NHANES August 2021-August 2023. Available at: wwwn.cdc.gov/nchs/nhanes/continuousnhanes/overviewbrief.aspx?Cycle = 2021–
15. Lampón N, Hermida-Cadahia EF, Riveiro A, Tutor JC (2012) Association between butyrylcholinesterase activity and low-grade systemic inflammation. Ann Hepatol 11(3):356–363
16. Munshaw S, Hwang HS, Torbenson M, Quinn J, Hansen KD, Astemborski J et al (2012) Laser captured hepatocytes show association of butyrylcholinesterase gene loss and fibrosis progression in hepatitis C-infected drug users. Hepatology 56(2):544–554

Figures

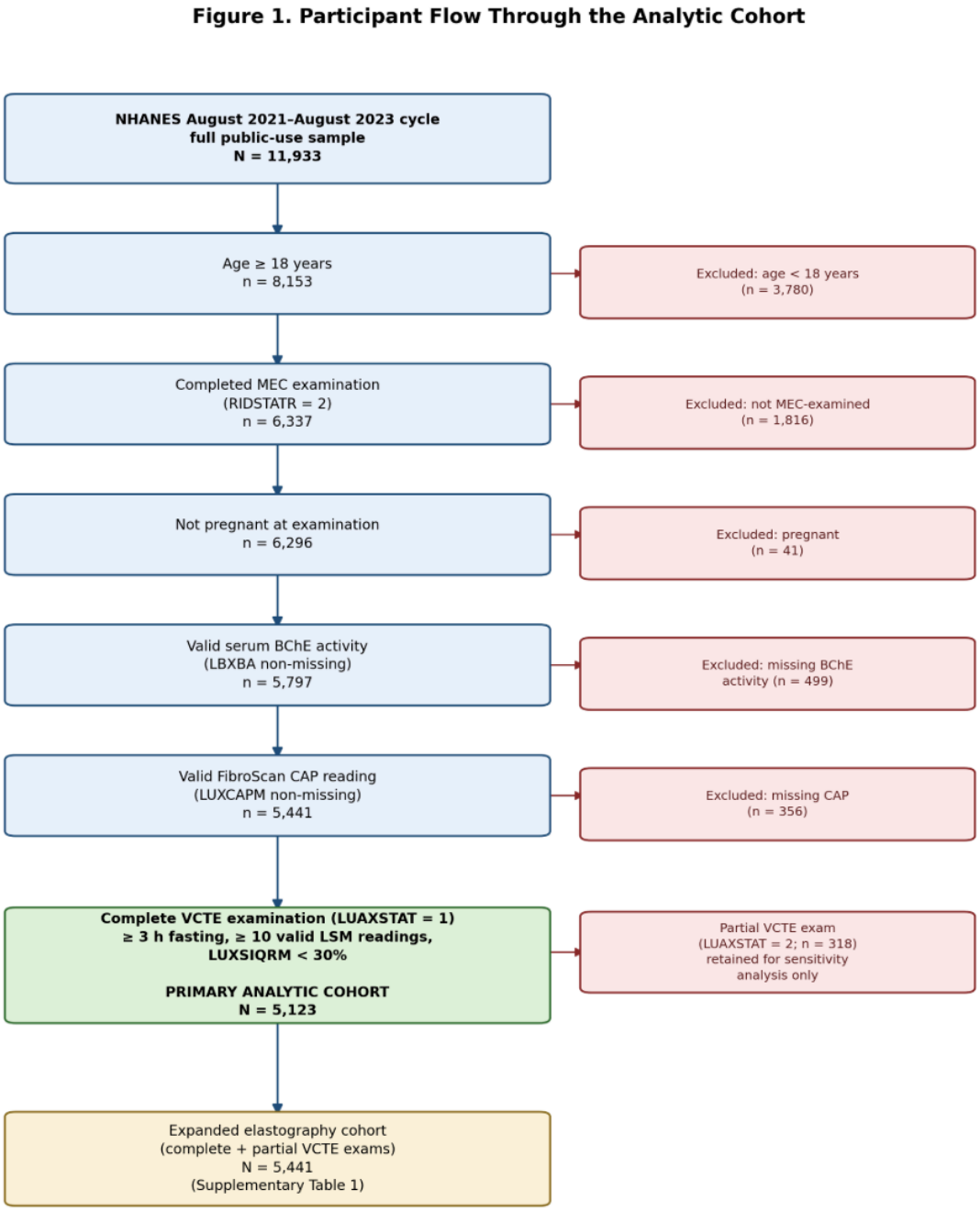


Figure 1

Participant flow through the analytic cohort.

From the full NHANES August 2021–2023 public-use sample (N=11,933), sequential eligibility restrictions yielded a primary analytic cohort of N=5,123 (complete VCTE examination, LUAXSTAT=1) and an expanded sensitivity cohort of N=5,441 (including 318 participants with a partial VCTE examination, LUAXSTAT=2).

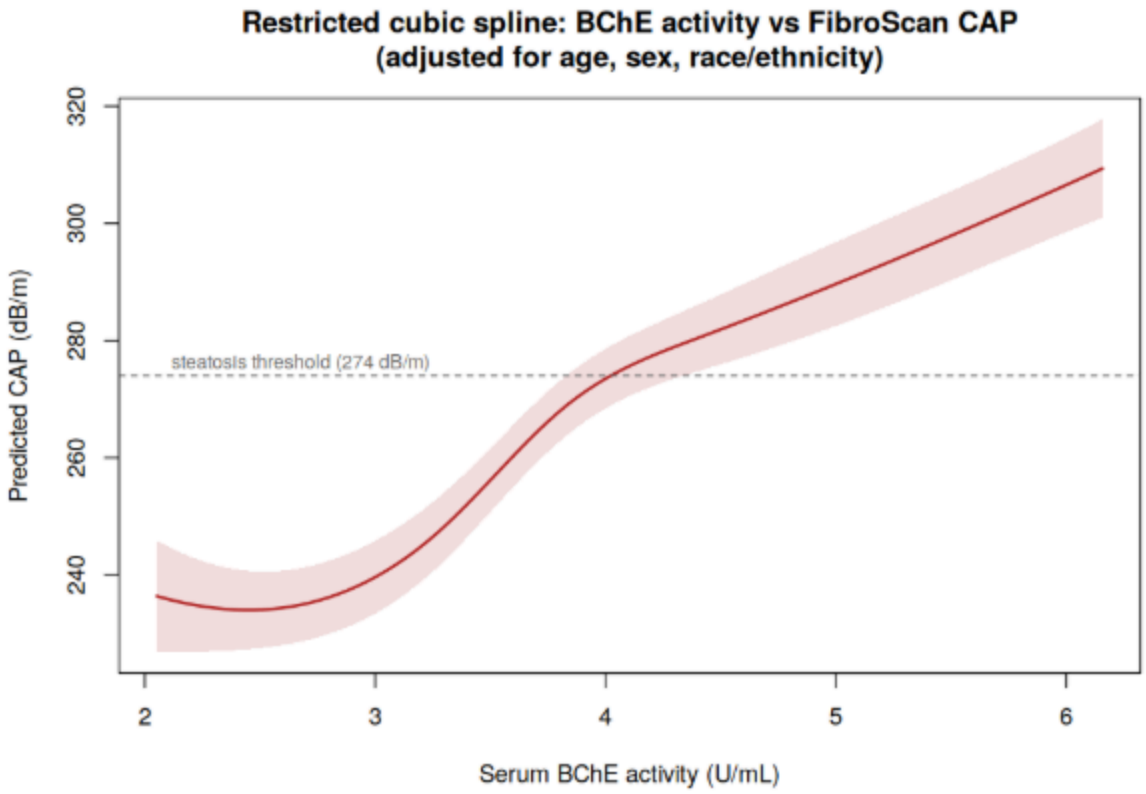


Figure 2

Restricted cubic spline of predicted FibroScan CAP across the range of serum BChE activity.

Primary analytic cohort (N=5,123). Natural cubic spline (4 df, quantile-placed knots) for BChE activity, adjusted for age, sex, and race/ethnicity; survey-weighted. Shaded band is the 95% confidence band. Dashed line marks the steatosis threshold (CAP=274 dB/m).

Supplementary Files

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- floatimage1.png