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Associations of Serum Butyrylcholinesterase Activity With Hepatic Steatosis and Liver Stiffness in U.S. Adults: NHANES 2021–2023

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Abstract

Background

Serum butyrylcholinesterase (BChE) has been linked to metabolic dysfunction and hepatic steatosis. We examined associations of BChE activity and concentration with liver phenotypes and their incremental discriminatory value.

Methods

We analyzed 4,298 adults aged ≥ 20 years from NHANES 2021–2023 in a survey-weighted cross-sectional study. Multivariable regression assessed associations between serum BChE activity and controlled attenuation parameter (CAP), CAP-defined steatosis (≥ 274 dB/m), and liver stiffness measurement (LSM). Sensitivity analyses addressed metabolic adjustment, alternative steatosis definitions, and joint modeling of activity and concentration. Discrimination was evaluated beyond clinical risk factors.

Results

Per 1 survey-weighted standard deviation higher BChE activity, CAP was 8.90 dB/m higher (95% CI, 6.74–11.07), and steatosis prevalence was greater (prevalence ratio, 1.19; 95% CI, 1.14–1.23). Associations persisted across subgroups, alternative CAP thresholds, and a metabolic dysfunction-associated steatotic liver disease phenotype. Additional adjustment for HbA1c and lipids attenuated the CAP association (N = 4,218; β = 5.95 dB/m; 95% CI, 3.49–8.42). In a joint model (N = 4,297), activity remained associated with CAP after adjustment for concentration (β = 8.70; 95% CI, 4.45–12.95), whereas concentration showed little conditional association (β = 0.25; 95% CI, − 3.22 to 3.73). Adding activity beyond concentration increased weighted in-sample R^2 by 0.00578. Adding activity to clinical factors increased the area under the curve from 0.820 to 0.829 (difference, 0.009; 95% CI, 0.005–0.013). Adjusted LSM associations were not consistently significant, although exploratory spline analysis suggested nonlinearity.

Conclusions

Higher BChE activity was associated with CAP-measured hepatic steatosis, but added discrimination was modest. Cross-sectional findings do not establish causality or clinical utility.

1. INTRODUCTION

Hepatic steatosis is a common metabolic liver phenotype and represents an early stage along a continuum that may, in susceptible individuals, progress toward steatohepatitis, fibrosis, cirrhosis, and

liver-related complications.[1] Because steatosis and fibrosis reflect biologically distinct aspects of liver disease, distinguishing factors associated primarily with hepatic fat accumulation from those associated with fibrotic remodeling may improve understanding of disease phenotype and biomarker interpretation. Vibration-controlled transient elastography provides two complementary noninvasive measurements for this purpose: the controlled attenuation parameter (CAP), which reflects ultrasound attenuation related to hepatic fat, and liver stiffness measurement (LSM), which is used as a surrogate of fibrosis-related tissue stiffness.[1, 2]

Butyrylcholinesterase (BChE), also known as pseudocholinesterase, is a glycoprotein synthesized predominantly by hepatocytes and released into the circulation.[3] Although historically used in the assessment of organophosphate exposure, atypical cholinesterase variants, and hepatic synthetic dysfunction, circulating BChE has also been investigated as a potential marker of metabolic status.[3–5] BChE activity has been associated with adiposity, insulin resistance, dyslipidemia, metabolic syndrome, and other cardiometabolic traits that are also closely related to hepatic lipid accumulation.[5]

The relationship between BChE and chronic liver disease is potentially complex. Advanced hepatic dysfunction may reduce synthesis of circulating cholinesterases, whereas individuals with obesity, insulin resistance, and uncomplicated hepatic steatosis may exhibit comparatively higher BChE activity.[3, 5] Small clinical studies have therefore suggested that circulating BChE may behave differently across the steatosis-to-fibrosis spectrum, with higher activity in fatty liver but lower values in patients with markers of more advanced hepatic fibrosis or impaired synthetic function.[6] Recent imaging-based work has further demonstrated a positive association between circulating BChE activity and quantitatively measured liver fat that persisted after adjustment for age, sex, and body mass index, supporting the possibility that BChE may reflect a hepatometabolic phenotype related to hepatic lipid accumulation.[7]

Although associations between circulating BChE and hepatic steatosis have been described, the relative contributions of enzymatic activity and circulating protein concentration to CAP variation remain incompletely characterized, particularly when both measures are considered in the same model. The sensitivity of the BChE–CAP association to additional glycemic and lipid covariate adjustment also warrants evaluation. We therefore conducted matched-sample post-hoc metabolic sensitivity analyses and an exploratory joint activity–concentration analysis, alongside the main CAP and LSM analyses, to better characterize these associations and their limitations.

Accordingly, we examined the associations of serum BChE activity with hepatic steatosis and liver stiffness among U.S. adults using National Health and Nutrition Examination Survey (NHANES) August 2021–August 2023 data. Continuous CAP was used as the primary liver outcome, while threshold-defined steatosis and multiple LSM-based outcomes were evaluated as supportive and secondary phenotypes. We additionally examined BChE concentration, exposure quartiles, potential nonlinearity, and several sensitivity analyses designed to assess the influence of adiposity, alcohol use, missing covariate data, and elevated liver stiffness. We also examined whether the association differed across

clinically relevant subgroups, whether BChE activity improved the identification of CAP-defined steatosis beyond established clinical risk factors, and whether the findings were robust to alternative CAP thresholds and a metabolic dysfunction-associated steatotic liver disease (MASLD) phenotype. We hypothesized that higher circulating BChE activity would be positively associated with CAP-measured hepatic steatosis, while its associations with LSM-derived outcomes might differ after adjustment for major metabolic and behavioral characteristics.

2. METHODS

1. **2.1 Study Design and Participants** – We conducted a cross-sectional analysis of publicly available data from the National Health and Nutrition Examination Survey (NHANES) August 2021–August 2023. NHANES is administered by the National Center for Health Statistics of the U.S. Centers for Disease Control and Prevention and uses a complex, stratified, multistage probability-sampling design to produce estimates representative of the civilian, noninstitutionalized U.S. population.[8] Adults aged ≥ 20 years were considered eligible for the present analysis. Demographic, serum butyrylcholinesterase, standard biochemistry, liver elastography, body-measurement, smoking, alcohol-use, diabetes, and blood-pressure datasets were linked using the unique participant identifier (SEQN). Among adults with a positive phlebotomy survey weight, 5,605 had available serum BChE activity measurements and 5,071 completed the liver elastography examination. A total of 4,914 participants had BChE activity together with valid CAP and LSM measurements. After requiring complete information for the covariates included in the primary multivariable model, 4,298 participants contributed to the primary adjusted analysis; the expanded socioeconomic model included 3,787 participants. NHANES protocols were reviewed and approved by the National Center for Health Statistics Research Ethics Review Board, and written informed consent was obtained from participants according to NHANES procedures.[9] The present study involved secondary analysis of publicly available, deidentified data and no direct participant contact.
2. **2.2 Serum Butyrylcholinesterase and Liver Elastography Assessment –** Serum BChE measurements were obtained from the NHANES Butyrylcholinesterase laboratory component.[10] BChE activity (LBXBA), expressed in U/mL, was the primary exposure, while serum BChE concentration (LBXCH), expressed in ng/mL, was evaluated as a complementary exposure because enzyme activity and circulating concentration represent related but distinct biochemical characteristics. BChE activity was quantified by absorption spectroscopy based on the rate of substrate hydrolysis, whereas BChE concentration was measured using immunomagnetic separation coupled with liquid chromatography–tandem mass spectrometry.[10] BChE activity was modeled continuously per 1 survey-weighted standard deviation (SD) and was additionally evaluated using survey-weighted quartiles and restricted cubic splines. Hepatic steatosis and liver stiffness were assessed using NHANES vibration-controlled transient elastography.[11] Analyses were restricted to participants with a complete elastography examination (LUAXSTAT = 1), defined by NHANES as a fasting time of at least 3 hours, at least 10 complete liver-stiffness measurements, and a liver-stiffness interquartile-range-to-median ratio < 30%.[11] The controlled attenuation

parameter (CAP; LUXCAPM), expressed in dB/m, was used as the objective measure of hepatic steatosis and served as the primary outcome. Continuous CAP was selected to preserve information across the observed distribution and avoid making the primary inference dependent on a single diagnostic threshold. CAP ≥ 274 dB/m was evaluated as a supportive categorical steatosis outcome, corresponding to a sensitivity-oriented threshold for detecting histologic steatosis (≥ S1) in a previous biopsy-based validation study.[12] This threshold was used for operational classification rather than definitive diagnosis. Liver stiffness measurement (LSM; LUXSMED), expressed in kPa, was evaluated as a secondary liver phenotype. Because LSM was right-skewed, continuous values were log2-transformed. Additional outcomes were LSM ≥ 8.0 kPa, LSM ≥ 12.0 kPa, and LSM ≥ 8.0 kPa among participants with CAP ≥ 274 dB/m.

3. **2.3 Covariates –** Covariates were selected based on plausible relationships with circulating BChE, hepatic steatosis, liver stiffness, or shared metabolic determinants. Demographic covariates included age, sex, and race/ethnicity, categorized as Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, or other/multiracial. Anthropometric and metabolic covariates included body mass index (BMI), diabetes, and hypertension, while behavioral covariates included smoking status and past-year alcohol use. Smoking was classified as never, former, or current, and alcohol use as no past-year use or past-year drinking in the primary model. A sensitivity analysis used a more granular alcohol measure incorporating drinking frequency and average drinks consumed on drinking days. Educational attainment and family poverty-income ratio were included in an expanded socioeconomic model; education was categorized as less than high school, high school or General Educational Development equivalent, or greater than high school. Waist circumference was examined in a sensitivity model replacing BMI. Serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and albumin were not included in the primary adjustment set because these biomarkers may reflect hepatic injury or synthetic function and could lie downstream of, or be closely intertwined with, the liver phenotype under investigation.

For an additional post-hoc metabolic sensitivity analysis, glycated hemoglobin (HbA1c; LBXGH, %), total cholesterol (LBXTC, mg/dL), and high-density lipoprotein cholesterol (HDL-C; LBDHDD, mg/dL) were obtained from the corresponding NHANES laboratory components.[13–15] These variables were examined to evaluate the extent to which the observed BChE–steatosis association was sensitive to further adjustment for glycemic status and circulating lipid measures. They were not included in the primary adjustment set because of their close biological relationships with the metabolic characteristics under investigation.

2.4 Statistical Analysis – All analyses incorporated the complex NHANES survey design. The survey-design object was created using the full eligible phlebotomy-weighted parent sample before restriction to individual analytic domains. The two-year phlebotomy weight (WTPH2YR) was used because serum BChE represented the more restrictive laboratory component, together with the masked variance strata (SDMVSTRA) and primary sampling units (SDMVPSU).[8] Statistical inference incorporated 15 survey design degrees of freedom. The primary exposure was serum BChE activity modeled continuously per 1

survey-weighted SD, and the primary outcome was continuous CAP. Survey-weighted linear regression was used to estimate adjusted CAP differences with 95% confidence intervals (CIs). Age was modeled flexibly using a natural cubic spline with three degrees of freedom. An initial model adjusted for age, sex, and race/ethnicity; the primary model additionally adjusted for BMI, diabetes, hypertension, smoking status, and alcohol use; and an expanded socioeconomic model further included educational attainment and family poverty-income ratio. Supportive analyses evaluated CAP ≥ 274 dB/m using survey-weighted Poisson regression with a log link and design-based robust variance to estimate prevalence ratios (PRs). BChE activity was also modeled using survey-weighted quartiles, with Q1 as the reference and linear trend assessed using quartile rank. Potential nonlinearity was examined using restricted cubic splines with four survey-weighted knots at the 5th, 35th, 65th, and 95th percentiles, using the survey-weighted median as the reference. Overall spline association and departure from linearity were evaluated using joint Wald tests, with additional analyses restricted to the weighted 1st–99th and 5th–95th percentile ranges.

Liver stiffness was evaluated as a secondary phenotype using survey-weighted linear regression for log2-transformed LSM, with results expressed as percentage differences in LSM, and survey-weighted Poisson models for LSM ≥ 8.0 and ≥ 12.0 kPa. LSM ≥ 8.0 kPa was also examined among participants with CAP ≥ 274 dB/m. Complementary and sensitivity analyses substituted BChE concentration for activity, cross-adjusted CAP for log2-transformed LSM and LSM for CAP to examine conditional associations with the two hepatic phenotypes, replaced BMI with waist circumference, omitted alcohol from the adjustment set to reduce complete-case loss, applied a more granular alcohol adjustment, and repeated the CAP analysis among participants with LSM < 8 and < 12 kPa. Missing covariate data were handled using complete-case analysis within each model; because missingness was concentrated primarily in alcohol use and family poverty-income ratio, we quantified covariate-specific missingness, compared participants included in and excluded from the primary complete-case model, and assessed whether the BChE–CAP association changed materially in analyses retaining a larger proportion of the eligible sample. All tests were two-sided. Continuous results are presented as regression coefficients or percentage differences with 95% CIs, and binary outcomes as PRs with 95% CIs. Analyses were conducted in R using the survey package for complex-survey estimation.

In a post-hoc exploratory metabolic sensitivity analysis, we examined the association of serum BChE activity with continuous CAP after additional adjustment for HbA1c, total cholesterol, and HDL-C. The supportive CAP ≥ 274 dB/m outcome was reassessed using survey-weighted Poisson regression with a log link and design-based robust variance. To distinguish changes in coefficient estimates attributable to additional covariate adjustment from those associated with changes in analytic sample composition, the original and metabolically expanded models were compared within identical complete-case samples. Additional models incorporating HbA1c alone or total cholesterol and HDL-C together were examined to characterize the attenuation of the association. All models retained the original BChE standardization and NHANES survey-design specification. The expanded model included the original primary covariates, with HbA1c and both cholesterol measures added. Changes in the BChE coefficient were summarized descriptively and were not interpreted as mediation effects or causal estimates. Single-coefficient

confidence intervals and P values were calculated using design-based standard errors and the 15 degrees of freedom of the full survey design. These analyses were exploratory and did not replace the primary models.

In an additional post-hoc exploratory analysis, we examined the conditional associations of serum BChE enzymatic activity and circulating BChE concentration with continuous CAP. Four survey-weighted linear regression models were fitted using an identical complete-case sample: the original covariate model without either BChE measure (M0), the covariate model with BChE activity (M1), the covariate model with BChE concentration (M2), and a joint model including both measures (M3). Both exposures were standardized using their original survey-weighted standard deviations. Changes in weighted in-sample R^2 were calculated descriptively to assess the additional model fit associated with each measure. These analyses were post-hoc and did not modify the primary analytical model. Because the joint model included 18 regression parameters while the survey design provided 15 degrees of freedom, coefficient-level inference was considered exploratory. Taylor-linearized estimates were compared with stratified jackknife variance estimates, and additional sensitivity analyses assessed the influence of extreme exposure values and individual survey strata.

1. **2.5 Subgroup and Effect-Modification Analyses** – In additional post-hoc analyses, to evaluate whether the association between BChE activity and continuous CAP differed across clinically relevant subgroups, the primary survey-weighted model was fitted within strata defined by sex, age (20–39, 40–59, and ≥ 60 years), obesity status (BMI < 30 vs ≥ 30 kg/m²), and diabetes status, with hypertension examined as an additional cardiometabolic subgroup. Each stratum was analyzed as a domain of the full survey design, and the stratifying variable was removed from the adjustment set; age was modeled linearly within age strata, and continuous BMI was retained within obesity strata. Effect modification was tested in the full primary analytic sample by adding to the primary model a product term between standardized BChE activity and each modifier, together with the modifier's main effect when it was not already represented. Interactions with BMI (per 5 kg/m²) and age (per 10 years) were also examined as continuous terms. P values for interaction were obtained from design-based Wald tests, using F statistics with 15 denominator degrees of freedom for modifiers with more than two categories. Because continuous CAP was the primary outcome, interaction on this scale was considered the principal assessment. For CAP ≥ 274 dB/m, interaction was examined on both the multiplicative scale (prevalence ratios from survey-weighted Poisson regression) and the additive scale (prevalence differences from survey-weighted linear probability models), because heterogeneity on a single scale can arise from differences in baseline prevalence alone.[16] BMI categories (< 25, 25–29.9, and ≥ 30 kg/m²) and race/ethnicity were examined in supplementary analyses. Given the number of comparisons, subgroup and interaction analyses were interpreted as exploratory.
2. **2.6 Incremental Predictive Value** – A clinical model including age, sex, BMI, diabetes, and hypertension was compared with the same model additionally including BChE activity (per 1 survey-weighted SD) for the identification of CAP ≥ 274 dB/m. Age and BMI were modeled with natural cubic splines (3 degrees of freedom), and both models were fitted with survey-weighted logistic

regression in the primary analytic sample. Performance measures were survey weighted so that they refer to the target population.[17] Discrimination was quantified with the weighted area under the receiver operating characteristic curve (AUC), defined as the weighted probability that a participant with steatosis had a higher predicted probability than a participant without steatosis. Calibration was assessed with calibration-in-the-large and the calibration slope, calibration plots comparing the mean predicted probability with the weighted observed prevalence across deciles of predicted risk (with weighted loess smoothing), the integrated calibration index (ICI),[18] and the Archer–Lemeshow F-adjusted goodness-of-fit test for complex survey data.[19] Overall performance was summarized with the Brier score, and incremental performance with the change in AUC (ΔAUC), the category-free net reclassification improvement (NRI), and the integrated discrimination improvement (IDI).[20, 21] Design-based 95% CIs and P values for each measure and for between-model differences were obtained by refitting both models within each of 30 stratified jackknife replicate weights, with t-based inference on 15 degrees of freedom. Optimism was estimated with 1,000 bootstrap resamples in which both models were refitted and evaluated in the original sample.[22] Clinical usefulness was explored with decision-curve analysis of weighted net benefit.[23] For continuous CAP, the change in weighted R^2 was examined analogously.

Sensitivity analyses of incremental predictive value used linear terms for age and BMI; an extended clinical model additionally including race/ethnicity, smoking, and alcohol use; the alternative CAP thresholds and MASLD phenotype described below; all participants with complete clinical-model data (N = 4,883); and unweighted models compared using DeLong's test, interpreted cautiously for nested models.[24, 25] To provide a reference for the magnitude of any improvement, the increment from adding log-transformed alanine aminotransferase (ALT), a routinely available liver test, was also estimated, as was the increment from BChE activity beyond the clinical model plus ALT.

2.7 Alternative Steatosis Definitions and MASLD Phenotype – The supportive binary analysis was repeated using alternative literature-based CAP thresholds: 248 dB/m (optimal cut-off for S ≥ S1 in an individual patient data meta-analysis),[26] 285 dB/m (cut-off optimizing sensitivity and specificity for S ≥ S1 in the NASH Clinical Research Network cohort),[27] 302 dB/m (Youden-optimal cut-off for S ≥ S1), and 331 dB/m (Youden-optimal cut-off for S ≥ S2, representing moderate-to-severe steatosis).[12] For each definition, the primary adjusted model was used to estimate prevalence ratios per 1 survey-weighted SD and for the highest versus lowest BChE quartile. Steatosis grade (S0, < 248; S1, 248–267; S2, 268–279; and S3, ≥ 280 dB/m)[26] was also analyzed as an ordinal outcome using survey-weighted proportional-odds regression.

A metabolic dysfunction-associated steatotic liver disease (MASLD) phenotype was constructed according to the 2023 multisociety Delphi consensus as CAP-defined steatosis with at least one cardiometabolic risk factor and alcohol intake below the MetALD range (< 140 g/week for women and < 210 g/week for men).[28] With the available variables, cardiometabolic criteria were operationalized as (1) BMI ≥ 25 kg/m² (≥ 23 kg/m² for non-Hispanic Asian participants) or waist circumference > 94 cm in men (> 90 cm in non-Hispanic Asian men) or > 80 cm in women; (2) self-reported diabetes or

prediabetes, current insulin or oral glucose-lowering medication, or serum glucose ≥ 200 mg/dL; (3) self-reported hypertension or current antihypertensive medication; and (4) serum triglycerides ≥ 150 mg/dL or current lipid-lowering medication. Average weekly alcohol intake was derived from past-year drinking frequency (ALQ121) and usual number of drinks per drinking day (ALQ130), assuming 14 g of ethanol per standard drink. The primary MASLD analysis used CAP ≥ 274 dB/m and compared participants with MASLD with those without steatosis; participants with steatosis who did not meet MASLD criteria (no cardiometabolic risk factor, alcohol intake in the MetALD range or higher, or unclassifiable alcohol intake) were excluded from this comparison. Sensitivity analyses applied the alternative CAP thresholds, compared MASLD with all other participants, and required at least two cardiometabolic risk factors. These additional analyses were conducted in R version 4.3.3 using the survey (version 4.2-1) and pROC packages.

3. RESULTS

3.1 Study Population and Participant Characteristics – Among 5,792 adults aged ≥ 20 years with a positive phlebotomy survey weight, 5,605 had available serum BChE activity measurements and 5,071 completed the liver elastography examination. A total of 4,914 participants had BChE activity together with valid CAP and LSM measurements. The age-, sex-, and race/ethnicity-adjusted analysis therefore included 4,914 participants, while 4,298 participants had complete data for the primary multivariable model and 3,787 for the expanded socioeconomic model.

Participant characteristics across survey-weighted BChE activity quartiles are summarized in Table 1. Mean BChE activity increased from 2.87 U/mL in Q1 to 5.12 U/mL in Q4, while mean BChE concentration increased from 45.2 to 77.4 ng/mL. Higher BChE quartiles were characterized by progressively higher BMI, waist circumference, and CAP. Mean CAP increased from 234.6 dB/m in Q1 to 286.6 dB/m in Q4, and the weighted prevalence of CAP ≥ 274 dB/m increased from 21.5% to 58.2%. In contrast, differences in LSM across BChE quartiles were comparatively modest: mean LSM ranged from 5.96 kPa in Q1 to 6.32 kPa in Q4, while the prevalence of LSM ≥ 12 kPa remained approximately 4% across quartiles.

Table 1
Survey-Weighted Characteristics of U.S. Adults According to Serum Butyrylcholinesterase Activity Quartile, NHANES August 2021–August 2023

Characteristic	Overall	Q1	Q2	Q3	Q4
Unweighted N	**4,298**	**1,167**	**1,088**	**1,072**	**971**
Age, years	48.3	48.1	48.7	48.8	47.6
Family poverty-income ratio	3.2	3.1	3.2	3.1	3.2
Body mass index, kg/m²	29.4	26.9	29.4	30.0	31.3
Waist circumference, cm	99.7	92.8	99.4	101.5	105.2
BChE activity, U/mL	3.94	2.87	3.62	4.16	5.12
BChE concentration, ng/mL	60.7	45.2	56.3	64.2	77.4
Albumin, g/dL	4.12	4.07	4.11	4.13	4.17
ALT, U/L	23.1	18.8	21.5	23.5	28.7
AST, U/L	22.8	22.7	21.9	22.3	24.3
GGT, U/L	30.0	26.0	25.6	30.6	37.9
CAP, dB/m	262.2	234.6	256.7	271.5	286.6
LSM, kPa	6.12	5.96	6.03	6.16	6.32
Female, %	49.5%	61.2%	50.2%	45.6%	40.6%
Race/ethnicity: Mexican American, %	6.7%	4.3%	5.6%	8.2%	8.6%
Race/ethnicity: Other Hispanic, %	9.2%	8.9%	9.1%	9.3%	9.6%
Race/ethnicity: Non-Hispanic White, %	62.7%	60.4%	62.2%	64.1%	64.1%
Race/ethnicity: Non-Hispanic Black, %	10.2%	14.9%	11.5%	7.3%	7.1%
Race/ethnicity: Non-Hispanic Asian, %	5.4%	4.5%	5.7%	5.1%	6.1%
Race/ethnicity: Other/multiracial, %	5.8%	7.0%	5.9%	6.0%	4.4%
Education > high school, %	66.8%	67.5%	69.6%	64.6%	65.6%
Current smoking, %	14.2%	15.0%	13.7%	13.2%	15.0%
Past-year alcohol use, %	79.5%	81.3%	77.2%	77.4%	81.9%
Diabetes, %	10.2%	7.4%	9.8%	10.8%	12.8%
Hypertension, %	29.1%	25.9%	26.9%	29.6%	34.0%
CAP ≥ 274 dB/m, %	40.4%	21.5%	36.5%	45.9%	58.2%

Characteristic	Overall	Q1	Q2	Q3	Q4
Unweighted N	**4,298**	**1,167**	**1,088**	**1,072**	**971**
LSM ≥ 8 kPa, %	11.4%	9.4%	11.3%	12.1%	13.0%
LSM ≥ 12 kPa, %	4.3%	4.3%	4.7%	4.2%	4.1%

Values are presented as survey-weighted means or percentages unless otherwise indicated. The primary analytic sample included 4,298 participants (Q1: 1,167; Q2: 1,088; Q3: 1,072; Q4: 971). Descriptive estimates were calculated using available nonmissing observations. Variable-specific available sample sizes were 3,787 for family poverty-income ratio, 4,233 for waist circumference, 4,297 for BChE concentration, 4,209 for albumin, 4,178 for ALT, 4,169 for AST, and 4,181 for GGT. Family poverty-income ratio was not required for inclusion in the primary adjusted model. BChE activity quartiles were defined using survey-weighted cutpoints of 3.33, 3.87, and 4.47 U/mL. BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

3.2 Association of BChE With Controlled Attenuation Parameter and Hepatic Steatosis – In the age-, sex-, and race/ethnicity-adjusted model, each 1 survey-weighted SD (0.8988 U/mL) higher BChE activity was associated with 18.82 dB/m higher CAP (95% CI, 16.16–21.49; P < 0.001). Adjustment for BMI, diabetes, hypertension, smoking, and alcohol use attenuated the magnitude of the association but it remained strong: each 1-SD higher BChE activity was associated with 8.90 dB/m higher CAP (95% CI, 6.74–11.07; P < 0.001). Further adjustment for educational attainment and family poverty-income ratio produced a nearly identical estimate of 8.81 dB/m (95% CI, 6.50–11.13; P < 0.001) (Table 2).

The categorical steatosis analysis yielded concordant findings. Each 1-SD higher BChE activity was associated with a 19% higher prevalence of CAP ≥ 274 dB/m in both the primary adjusted model (PR, 1.19; 95% CI, 1.14–1.23; P < 0.001) and the expanded socioeconomic model (PR, 1.19; 95% CI, 1.14–1.25; P < 0.001). A graded pattern was also observed across BChE activity quartiles. Compared with Q1, adjusted CAP was higher by 6.47 dB/m (95% CI, 3.81–9.13) in Q2, 16.37 dB/m (95% CI, 10.65–22.09) in Q3, and 23.90 dB/m (95% CI, 19.37–28.44) in Q4, with a strong linear trend across quartiles (P for trend < 0.001) (Table 2; Fig. 1).

The complementary analysis using serum BChE concentration produced a similar pattern. Each 1 weighted-SD higher BChE concentration was associated with 7.31 dB/m higher CAP (95% CI, 5.44–9.17; P < 0.001) in the primary model and 7.20 dB/m higher CAP (95% CI, 5.30–9.09; P < 0.001) after additional socioeconomic adjustment.

Table 2
Survey-Weighted Associations of Serum Butyrylcholinesterase With Controlled Attenuation Parameter and Hepatic Steatosis

Exposure / contrast	Model	Outcome	Estimate (95% CI)	P value
BChE activity per 1 weighted SD	Age/sex/race adjusted	Continuous CAP	18.82 (16.16–21.49)	< 0.001
BChE activity per 1 weighted SD	Primary adjusted	Continuous CAP	8.90 (6.74–11.07)	< 0.001
BChE activity per 1 weighted SD	Expanded socioeconomic	Continuous CAP	8.81 (6.50–11.13)	< 0.001
BChE activity per 1 weighted SD	Primary adjusted	CAP ≥ 274 dB/m	1.19 (1.14–1.23)	< 0.001
BChE activity per 1 weighted SD	Expanded socioeconomic	CAP ≥ 274 dB/m	1.19 (1.14–1.25)	< 0.001
BChE activity Q1	Primary adjusted	Continuous CAP	0 (reference)	—
BChE activity Q2 vs Q1	Primary adjusted	Continuous CAP	6.47 (3.81–9.13)	< 0.001
BChE activity Q3 vs Q1	Primary adjusted	Continuous CAP	16.37 (10.65–22.09)	< 0.001
BChE activity Q4 vs Q1	Primary adjusted	Continuous CAP	23.90 (19.37–28.44)	< 0.001
BChE activity, per one-quartile increment	Primary adjusted	Continuous CAP	8.17 (6.49–9.84)	< 0.001
BChE concentration per 1 weighted SD	Primary adjusted	Continuous CAP	7.31 (5.44–9.17)	< 0.001
BChE concentration per 1 weighted SD	Expanded socioeconomic	Continuous CAP	7.20 (5.30–9.09)	< 0.001

For continuous CAP, estimates are adjusted mean differences in dB/m. For CAP ≥ 274 dB/m, estimates are prevalence ratios (PRs). The primary model adjusted for age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking, and alcohol use; the expanded socioeconomic model additionally adjusted for education and family poverty-income ratio. One survey-weighted SD corresponded to 0.8988 U/mL for BChE activity and 15.0717 ng/mL for BChE concentration. Q1 served as the reference quartile. CI, confidence interval; CAP, controlled attenuation parameter; BChE, butyrylcholinesterase.

Adjusted differences in controlled attenuation parameter (CAP) are shown across survey-weighted serum butyrylcholinesterase activity quartiles, with Q1 as the reference. The model was adjusted for age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking status, and alcohol use. P for trend < 0.001.

3.3 BChE and Liver Stiffness – In contrast to the CAP findings, BChE activity showed no statistically significant association with liver stiffness in the linear or threshold-based models after multivariable adjustment. Each 1-SD higher BChE activity was associated with a − 1.90% difference in LSM in the primary adjusted model (95% CI, − 4.51% to 0.79%; P = 0.152) and a − 1.27% difference in the expanded socioeconomic model (95% CI, − 4.35% to 1.91%; P = 0.403). Similarly, no significant associations were observed with LSM ≥ 8 kPa (primary-model PR, 0.98; 95% CI, 0.88–1.09; P = 0.714) or LSM ≥ 12 kPa (PR, 0.84; 95% CI, 0.63–1.13; P = 0.233). These associations also remained statistically nonsignificant after additional socioeconomic adjustment.

Among participants with CAP ≥ 274 dB/m, BChE activity was also not associated with LSM ≥ 8 kPa. The primary adjusted PR was 1.00 (95% CI, 0.85–1.17; P = 0.972), with a similar estimate in the expanded model (PR, 1.02; 95% CI, 0.89–1.17; P = 0.803). These findings contrasted with the consistently positive associations observed for CAP.

Exploratory cross-adjustment analyses were performed to examine the association of BChE activity with each hepatic measure conditional on the other. After additional adjustment for log2-transformed LSM, each 1-SD higher BChE activity remained associated with 9.22 dB/m higher CAP (95% CI, 7.24–11.20; P < 0.001). Conversely, after additional adjustment for CAP, BChE activity was associated with a modest − 2.90% difference in LSM (95% CI, − 5.34% to − 0.39%; P = 0.027). Because these cross-adjusted models condition on related hepatic phenotypes, the inverse LSM association was interpreted as an exploratory conditional finding rather than evidence of a protective association with fibrosis.

3.4 Dose–Response Shape and Robustness Analyses – Restricted cubic spline analysis demonstrated a strong overall association between BChE activity and CAP (Fig. 2; P for overall association < 0.001), with evidence of departure from linearity in the full primary complete-case sample (P for nonlinearity = 0.0046). However, evidence for nonlinearity was attenuated after restricting the exposure distribution to the weighted 1st–99th percentiles (P for nonlinearity = 0.077) and 5th–95th percentiles (P = 0.062), whereas the overall association remained highly significant in both restricted analyses. Thus, the data supported a robust positive association across the BChE distribution more strongly than they supported a specific nonlinear threshold pattern.

For liver stiffness, restricted cubic spline analysis provided evidence of an overall association with a nonlinear component (P for overall association = 0.006; P for nonlinearity = 0.009). The spline curve suggested a nonmonotonic pattern, but confidence intervals widened at the extremes of BChE activity, and the linear and threshold-based LSM models did not show statistically significant associations. Accordingly, this finding was considered exploratory (Supplementary Table S2; Supplementary Figure S2).

The solid line represents the adjusted difference in CAP and the shaded region represents the 95% CI. The survey-weighted median BChE activity (3.87 U/mL) was used as the reference. Knots were positioned at the survey-weighted 5th, 35th, 65th, and 95th percentiles. The model was adjusted for age, sex, race/ethnicity,

body mass index, diabetes, hypertension, smoking status, and alcohol use. P for overall association < 0.001; P for nonlinearity = 0.0046.

The primary association was also stable across multiple sensitivity analyses. Replacing BMI with waist circumference yielded an adjusted CAP difference of 7.84 dB/m per 1-SD higher BChE activity (95% CI, 6.27–9.41; P < 0.001). Omitting alcohol use from the adjustment set increased the analytic sample to 4,878 participants and produced an estimate of 9.17 dB/m (95% CI, 7.22–11.12; P < 0.001), while a more granular alcohol adjustment based on drinking frequency and average drinks per drinking day yielded an almost identical estimate of 9.17 dB/m (95% CI, 6.82–11.52; P < 0.001; N = 4,275).

The association was similarly preserved after restricting the analysis according to liver stiffness. Among participants with LSM < 8 kPa, each 1-SD higher BChE activity was associated with 8.87 dB/m higher CAP (95% CI, 6.65–11.09; P < 0.001; N = 3,768); among those with LSM < 12 kPa, the corresponding estimate was 8.94 dB/m (95% CI, 6.94–10.95; P < 0.001; N = 4,101).

Missingness was concentrated primarily in alcohol use (12.1%) and family poverty-income ratio (12.9%), whereas missingness for the remaining primary covariates was ≤ 0.6%. Participants included in and excluded from the primary complete-case model had similar weighted mean BChE activity (3.94 vs 3.94 U/mL; P = 0.998), CAP (262.2 vs 263.1 dB/m; P = 0.755), LSM (6.12 vs 5.91 kPa; P = 0.388), BMI, and age. The proportion of women was higher among excluded participants (56.3% vs 49.5%; P = 0.034). The BChE–CAP estimate remained similar in the larger-sample analysis that omitted alcohol from the adjustment set. However, because this analysis also changed the covariate specification, it cannot independently exclude bias associated with complete-case selection.

Table 3
Associations With Liver Stiffness and Robustness Analyses of the BChE–CAP Association

Analysis / sample	Outcome	Estimate (95% CI)	P value	N
Primary adjusted	log2 LSM	-1.90% (-4.51% to 0.79%)	0.152	4,298
Expanded socioeconomic	log2 LSM	-1.27% (-4.35% to 1.91%)	0.403	3,787
Primary adjusted	LSM ≥ 8 kPa	PR 0.98 (0.88–1.09)	0.714	4,298
Expanded socioeconomic	LSM ≥ 8 kPa	PR 1.02 (0.92–1.13)	0.755	3,787
Primary adjusted	LSM ≥ 12 kPa	PR 0.84 (0.63–1.13)	0.233	4,298
Expanded socioeconomic	LSM ≥ 12 kPa	PR 0.89 (0.63–1.24)	0.450	3,787
Primary adjusted	LSM ≥ 8 among CAP ≥ 274	PR 1.00 (0.85–1.17)	0.972	1,745
Expanded socioeconomic	LSM ≥ 8 among CAP ≥ 274	PR 1.02 (0.89–1.17)	0.803	1,529
CAP additionally adjusted for log2 LSM	CAP beta, dB/m	9.22 dB/m (7.24–11.20)	< 0.001	4,298
log2 LSM additionally adjusted for CAP	Percent LSM difference	-2.90% (-5.34% to -0.39%)	0.027	4,298
Waist-circumference sensitivity	Continuous CAP	7.84 dB/m (6.27–9.41)	< 0.001	4,244
Primary model without alcohol	Continuous CAP	9.17 dB/m (7.22–11.12)	< 0.001	4,878
Granular alcohol adjustment	Continuous CAP	9.17 dB/m (6.82–11.52)	< 0.001	4,275
Restricted to LSM < 8 kPa	Continuous CAP	8.87 dB/m (6.65–11.09)	< 0.001	3,768
Restricted to LSM < 12 kPa	Continuous CAP	8.94 dB/m (6.94–10.95)	< 0.001	4,101

Continuous LSM was log2-transformed; estimates are presented as the percentage difference in LSM per 1-SD higher BChE activity. Binary LSM outcomes are reported as prevalence ratios. CAP sensitivity estimates are adjusted mean differences in dB/m. Cross-adjusted CAP and LSM models are phenotypic-specificity analyses and should not be interpreted as mediation or causal models. Primary adjusted model: age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking, and alcohol use. Expanded socioeconomic model additionally included education and family poverty-income ratio. CI, confidence interval; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; PR, prevalence ratio; BChE, butyrylcholinesterase.

3.5 Post-hoc Metabolic Sensitivity Analysis – In the additional exploratory analysis, 4,218 of the 4,298 participants in the primary complete-case sample had available HbA1c, total cholesterol, and HDL-C measurements. Within this identical analytic sample, the original primary adjustment model yielded a CAP difference of 8.88 dB/m per 1-SD higher BChE activity (95% CI, 6.85–10.91; P < 0.001). After further adjustment for HbA1c, total cholesterol, and HDL-C, the association was attenuated but remained statistically significant (adjusted difference, 5.95 dB/m; 95% CI, 3.49–8.42; P < 0.001), corresponding to a descriptive 33.0% reduction in the BChE regression coefficient.

A similar pattern was observed for the supportive CAP ≥ 274 dB/m outcome. In the same 4,218 participants, the prevalence ratio decreased from 1.18 (95% CI, 1.14–1.23) in the original adjustment model to 1.12 (95% CI, 1.08–1.16; P < 0.001) after additional metabolic adjustment. Models separately incorporating HbA1c or the two cholesterol measures showed attenuation of varying magnitude, with greater attenuation observed following adjustment for total cholesterol and HDL-C than following adjustment for HbA1c alone (Supplementary Table S9). These post-hoc findings indicate that the positive BChE–CAP association persisted under the expanded covariate specification, although its estimated magnitude was reduced. The coefficient changes were descriptive and were not interpreted as evidence of mediation or causal independence.

1. **3.6 Post-hoc Joint Analysis of BChE Activity and Concentration** – In a matched sample of 4,297 participants with complete information on both BChE measures and the original adjustment covariates, higher BChE activity and concentration were separately associated with higher CAP. The adjusted CAP differences per 1 survey-weighted SD were 8.91 dB/m (95% CI, 6.75–11.07) for activity and 7.31 dB/m (95% CI, 5.44–9.17) for concentration. When both measures were included simultaneously, the association for BChE activity remained positive (β = 8.70 dB/m; 95% CI, 4.45–12.95), whereas the conditional association for concentration was close to zero (β = 0.25 dB/m; 95% CI, − 3.22 to 3.73). Adding activity to a model already containing concentration increased weighted in-sample R^2 by 0.00578, compared with an increase of 0.000005 when concentration was added to the activity model. Taylor-linearized and jackknife standard errors were similar, and the qualitative pattern persisted after restricting both exposure distributions to their weighted 1st–99th percentile ranges. These post-hoc findings suggest a stronger conditional association for enzymatic activity than concentration in the fitted models, without establishing incremental clinical utility (Supplementary Table S10).
2. **3.7 Subgroup and Interaction Analyses** – The positive association between BChE activity and CAP was present in every subgroup examined (Fig. 3). Per 1-SD higher BChE activity, adjusted CAP was higher by 7.56 dB/m (95% CI, 4.77–10.35) in men and 9.97 dB/m (95% CI, 7.10–12.85) in women (P for interaction = 0.825); by 7.32, 10.71, and 8.18 dB/m among participants aged 20–39, 40–59, and ≥ 60 years, respectively (P for interaction = 0.274); by 8.87 dB/m (95% CI, 6.35–11.39) and 7.21 dB/m (95% CI, 3.76–10.67) among participants without and with obesity (P for interaction = 0.640); and by 8.68 dB/m (95% CI, 6.67–10.69) and 8.88 dB/m (95% CI, 2.65–15.10) among participants without and with diabetes (P for interaction = 0.653). There was likewise no evidence of effect modification by hypertension (P = 0.390), by BMI modeled continuously (interaction coefficient per 5

kg/m², − 0.02 dB/m; 95% CI, − 1.13 to 1.08; P = 0.965), or by age modeled continuously (per 10 years, 0.15 dB/m; 95% CI, − 0.83 to 1.13; P = 0.751). Race/ethnicity did not significantly modify the association (P = 0.062), although estimates in the smaller racial and ethnic groups were imprecise (Supplementary Table S11).

For CAP ≥ 274 dB/m, the prevalence ratio per 1-SD higher BChE activity was smaller among participants with obesity (PR, 1.08; 95% CI, 1.04–1.12) than among those without obesity (PR, 1.26; 95% CI, 1.18–1.34; P for multiplicative interaction < 0.001), with a corresponding interaction for continuous BMI (P < 0.001). However, the absolute prevalence differences were nearly identical (5.3 and 5.5 percentage points per 1 SD; P for additive interaction = 0.830), consistent with the much higher baseline prevalence of steatosis among participants with obesity (67.3% vs 23.2%) rather than a different BChE–CAP gradient. Conversely, on the additive scale, the prevalence difference was smaller among adults aged 20–39 years (2.4 percentage points; 95% CI, − 0.3 to 5.0) than in older age groups (P for interaction = 0.012), whose CAP distribution lay closer to the 274 dB/m threshold (weighted mean CAP, 248.1 dB/m at 20–39 years vs 272.1 and 267.9 dB/m in older groups); no age interaction was evident for continuous CAP or on the multiplicative scale (P = 0.479). A nominal multiplicative interaction with hypertension (P = 0.046) was not supported on the additive scale (P = 0.172) or for continuous CAP (Supplementary Table S11; Supplementary Figure S3).

Squares (diamond for all participants) represent adjusted mean differences in controlled attenuation parameter (CAP) per 1 survey-weighted SD (0.8988 U/mL) higher BChE activity, and horizontal lines represent 95% CIs; the dashed vertical line marks the overall estimate. Estimates were obtained from survey-weighted linear regression fitted within each subgroup as a survey domain and adjusted for age, sex, race/ethnicity, body mass index (BMI), diabetes, hypertension, smoking, and alcohol use, excluding the stratifying variable (age was modeled linearly within age strata). P values for interaction are design-based Wald tests of BChE × subgroup product terms in the full primary analytic sample (N = 4,298), with 15 survey degrees of freedom.

3.8 Incremental Predictive Value of BChE Activity – The clinical model discriminated CAP ≥ 274 dB/m with a weighted AUC of 0.820 (95% CI, 0.798–0.842). Adding BChE activity increased the AUC to 0.829 (95% CI, 0.809–0.849), corresponding to a ΔAUC of 0.009 (95% CI, 0.005–0.013; P < 0.001) (Table 4; Fig. 4A). Optimism-corrected AUCs were 0.818 and 0.827, respectively, and BChE activity remained independently associated with steatosis in the augmented model (odds ratio per 1 SD, 1.47; 95% CI, 1.37–1.58). Both models were well calibrated (Fig. 4B): optimism-corrected calibration slopes were 0.988 and 0.987, ICIs were 0.008 and 0.004, and the Archer–Lemeshow test did not indicate lack of fit (P = 0.333 and P = 0.405). The Brier score decreased from 0.169 to 0.165 (difference, − 0.004; 95% CI, − 0.006 to − 0.003), and the IDI was 0.018 (95% CI, 0.011–0.025). The category-free NRI was 0.31 (95% CI, 0.21–0.42), arising predominantly from participants without steatosis (0.23) rather than those with steatosis (0.09). In decision-curve analysis, the BChE-augmented model yielded slightly higher net benefit at most threshold probabilities between 0.20 and 0.70 (median gain, 0.006; maximum, 0.020)

(Fig. 4C). For continuous CAP, adding BChE activity increased the weighted R^2 from 0.392 to 0.412 (ΔR^2, 0.020; 95% CI, 0.008–0.032).

The incremental gain was similar with linear age and BMI terms (ΔAUC, 0.010), in an extended clinical model additionally including race/ethnicity, smoking, and alcohol use (ΔAUC, 0.007; 95% CI, 0.004–0.010), across alternative CAP thresholds (ΔAUC range, 0.006–0.011) and the MASLD phenotype (0.009), in the broader sample of 4,883 participants (0.009), and in unweighted analyses (AUC, 0.816 vs 0.826; DeLong $P < 0.001$) (Supplementary Table S12). For comparison, adding ALT to the clinical model increased the AUC by 0.011 (95% CI, 0.005–0.017), and BChE activity added 0.005 (95% CI, 0.003–0.008) beyond the clinical model plus ALT.

Table 4
Incremental Predictive Performance of Serum Butyrylcholinesterase Activity Beyond a Clinical Model for CAP-Defined Hepatic Steatosis

Measure	Clinical model	Clinical model + BChE	Difference (95% CI)	P value
Discrimination: CAP ≥ 274 dB/m				
Weighted AUC (95% CI)	0.820 (0.798–0.842)	0.829 (0.809–0.849)	0.009 (0.005–0.013)	< 0.001
Optimism-corrected AUC	0.818	0.827	0.009	—
Discrimination slope (difference = IDI)	0.300	0.318	0.018 (0.011–0.025)	< 0.001
Calibration				
Calibration-in-the-large, optimism-corrected	0.001	0.001	—	—
Calibration slope, optimism-corrected	0.988	0.987	—	—
Integrated calibration index	0.008	0.004	—	—
Archer–Lemeshow F test, P value	0.333	0.405	—	—
Overall performance and reclassification				
Brier score (95% CI)	0.169 (0.158–0.180)	0.165 (0.153–0.176)	−0.004 (− 0.006 to − 0.003)	< 0.001
Scaled Brier score, %	29.8	31.7	—	—
Category-free NRI	—	—	0.313 (0.209–0.418)	< 0.001
Participants with steatosis	—	—	0.087 (0.039–0.134)	0.001
Participants without steatosis	—	—	0.227 (0.131–0.322)	< 0.001
Continuous CAP (linear regression)				
Weighted R^2 (95% CI)	0.392 (0.357–0.426)	0.412 (0.381–0.443)	0.020 (0.008–0.032)	0.003
Optimism-corrected R^2	0.388	0.408	0.020	—
Root mean squared error, dB/m	48.7	47.9	−0.8	—

The clinical model included age and body mass index (natural cubic splines, 3 degrees of freedom each), sex, diabetes, and hypertension; the augmented model additionally included BChE activity per 1 survey-weighted SD (0.8988 U/mL). Models were fitted in the primary analytic sample (N = 4,298; 1,745 participants with CAP ≥ 274 dB/m; weighted prevalence, 40.4%) with survey-weighted logistic regression or, for continuous CAP, survey-weighted linear regression. All performance measures were survey weighted. 95% CIs and P values were derived from 30 stratified jackknife replicate weights (15 degrees of freedom), in which both models were refitted; optimism-corrected values were derived from 1,000 bootstrap resamples. Apparent calibration-in-the-large and slope equal 0 and 1 by construction for in-sample weighted maximum-likelihood fits and are therefore reported after optimism correction. The Archer–Lemeshow test used 10 weighted deciles of predicted risk (F with 9 and 7 degrees of freedom). Odds ratio for BChE activity in the augmented model, 1.47 (95% CI, 1.37–1.58). AUC, area under the receiver operating characteristic curve; BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; IDI, integrated discrimination improvement; NRI, net reclassification improvement.

1. **3.9 Alternative Definitions of Hepatic Steatosis and MASLD Phenotype** – The weighted prevalence of steatosis ranged from 55.9% with CAP ≥ 248 dB/m to 15.2% with CAP ≥ 331 dB/m (Table 5). Higher BChE activity was associated with a higher prevalence of steatosis under every definition, with PRs per 1 SD of 1.15 (95% CI, 1.12–1.18) at 248 dB/m, 1.21 (95% CI, 1.16–1.26) at 285 dB/m, 1.23 (95% CI, 1.15–1.30) at 302 dB/m, and 1.21 (95% CI, 1.13–1.30) at 331 dB/m, compared with 1.19 (95% CI, 1.14–1.23) for the primary threshold. Graded associations across BChE quartiles were observed for all thresholds (Q4 vs Q1 PR range, 1.65–2.08; all P for trend < 0.001), and higher BChE activity was associated with higher steatosis grade in ordinal analysis (cumulative odds ratio per 1 SD, 1.50; 95% CI, 1.38–1.62).

Of participants with CAP ≥ 274 dB/m, 98.8% (weighted) met at least one cardiometabolic criterion, almost entirely through the adiposity criterion (98.0%). The weighted prevalence of MASLD was 35.9%, with an additional 2.6% meeting MetALD criteria and 1.2% reporting alcohol intake above the MetALD range (Supplementary Table S13). Each 1-SD higher BChE activity was associated with a higher prevalence of MASLD compared with no steatosis (PR, 1.21; 95% CI, 1.15–1.26; Q4 vs Q1 PR, 1.99; 95% CI, 1.78–2.23). Results were similar when MASLD was defined using CAP thresholds of 248, 285, or 302 dB/m (PRs, 1.17–1.25), when MASLD was compared with all other participants (PR, 1.19; 95% CI, 1.14–1.23), and when at least two cardiometabolic risk factors were required (PR, 1.28; 95% CI, 1.21–1.36). Among participants with alcohol intake below the MetALD range (N = 3,907), each 1-SD higher BChE activity was associated with 9.54 dB/m (95% CI, 7.12–11.95) higher CAP.

Table 5
Associations of Serum Butyrylcholinesterase Activity With Hepatic Steatosis Under Alternative CAP Thresholds and a MASLD Phenotype

Definition	Rationale (source)	Cases/N	Weighted prevalence, %	PR per 1 SD (95% CI)	PR, Q4 vs Q1 (95% CI)	P for trend
CAP-defined steatosis						
CAP ≥ 248 dB/m	Optimal cut-off for S ≥ S1 (Karlas et al.[26])	2,401/4,298	55.9	1.15 (1.12–1.18)	1.65 (1.52–1.79)	< 0.001
CAP ≥ 274 dB/m (primary)	90% sensitivity for S ≥ S1 (Eddowes et al.[12])	1,745/4,298	40.4	1.19 (1.14–1.23)	1.87 (1.67–2.08)	< 0.001
CAP ≥ 285 dB/m	Optimized sensitivity and specificity for S ≥ S1 (Siddiqui et al.[27])	1,485/4,298	34.3	1.21 (1.16–1.26)	1.98 (1.77–2.21)	< 0.001
CAP ≥ 302 dB/m	Youden-optimal cut-off for S ≥ S1 (Eddowes et al.[12])	1,154/4,298	26.7	1.23 (1.15–1.30)	1.97 (1.63–2.38)	< 0.001
CAP ≥ 331 dB/m	Youden-optimal cut-off for S ≥ S2 (Eddowes et al.[12])	654/4,298	15.2	1.21 (1.13–1.30)	2.08 (1.57–2.74)	< 0.001
Steatosis grade S0–S3, ordinal	S1 248–267, S2 268–279, S3 ≥ 280 dB/m (Karlas et al.[26])	S1–S3: 2,401/4,298	55.9 (S1–S3)	cOR 1.50 (1.38–1.62)	—	—
MASLD phenotype (comparator: no steatosis unless stated)						
MASLD, CAP ≥ 274 dB/m (primary)	Steatosis + ≥ 1 cardiometabolic criterion + alcohol below MetALD range (Rinella et al.[28])	1,568/4,109	35.9	1.21 (1.15–1.26)	1.99 (1.78–2.23)	< 0.001
MASLD, CAP ≥ 248 dB/m	As above, alternative CAP threshold	2,126/4,013	49.0	1.17 (1.13–1.21)	1.77 (1.60–1.96)	< 0.001
MASLD, CAP ≥ 285 dB/m	As above, alternative CAP threshold	1,338/4,135	30.4	1.23 (1.17–1.29)	2.07 (1.82–2.36)	< 0.001
MASLD, CAP ≥ 302 dB/m	As above, alternative CAP	1,032/4,156	23.5	1.25 (1.17–	2.12 (1.72–	< 0.001

Definition	Rationale (source)	Cases/N	Weighted prevalence, %	PR per 1 SD (95% CI)	PR, Q4 vs Q1 (95% CI)	P for trend
CAP-defined steatosis						
	threshold			1.35)	2.60)	
MASLD vs all other participants	CAP ≥ 274 dB/m; comparator includes other steatotic liver disease	1,568/4,275	35.9	1.19 (1.14–1.23)	1.93 (1.73–2.16)	< 0.001
MASLD with ≥ 2 cardiometabolic criteria	CAP ≥ 274 dB/m; stricter metabolic phenotype	1,216/3,757	26.4	1.28 (1.21–1.36)	2.43 (2.12–2.79)	< 0.001

Prevalence ratios (PRs) were estimated with survey-weighted Poisson regression (log link) per 1 survey-weighted SD (0.8988 U/mL) higher BChE activity and for the highest vs lowest survey-weighted quartile, adjusted for age (natural spline), sex, race/ethnicity, body mass index, diabetes, hypertension, smoking, and alcohol use; P for trend used the quartile rank. The ordinal model was a survey-weighted proportional-odds model (cOR, cumulative odds ratio). Cases/N are unweighted counts; weighted prevalence refers to the primary analytic sample (for MASLD rows, the proportion of the primary analytic sample meeting the definition). MASLD analyses were restricted to participants with classifiable alcohol intake; except in the comparison with all other participants, participants with steatosis not meeting MASLD criteria were excluded. Cardiometabolic criteria were operationalized as described in the Methods and Supplementary Table S13. BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, MASLD with increased alcohol intake; Q, quartile.

4. DISCUSSION

In this survey-weighted cross-sectional analysis of U.S. adults participating in NHANES 2021–2023, higher serum BChE activity was consistently associated with higher CAP, whereas the adjusted linear and threshold-based LSM models did not yield statistically significant associations. Each 1 survey-weighted SD higher BChE activity was associated with approximately 8.9 dB/m higher CAP in the primary adjusted model, and the estimate was essentially unchanged after additional socioeconomic adjustment. The association was also evident when steatosis was defined categorically, with a 19% higher prevalence of CAP ≥ 274 dB/m per 1-SD higher BChE activity, and a clear graded increase in CAP across BChE quartiles. In contrast, BChE activity was not significantly associated with LSM in the linear model, with LSM ≥ 8 kPa or ≥ 12 kPa, or with elevated LSM among participants with steatosis, although exploratory spline analysis suggested a nonlinear relationship with continuous LSM. Taken together, these findings suggest that circulating BChE activity is more consistently associated with CAP-measured hepatic steatosis than with the linear and threshold-based LSM outcomes examined in this study. Additional analyses showed that the association was consistent across clinically relevant subgroups

and alternative steatosis definitions, including a MASLD phenotype, whereas adding BChE activity to established clinical risk factors produced only a small improvement in discrimination.

The positive association between BChE and hepatic steatosis is biologically plausible and consistent with prior clinical observations linking circulating BChE with metabolic dysfunction. BChE is synthesized predominantly by hepatocytes, but its circulating activity is influenced not only by hepatic synthetic capacity but also by obesity, insulin resistance, dyslipidemia, and other features of metabolic syndrome that are closely linked to hepatic lipid accumulation.[3, 5] Previous studies have reported higher BChE activity in individuals with uncomplicated fatty liver or greater liver fat, whereas lower activity may emerge in more advanced liver disease when impaired hepatic synthetic function becomes increasingly important.[3, 6, 7] More recent imaging-based evidence has also demonstrated positive associations between circulating BChE and quantitatively measured hepatic fat, supporting the concept that elevated BChE may accompany a metabolically active steatotic phenotype rather than simply serving as a nonspecific marker of liver dysfunction.[7]

The differing patterns of association observed for CAP and LSM warrant careful consideration when interpreting BChE in relation to distinct hepatic phenotypes. Although BChE activity showed a strong and graded association with CAP, the linear and threshold-based liver-stiffness analyses did not show a consistent association. Exploratory spline analysis suggested a nonmonotonic association with continuous LSM. Because confidence intervals widened at the extremes of BChE activity and the linear and threshold-based models did not show statistically significant associations, the clinical significance of this exploratory finding remains uncertain. Furthermore, among participants with CAP-defined steatosis, higher BChE activity was not associated with LSM ≥ 8 kPa. The CAP association also remained essentially unchanged after additional adjustment for log2-transformed LSM and after restricting analyses to participants with LSM < 8 or < 12 kPa. These findings indicate that the positive BChE–CAP association was not confined to participants with elevated liver stiffness, although they do not establish that the association is independent of underlying fibrotic liver disease. Conversely, BChE activity was modestly inversely associated with LSM after additional adjustment for CAP. This conditional estimate should be interpreted cautiously because CAP and LSM represent related hepatic phenotypes, and cross-adjustment may alter the underlying covariate structure or introduce overadjustment. We therefore do not interpret this secondary result as evidence that higher BChE protects against fibrosis. Rather, the overall pattern indicates a more consistent association of BChE activity with CAP than with the LSM outcomes examined, without establishing a formal statistical difference between these associations.

Several additional findings strengthen the consistency of the primary association. Although the association was statistically robust, the adjusted CAP difference of approximately 8.9 dB/m per 1-SD higher BChE activity does not, by itself, establish clinical utility. In the incremental-value analysis, BChE activity improved the identification of CAP-defined steatosis beyond established clinical risk factors only modestly, as discussed below. Serum BChE concentration showed a concordant positive association with CAP, suggesting that the finding was not unique to the enzymatic activity measure. The quartile analysis demonstrated a graded pattern, with progressively higher adjusted CAP across Q2, Q3, and Q4

compared with the lowest BChE quartile. Restricted cubic spline analysis also supported an overall BChE–CAP association; however, evidence of nonlinearity in the full sample weakened after restriction to the central 98% and 90% of the weighted exposure distribution. The overall BChE–CAP association persisted in both tail-restricted analyses, but the evidence was insufficient to establish a stable nonlinear shape or a clinically meaningful BChE threshold. Accordingly, BChE should not be interpreted from these data as having a defined diagnostic threshold for hepatic steatosis.

The exploratory joint analysis of BChE activity and concentration provides additional insight into the biochemical characterization of these associations. Although both measures were positively associated with CAP when examined separately, the activity coefficient remained positive after mutual adjustment, whereas concentration showed little additional linear association after accounting for activity. This pattern may indicate that the enzymatic activity measure captures variation relevant to CAP beyond circulating protein concentration. However, activity and concentration were strongly correlated, and their simultaneous inclusion increased coefficient uncertainty. Furthermore, adding enzymatic activity to a model already containing BChE concentration increased weighted in-sample R^2 by only 0.00578, corresponding to approximately 0.58 percentage points of additional explained variation. This improvement was not assessed using internal or external predictive validation and should not be interpreted as evidence of incremental clinical utility. Potential measurement error, residual confounding, and limitations of complex-survey inference also warrant caution. These findings should therefore be considered hypothesis-generating rather than evidence that enzymatic activity is biologically independent of concentration or clinically superior as a marker of hepatic steatosis.

The primary BChE–CAP association demonstrated consistency across several sensitivity analyses, although the additional post-hoc metabolic models revealed meaningful attenuation. Replacing BMI with waist circumference, modifying alcohol adjustment, and restricting the analysis to participants without elevated liver stiffness yielded estimates broadly comparable to the primary result. Furthermore, participants included in and excluded from the primary complete-case model had similar mean BChE activity, CAP, LSM, BMI, and age, although the proportion of women differed between groups. In the additional metabolic sensitivity analysis, further adjustment for HbA1c, total cholesterol, and HDL-C reduced the BChE coefficient from 8.88 to 5.95 dB/m within the same analytic sample, while the association remained statistically significant. This attenuation suggests that the observed association is sensitive to additional metabolic covariate adjustment and may reflect shared metabolic characteristics not fully represented in the primary model. However, because glycemic measures, circulating lipids, adiposity, and hepatic steatosis are biologically interconnected, changes in regression coefficients cannot establish how much of the association is attributable to confounding or mediation. The additional analysis therefore strengthens the characterization of the observed association without demonstrating causal or metabolic independence. Residual confounding, potential overadjustment, and selection bias remain possible.

The additional analyses address the consistency and potential clinical relevance of the BChE–CAP association. First, the association was similar in men and women, across age groups, and in participants

with and without obesity, diabetes, or hypertension, with no evidence that continuous BMI or age modified the BChE–CAP gradient. Apparent heterogeneity for the binary steatosis outcome was scale-dependent: the smaller prevalence ratio among participants with obesity reflected their high baseline prevalence, whereas absolute prevalence differences were nearly identical, and the smaller prevalence difference among younger adults reflected their lower CAP distribution relative to the threshold. These findings suggest that BChE activity tracks hepatic fat across metabolic strata rather than acting solely as a proxy for adiposity or diabetes. Second, the association was not an artifact of the 274 dB/m threshold: it persisted across sensitive and specific cut-offs for any steatosis, at the Youden-optimal cut-off for moderate-to-severe steatosis, for steatosis grade, and for a MASLD phenotype aligned with current nomenclature. Third, BChE activity added statistically significant but small discriminative information beyond age, sex, BMI, diabetes, and hypertension: the AUC increased by approximately 0.009 from an already high baseline of 0.82, calibration was preserved, and gains in Brier score, IDI, and net benefit were modest. The comparatively large category-free NRI arose mainly from participants without steatosis and should be interpreted cautiously, because this measure can be substantial even when improvements in discrimination are small.[29] The increment from BChE activity was similar in magnitude to that from ALT and only partly overlapping with it. Taken together, these results support BChE activity as a correlate of a hepatometabolic phenotype rather than as a standalone screening marker; whether it adds clinically meaningful information in combination with other biomarkers or in specific clinical settings requires external validation.

This study has several strengths. BChE activity and concentration were objectively measured, and both hepatic steatosis and liver stiffness were assessed using vibration-controlled transient elastography rather than self-reported liver disease or indirect surrogate indices. Using continuous CAP as the primary outcome preserved information across the full distribution and reduced dependence on a single steatosis threshold. The analysis incorporated the NHANES complex survey design and phlebotomy weights, evaluated BChE using continuous, categorical, and spline parameterizations, and distinguished hepatic steatosis from liver stiffness within the same population. The consistency of the association across activity and concentration measures, adiposity definitions, alcohol-adjustment strategies, missing-data analyses, and liver-stiffness restrictions provides additional support for the robustness of the observed BChE–CAP relationship.

Several limitations should also be considered. First, the cross-sectional design precludes establishing temporality or causality. Higher BChE may contribute to, result from, or simply accompany the metabolic processes associated with hepatic fat accumulation. Second, residual confounding remains possible despite adjustment for major demographic, metabolic, anthropometric, and behavioral factors. Although the post-hoc metabolic sensitivity models additionally accounted for HbA1c, total cholesterol, and HDL-C, residual confounding by dietary patterns, medication use, insulin resistance severity, inflammatory status, and other unmeasured or incompletely measured metabolic characteristics remains possible. Moreover, these metabolic variables are biologically intertwined with hepatic steatosis, and additional adjustment may introduce overadjustment. Consequently, the observed attenuation in the BChE coefficient cannot be interpreted as a quantified mediation effect or evidence of causal independence.

The post-hoc nature of these analyses and the limited survey design degrees of freedom also warrant caution when interpreting the expanded models. The exploratory joint activity–concentration model also included more regression parameters than the available survey design degrees of freedom, resulting in a rank-deficient design-based covariance matrix. Although single-coefficient estimates showed agreement between Taylor linearization and jackknife variance estimation, this limitation warrants caution in statistical inference and interpretation. Third, CAP and LSM are noninvasive surrogate measures and do not provide histologic confirmation of steatosis, steatohepatitis, or fibrosis stage. The CAP ≥ 274 dB/m and LSM thresholds used in supportive analyses should therefore be interpreted as operational imaging thresholds rather than definitive clinical diagnoses. The absence of statistically significant associations in the linear and threshold-based LSM models should not be interpreted as evidence of no relationship, particularly given the exploratory nonlinear spline finding and the relatively small proportion of participants with markedly elevated liver stiffness. Fourth, BChE and elastography measurements were obtained at a single examination and may not reflect long-term trajectories. Fifth, the MASLD phenotype was constructed with partially available cardiometabolic criteria: measured blood pressure, HbA1c, fasting glucose, and HDL-C were not incorporated, triglycerides and glucose were measured in predominantly nonfasting samples, alcohol intake was self-reported, and viral hepatitis and other potential causes of steatosis were not systematically excluded in the present analysis. Because nearly all participants with steatosis met the adiposity criterion, misclassification of cardiometabolic status is likely limited. Sixth, the predictive analyses were developed and evaluated in the same sample with internal bootstrap validation only, against an imaging-based reference standard that is itself an imperfect surrogate of histologic steatosis, so performance estimates may not transport to other populations or clinical settings. Seventh, the subgroup and interaction analyses involved multiple comparisons and had limited precision in smaller subgroups, such as participants with diabetes and some racial and ethnic groups, and should be regarded as exploratory. Finally, although NHANES weighting procedures address the complex sampling design and nonresponse, residual nonresponse and complete-case selection bias remain possible. The validity of complete-case estimates depends on assumptions regarding the missing-data mechanism that cannot be fully verified. Similarity between included and excluded participants on measured characteristics, together with consistency across sensitivity analyses, does not eliminate the possibility of selection bias.

Overall, the present findings demonstrate a consistent positive association between higher serum BChE activity and CAP-measured hepatic steatosis in this NHANES sample, whereas the linear and threshold-based LSM models did not yield statistically significant associations. This pattern suggests that BChE activity may be a correlate of CAP-measured hepatic fat accumulation, while its relationship with liver stiffness requires further investigation. Prospective studies incorporating repeated BChE measurements, longitudinal imaging, and more detailed metabolic and histologic characterization are needed to determine temporality, clarify the mechanisms underlying this association, and establish whether BChE provides information beyond established metabolic risk factors in the assessment of hepatic steatosis.

5. CONCLUSION

Among U.S. adults participating in NHANES 2021–2023, higher serum butyrylcholinesterase activity was consistently associated with greater CAP-measured hepatic steatosis after multivariable adjustment, whereas linear and threshold-based liver stiffness analyses did not demonstrate consistent associations. This association was consistent across sex, age, obesity, and diabetes subgroups and across alternative steatosis definitions, including a MASLD phenotype. The positive BChE–CAP association persisted, although attenuated, after additional post-hoc adjustment for glycemic and lipid measures. In an exploratory joint analysis, enzymatic activity retained a positive conditional association with CAP after accounting for circulating BChE concentration, whereas concentration contributed little additional in-sample explanatory information beyond activity. However, the incremental explained variation and the improvement in discrimination beyond established clinical risk factors were modest, and these findings do not establish biological independence, causality, or clinical utility. Longitudinal studies and external validation are needed to clarify the hepatometabolic significance of BChE activity and determine whether it provides clinically meaningful information beyond established metabolic risk factors.

Declarations

Disclosures:

None.

Ethics Approval and Consent to Participate –

The National Health and Nutrition Examination Survey (NHANES) protocols were reviewed and approved by the National Center for Health Statistics Research Ethics Review Board. Written informed consent was obtained from all participants in accordance with NHANES procedures. The present study used publicly available, deidentified data and involved no direct contact with participants; therefore, no additional institutional review board approval was required for this secondary analysis.

Consent for Publication –

Not applicable.

Competing Interests –

The authors declare that they have no competing interests.

Use of Artificial Intelligence

– During the preparation of this manuscript, the authors used ChatGPT 5.6 Sol (OpenAI) solely to assist with improving language and grammar. The authors reviewed and approved the final text and take full

responsibility for the content of the manuscript.

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AUTHORS CONTRIBUTIONS:

Abdulrhman H.M. Muhaisen: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Validation, Resources, Writing – original draft, Writing – review & editing, Project administration, and Software. Samah Rjoub: Methodology, Investigation, Validation, Resources, Supervision, and Writing – review & editing. Wejdan Y Alquran: Methodology, Investigation, Validation, Resources, Supervision, and Writing – review & editing. Elsayed Balbaa: Formal analysis, Visualization, and Writing – review & editing. All authors reviewed the manuscript and approved the final version for publication.

Data Availability:

All data analyzed in this study are publicly available from the National Center for Health Statistics through the NHANES August 2021–August 2023 public-use datasets. The datasets used in the present analysis include demographic, butyrylcholinesterase laboratory, liver ultrasound transient elastography, standard biochemistry, body-measurement, smoking, alcohol-use, diabetes, blood-pressure, education, and income data files.

Code Availability –

The statistical code used for the analyses is available from the corresponding author upon reasonable request.

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Figures

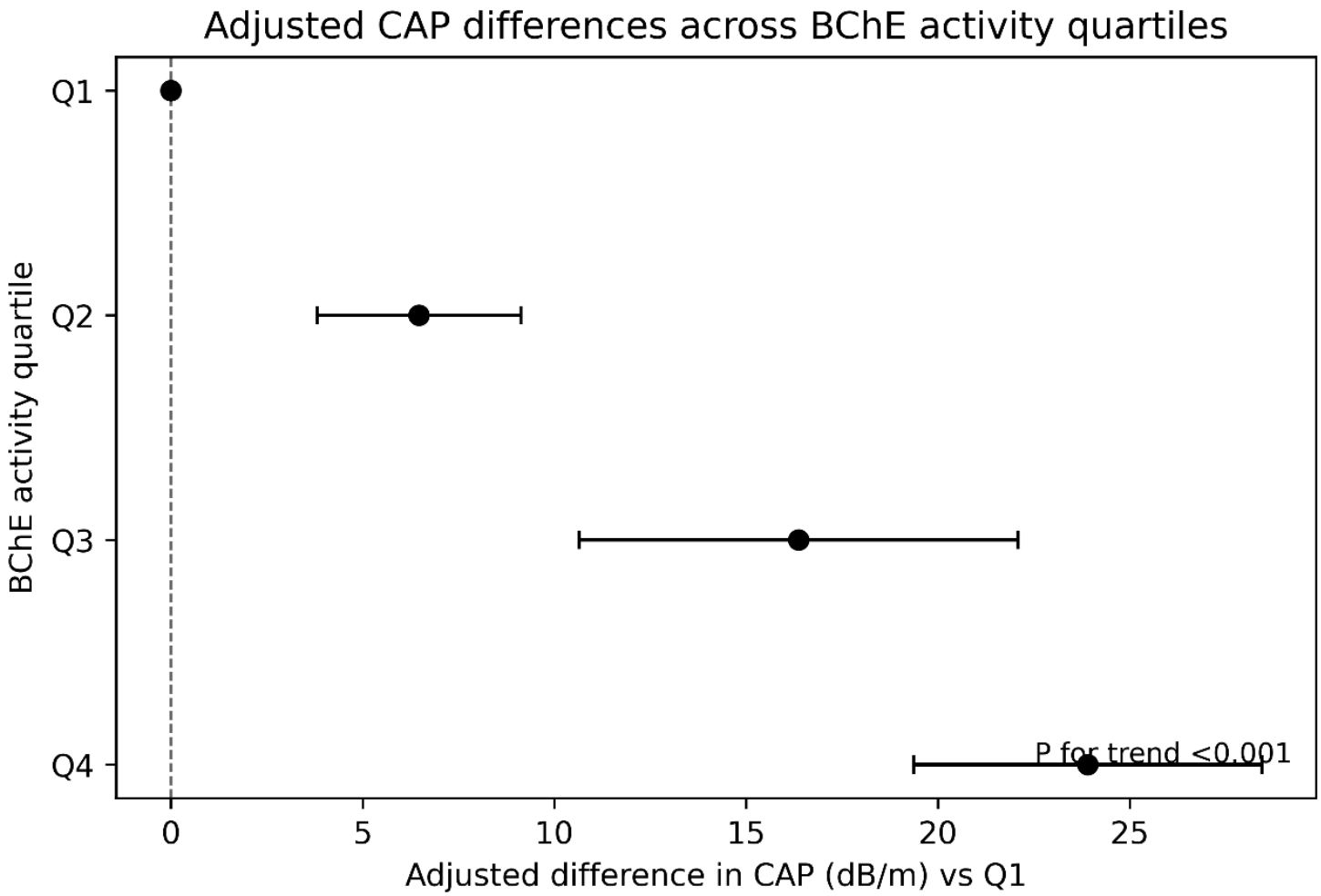


Figure 1

Adjusted Controlled Attenuation Parameter Across Serum Butyrylcholinesterase Activity Quartiles.

Adjusted differences in controlled attenuation parameter (CAP) are shown across survey-weighted serum butyrylcholinesterase activity quartiles, with Q1 as the reference. The model was adjusted for age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking status, and alcohol use. P for trend <0.001.

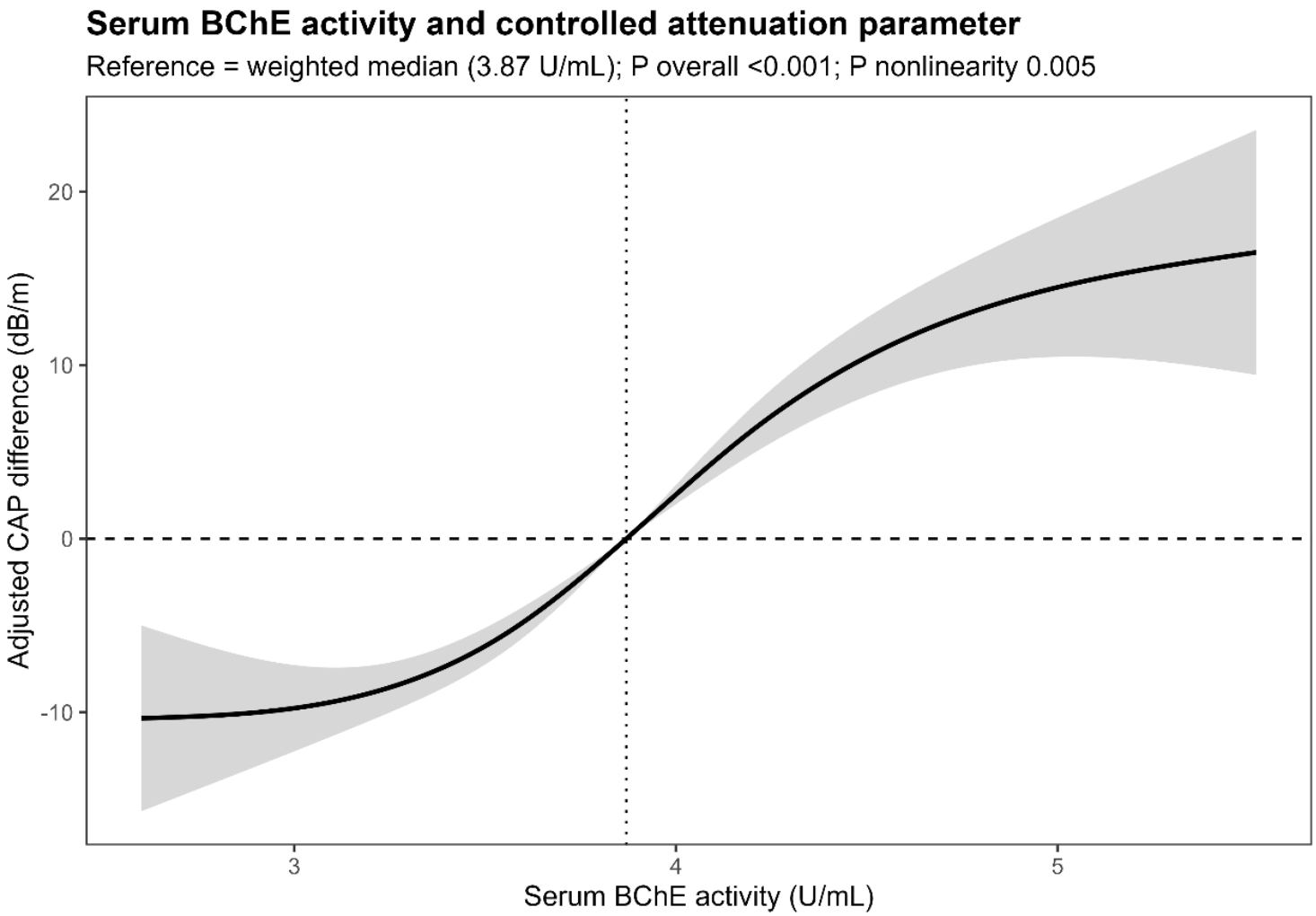


Figure 2

Restricted Cubic Spline Association Between Serum Butyrylcholinesterase Activity and Controlled Attenuation Parameter.

The solid line represents the adjusted difference in CAP and the shaded region represents the 95% CI. The survey-weighted median BChE activity (3.87 U/mL) was used as the reference. Knots were positioned at the survey-weighted 5th, 35th, 65th, and 95th percentiles. The model was adjusted for age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking status, and alcohol use. P for overall association <0.001; P for nonlinearity=0.0046.

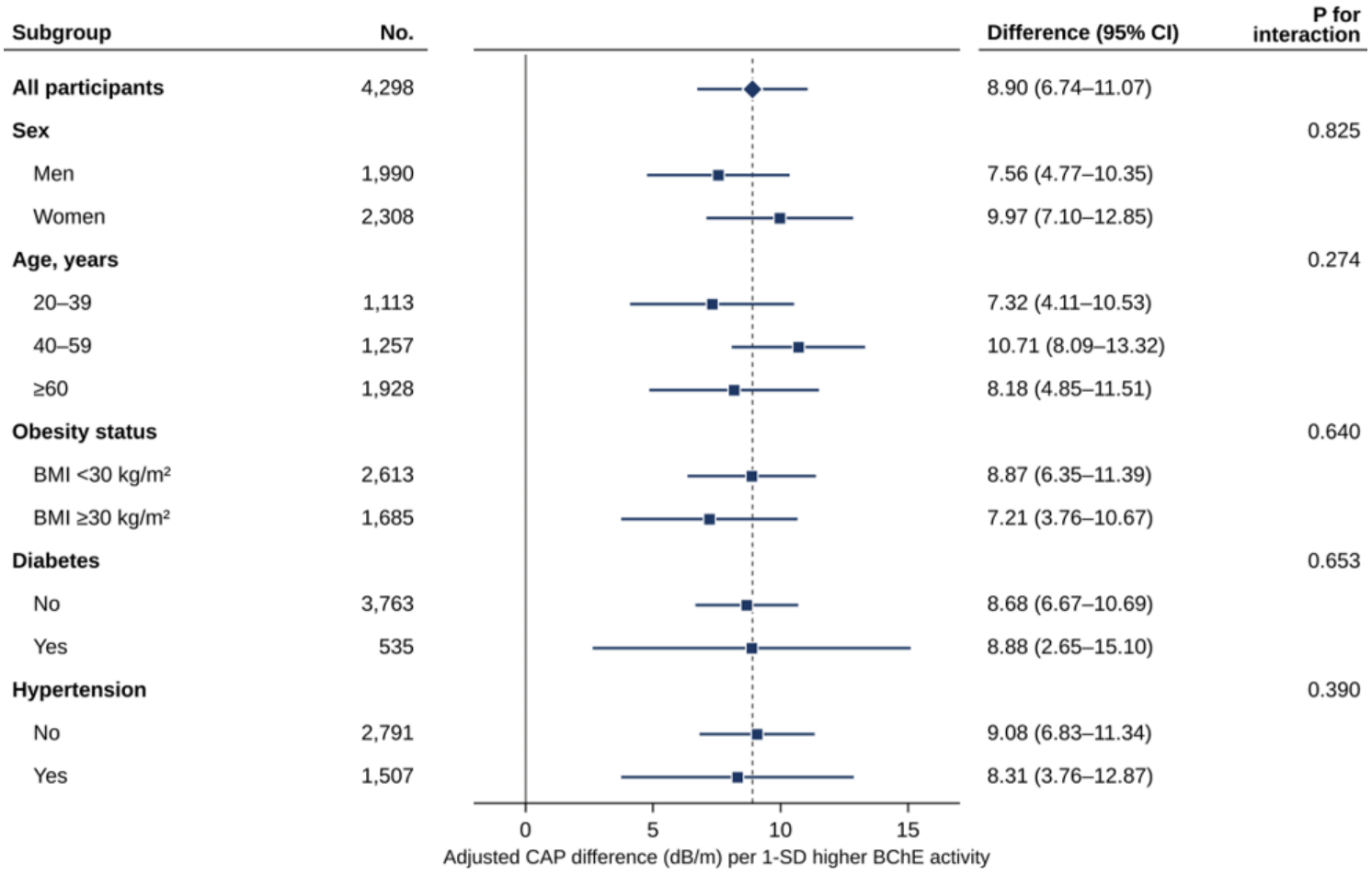


Figure 3

Association Between Serum Butyrylcholinesterase Activity and Controlled Attenuation Parameter Across Clinically Relevant Subgroups.

Squares (diamond for all participants) represent adjusted mean differences in controlled attenuation parameter (CAP) per 1 survey-weighted SD (0.8988 U/mL) higher BChE activity, and horizontal lines represent 95% CIs; the dashed vertical line marks the overall estimate. Estimates were obtained from survey-weighted linear regression fitted within each subgroup as a survey domain and adjusted for age, sex, race/ethnicity, body mass index (BMI), diabetes, hypertension, smoking, and alcohol use, excluding the stratifying variable (age was modeled linearly within age strata). P values for interaction are design-based Wald tests of BChE × subgroup product terms in the full primary analytic sample (N=4,298), with 15 survey degrees of freedom.

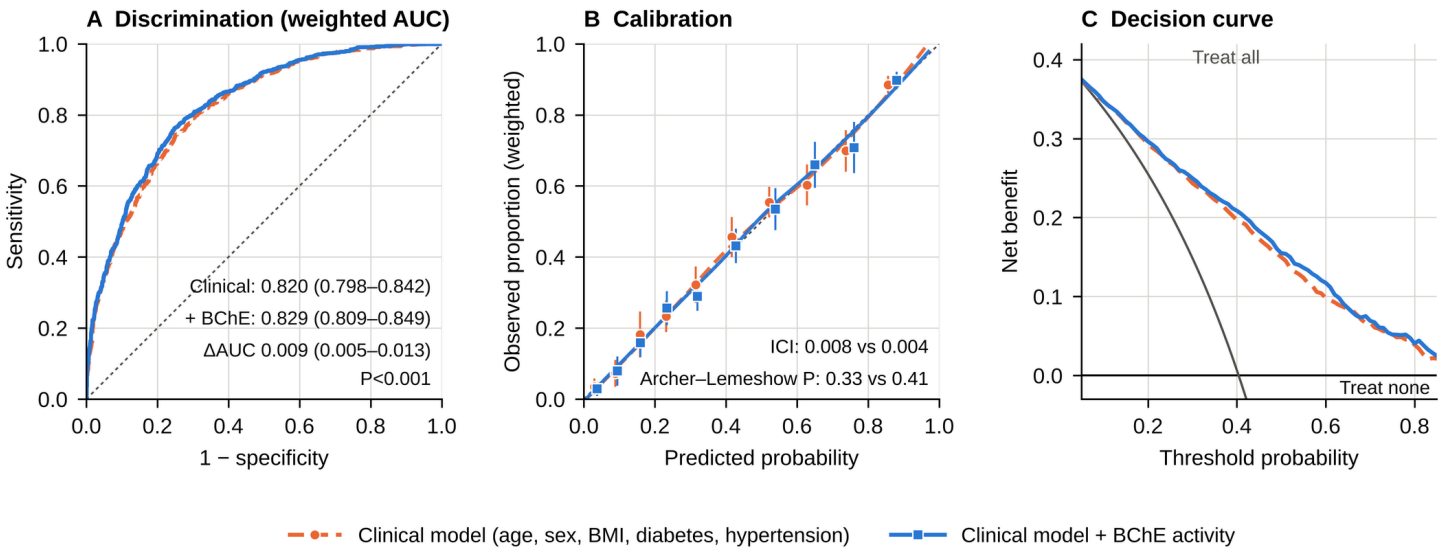


Figure 4

Discrimination, Calibration, and Net Benefit of the Clinical Model With and Without Serum Butyrylcholinesterase Activity for CAP ≥274 dB/m.

(A) Survey-weighted receiver operating characteristic curves with weighted AUCs (95% CIs). (B) Calibration: points show the mean predicted probability and weighted observed prevalence (95% CI) within weighted deciles of predicted risk, and lines show weighted loess calibration curves; the dotted diagonal indicates perfect calibration. ICI, integrated calibration index. (C) Decision curves showing weighted net benefit across threshold probabilities, compared with treating all or no participants as having steatosis. The clinical model included age, sex, body mass index, diabetes, and hypertension. Estimates are apparent (in-sample); optimism-corrected values are reported in Table 4.

Supplementary Files

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