SUPPLEMENTARY MATERIAL

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

Supplementary Methods

Survey design and analytic domains – Analyses used the NHANES August 2021–August 2023 complex survey design with the 2-year phlebotomy weight (WTPH2YR), masked variance strata (SDMVSTRA), and primary sampling units (SDMVPSU). The survey design object was specified in the full positive-phlebotomy-weight parent sample before age, exposure, liver-examination, or complete-case restrictions; analytic restrictions were then applied as survey domains. Statistical inference incorporated 15 survey design degrees of freedom.

Exposure and outcome parameterization – Serum BChE activity (LBXBA, U/mL) was the primary exposure and was expressed per 1 survey-weighted SD (0.8988 U/mL), by survey-weighted quartiles (cut points 3.33, 3.87, and 4.47 U/mL), and using restricted cubic splines. The survey-weighted BChE activity median was 3.87 U/mL; the 1st, 5th, 95th, and 99th percentiles were 2.07, 2.60, 5.52, and 6.30 U/mL, respectively. Four-knot spline models used the survey-weighted 5th, 35th, 65th, and 95th percentiles (2.60, 3.57, 4.21, and 5.52 U/mL). Serum BChE concentration (LBXCH, ng/mL) was examined as a complementary exposure. Continuous CAP (LUXCAPM, dB/m) was the primary outcome; CAP ≥274 dB/m was supportive. LSM (LUXSMED, kPa) was evaluated on the log2 scale and using thresholds of ≥8 and ≥12 kPa.

Adjustment and robustness strategy – The primary model included a natural spline for age with 3 degrees of freedom, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking status, and alcohol use. The expanded socioeconomic model additionally included education and family poverty-income ratio. Robustness analyses replaced BMI with waist circumference, omitted alcohol from the covariate set, used more granular alcohol adjustment, restricted the sample to LSM <8 or <12 kPa, and cross-adjusted CAP and LSM to assess phenotypic specificity. Spline robustness was examined after restricting BChE activity to the weighted 1st–99th and 5th–95th percentile ranges. Missing data were handled by complete-case analysis within each specified model.

Post-hoc metabolic sensitivity analysis – Additional survey-weighted models incorporated HbA1c (LBXGH), total cholesterol (LBXTC), and HDL-C (LBDHDD) as continuous covariates. Three nested adjustment comparisons were evaluated: the original primary model versus the same model additionally adjusted for HbA1c; the original primary model versus the same model additionally adjusted for total cholesterol and HDL-C; and the original primary model versus the model incorporating all three additional laboratory measures. Each comparison was performed on an identical complete-case sample, comprising 4,295, 4,221, and 4,218 participants, respectively. The original survey-weighted BChE standard deviation (0.8988 U/mL) was retained throughout. Survey-weighted linear regression was used for continuous CAP, and survey-weighted Poisson regression with a log link and robust variance was used to estimate prevalence ratios for CAP ≥274 dB/m. The survey design was specified using the original positive-phlebotomy-weight parent sample, retaining WTPH2YR, SDMVSTRA, and SDMVPSU. Design-based standard errors and 15 survey design degrees of freedom were used for single-coefficient inference. Changes in regression coefficients were assessed descriptively and were not subjected to formal mediation analysis.

Post-hoc joint activity–concentration analysis – A supplementary exploratory analysis compared four survey-weighted linear models for continuous CAP in the same 4,297 complete cases: an original-covariate-only model (M0), that model plus standardized BChE activity (M1), plus standardized BChE concentration (M2), and with both measures (M3). The original adjustment covariates, full positive-phlebotomy-weight survey design, and weighted SD scales (0.8988 U/mL for activity; 15.0717 ng/mL for concentration) were maintained. Marginal correlation and conditional collinearity were assessed before joint modeling. Changes in weighted in-sample R^2 and weighted mean squared error were descriptive measures of model fit, not internally validated prediction. For M3, Taylor-linearized design-based standard errors were compared with stratified two-PSU jackknife estimates; sensitivity checks restricted both exposures to their weighted 1st–99th percentiles and repeated fits omitting one survey stratum at a time. Individual-coefficient intervals used 15 design degrees of freedom, with limited-df stress tests treated as diagnostic only. The joint model contained 18 parameters and a rank-deficient Taylor covariance matrix; resulting inference warrants caution. These analyses were post-hoc and do not imply causal independence, diagnostic utility, or externally validated incremental prediction.

Subgroup and interaction analyses – Subgroup-specific estimates were obtained by fitting the primary model within each subgroup as a domain of the full parent survey design (WTPH2YR, SDMVSTRA, SDMVPSU), omitting the stratifying covariate; within age strata, the age spline was replaced by a linear age term, and within obesity strata, continuous BMI was retained. Interaction models added BChE × modifier product terms (and the modifier main effect for age group, obesity, and BMI category) to the primary model in the full primary analytic sample (N=4,298). Continuous modifiers were centered (BMI at 30 kg/m², scaled per 5 kg/m²; age at 50 years, scaled per 10 years). Interaction terms with more than one degree of freedom were tested with design-based Wald F tests using 15 denominator degrees of freedom. For CAP ≥274 dB/m, multiplicative interaction was assessed with survey-weighted Poisson models (log link) and additive interaction with survey-weighted linear probability models (identity link), the latter yielding prevalence differences per 1 SD.

Incremental predictive value – Natural cubic spline bases for age (interior knots, 44 and 64 years; boundary knots, 20 and 80 years) and BMI (interior knots, 25.8 and 31.0 kg/m²; boundary knots at the sample minimum and maximum) were fixed in the primary analytic sample so that every refit used identical basis functions. Models were fitted by survey-weighted pseudo-maximum likelihood. The weighted AUC was computed as $\Sigma_i\Sigma_j\, w_i w_j[I(p_i > p_j) + 0.5\cdot I(p_i = p_j)] / (\Sigma_i w_i \cdot \Sigma_j w_j)$, with *i* indexing participants with and *j* participants without CAP ≥274 dB/m. Calibration-in-the-large was the intercept of a weighted logistic model of the outcome with the linear predictor as an offset, and the calibration slope was the coefficient of the linear predictor. The integrated calibration index (ICI) was the weighted mean absolute difference between predicted probabilities and a weighted loess calibration curve (span, 0.75). The Archer–Lemeshow test compared design-based weighted mean residuals across 10 weighted deciles of predicted risk; the Wald statistic W was F-adjusted as $(f - g + 2)W/(fg)$ and referred to an F distribution with $g - 1$ and $f - g + 2$ degrees of freedom ($g = 10$ groups; $f = 15$ design degrees of freedom). The category-free NRI summed the net weighted proportions of participants with and without steatosis whose predicted risk moved in the appropriate direction, and the IDI was the difference between models in discrimination slope (weighted mean predicted risk among participants with minus without steatosis). For design-based inference, 30 stratified jackknife (JKn) replicate weights were created from the parent design; in each replicate, both models were refitted with the replicate weights and all measures recomputed, and variances were estimated as $\Sigma_r[(n_h - 1)/n_h](\theta_r - \theta)^2$, with t-based intervals on 15 degrees of freedom. Optimism was estimated by resampling participants with replacement (1,000 resamples), refitting both models with survey weights in each resample, and subtracting the mean difference between resample and original-sample performance from the apparent estimates. Net benefit at threshold probability p_t was $(TP - FP \times p_t/[1 - p_t])/N$, with weighted counts.

Alternative steatosis definitions and MASLD phenotype – Past-year drinking frequency (ALQ121) was converted to drinking days per year (every day, 365; nearly every day, 300; 3–4 times per week, 182; twice per week, 104; once per week, 52; 2–3 times per month, 30; once per month, 12; 7–11 times per year, 9; 3–6 times per year, 4.5; 1–2 times per year, 1.5; none in the past year, 0), and lifetime abstainers (ALQ111=2) were assigned 0 g/week. Weekly ethanol intake was calculated as drinking days per year ÷ 52 × usual drinks per drinking day (ALQ130; values of 1–15, where 15 denotes 15 or more) × 14 g; refused or unknown responses were treated as missing. Intake below 140 g/week (women) or 210 g/week (men) was compatible with MASLD, 140–350 and 210–420 g/week defined the MetALD range, and higher intake defined the alcohol-related liver disease (ALD) range. Cardiometabolic criteria used BMXBMI, BMXWAIST, DIQ010, DIQ160, DIQ050, DIQ070, serum glucose (LBXSGL), BPQ020, BPQ150, serum triglycerides (LBXSTR), and BPQ101D, as summarized in Supplementary Table S13. Measured blood pressure, HbA1c, fasting glucose, and HDL-C were not incorporated, so the HDL-C criterion was captured only through lipid-lowering medication. Ordinal steatosis grade was analyzed with survey-weighted proportional-odds regression; the proportional-odds assumption was examined by comparing cut-specific survey-weighted logistic odds ratios at 248, 268, and 280 dB/m (1.48, 1.43, 1.47, respectively).

Supplementary Table S1. Analytic Sample Flow and Covariate Missingness

Section	Item	N or missing N (%)
Sample flow	Adults ≥20 years with positive phlebotomy weight	5,792
Sample flow	BChE + complete elastography + valid CAP + valid LSM	4,914
Sample flow	Age/sex/race adjusted model complete cases	4,914
Sample flow	Primary adjusted model complete cases	4,298
Sample flow	Expanded socioeconomic model complete cases	3,787
Covariate missingness	Sex	0 (0.0%)
Covariate missingness	Race/ethnicity	0 (0.0%)
Covariate missingness	Body mass index	28 (0.6%)
Covariate missingness	Diabetes	0 (0.0%)
Covariate missingness	Hypertension	3 (0.1%)
Covariate missingness	Smoking status	5 (0.1%)
Covariate missingness	Alcohol use	593 (12.1%)
Covariate missingness	Education	2 (0.0%)
Covariate missingness	Family poverty-income ratio	633 (12.9%)

Covariate missingness counts and percentages were calculated among the 4,914 participants with available BChE activity and valid CAP and LSM measurements. Of these, 633 participants (12.9%) had missing family poverty-income ratio data. Within the primary analytic sample of 4,298 participants used for Table 1, family poverty-income ratio was available for 3,787 participants and missing for 511 participants. Accordingly, income-related descriptive estimates were calculated using available nonmissing observations. Family poverty-income ratio was not required for inclusion in the primary adjusted model. BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Supplementary Table S2. Restricted Cubic Spline Tests and Tail-Robustness Analyses

Outcome	Test	Sample	N	F	P value
CAP	Overall spline association	Full primary complete-case sample	4,298	71.29	<0.001
CAP	Departure from linearity	Full primary complete-case sample	4,298	7.87	0.0046
log2 LSM	Overall spline association	Full primary complete-case sample	4,298	6.19	0.0060
log2 LSM	Departure from linearity	Full primary complete-case sample	4,298	6.53	0.0091
CAP	Overall association / nonlinearity	Weighted 1st–99th percentile range	4,218	—	<0.001 / 0.077
CAP	Overall association / nonlinearity	Weighted 5th–95th percentile range	3,908	—	<0.001 / 0.062

Spline models used four survey-weighted knots at the 5th, 35th, 65th, and 95th percentiles. Full-sample CAP overall $P=4.18\times10^{-9}$ and nonlinearity $P=0.00459$; 1st–99th percentile overall $P=4.06\times10^{-8}$; 5th–95th percentile overall $P=2.24\times10^{-9}$. Tail-restricted analyses were diagnostic sensitivity checks rather than alternative primary models.

Supplementary Table S3. Survey-Weighted Comparison of Participants Included and Excluded From the Primary Complete-Case Model

Characteristic	Included	Excluded	P value
Age, years	48.30 (0.56)	48.79 (1.15)	0.677
BChE activity, U/mL	3.94 (0.06)	3.94 (0.06)	0.998
CAP, dB/m	262.19 (1.72)	263.07 (2.64)	0.755
LSM, kPa	6.12 (0.15)	5.91 (0.25)	0.388
BMI, kg/m²	29.36 (0.25)	29.20 (0.53)	0.718
Female, %	49.5 (0.9)	56.3 (2.6)	0.034

Values are survey-weighted mean (SE) or weighted percentage (SE). Included participants had complete data for the primary multivariable model. BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; BMI, body mass index.

Supplementary Table S4. Robustness of the Association Between BChE Activity and Continuous CAP

Analysis	N	CAP difference, dB/m	95% CI	P value
Age/sex/race adjusted	4,914	18.82	16.16 to 21.49	<0.001
Primary adjusted	4,298	8.90	6.74 to 11.07	<0.001
Expanded socioeconomic	3,787	8.81	6.50 to 11.13	<0.001

Analysis	N	CAP difference, dB/m	95% CI	P value
Waist circumference replacing BMI	4,244	7.84	6.27 to 9.41	<0.001
Primary model without alcohol	4,878	9.17	7.22 to 11.12	<0.001
Granular alcohol adjustment	4,275	9.17	6.82 to 11.52	<0.001
Restricted to LSM <8 kPa	3,768	8.87	6.65 to 11.09	<0.001
Restricted to LSM <12 kPa	4,101	8.94	6.94 to 10.95	<0.001

Estimates are adjusted mean differences in CAP per 1 survey-weighted SD (0.8988 U/mL) higher BChE activity. The primary model adjusted for age, sex, race/ethnicity, BMI, diabetes, hypertension, smoking, and alcohol use; the expanded model additionally adjusted for education and family poverty-income ratio.

Supplementary Table S5. Association Between BChE Activity and Continuous Liver Stiffness

Model	Percent LSM difference	95% CI	P value
Age/sex/race adjusted	+3.82%	+1.50% to +6.20%	0.003
Primary adjusted	−1.90%	−4.51% to +0.79%	0.152
Expanded socioeconomic	−1.27%	−4.35% to +1.91%	0.403

LSM was log2-transformed for modeling; estimates are back-transformed percentage differences in LSM per 1 survey-weighted SD higher BChE activity. LSM, liver stiffness measurement.

Supplementary Table S6. Prevalence Ratios for Elevated Liver Stiffness

Outcome	Model	PR	95% CI	P value
LSM ≥8 kPa	Primary adjusted	0.981	0.881–1.093	0.714
LSM ≥8 kPa	Expanded socioeconomic	1.016	0.916–1.126	0.755
LSM ≥12 kPa	Primary adjusted	0.842	0.628–1.130	0.233
LSM ≥12 kPa	Expanded socioeconomic	0.885	0.633–1.237	0.450

Prevalence ratios are reported per 1 survey-weighted SD higher BChE activity. PR, prevalence ratio; LSM, liver stiffness measurement.

Supplementary Table S7. Association With LSM ≥8 kPa Among Participants With CAP ≥274 dB/m

Model	N	PR	95% CI	P value
Primary adjusted	1,745	0.997	0.854–1.165	0.972
Expanded socioeconomic	1,529	1.017	0.886–1.166	0.803

The analysis was restricted to participants meeting the supportive CAP-defined steatosis threshold. Estimates are prevalence ratios per 1 survey-weighted SD higher BChE activity.

Supplementary Table S8. Phenotypic-Specificity Cross-Adjusted Models

Model	Outcome	Estimate	95% CI	P value
CAP additionally adjusted for log2 LSM	Continuous CAP	+9.22 dB/m	7.24 to 11.20	<0.001
log2 LSM additionally adjusted for CAP	Continuous LSM	−2.90%	−5.34% to −0.39%	0.027

Estimates are per 1 survey-weighted SD higher BChE activity. These cross-adjusted models condition on related hepatic phenotypes and are intended as descriptive phenotypic-specificity analyses, not mediation or causal models.

Supplementary Table S9. Post-hoc Metabolic Sensitivity Analyses of the Association Between Serum BChE Activity and Hepatic Steatosis

Outcome and model	N	Estimate (95% CI)
Continuous CAP (dB/m)		
HbA1c matched original	4,295	8.91 (6.74–11.07)
Additional HbA1c	4,295	8.20 (5.96–10.43)
Lipids matched original	4,221	8.88 (6.85–10.91)
Additional total cholesterol and HDL-C	4,221	6.41 (3.98–8.83)
Combined matched original	4,218	8.88 (6.85–10.91)
Additional HbA1c, total cholesterol, and HDL-C	4,218	5.95 (3.49–8.42)
CAP ≥274 dB/m (PR)		
HbA1c matched original	4,295	1.187 (1.144–1.232)
Additional HbA1c	4,295	1.179 (1.135–1.225)
Lipids matched original	4,221	1.183 (1.138–1.229)
Additional total cholesterol and HDL-C	4,221	1.122 (1.086–1.160)
Combined matched original	4,218	1.182 (1.138–1.229)
Additional HbA1c, total cholesterol, and HDL-C	4,218	1.121 (1.084–1.159)

Values represent adjusted CAP differences in dB/m or prevalence ratios (PRs) for CAP ≥274 dB/m per 1 survey-weighted SD (0.8988 U/mL) higher serum BChE activity. The original adjustment model included age, sex, race/ethnicity, BMI, diabetes, hypertension, smoking, and alcohol use. Each original model was refitted within the same complete-case sample as its corresponding metabolically expanded model. The additional metabolic covariates were HbA1c, total cholesterol, and HDL-C, as indicated. All analyses incorporated the original NHANES survey design and phlebotomy weights. These analyses were post-hoc and exploratory. Coefficient attenuation was assessed descriptively and should not be interpreted as evidence of mediation or causal independence. CAP, controlled attenuation parameter; BChE, butyrylcholinesterase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; CI, confidence interval.

Supplementary Table S10. Post-hoc Joint Associations of Serum BChE Activity and Concentration With Continuous CAP

Panel A. Survey-weighted linear regression using an identical complete-case sample

Model	Exposure	N	CAP difference, dB/m (95% CI)	P value	Weighted R^2
M0	Covariates only	4,297	—	—	0.404824
M1	Activity, per 0.8988 U/mL	4,297	8.91 (6.75 to 11.07)	<0.001	0.423121
M2	Concentration, per 15.0717 ng/mL	4,297	7.31 (5.44 to 9.17)	<0.001	0.417344
M3	Activity, adjusted for concentration	4,297	8.70 (4.45 to 12.95)	0.00056	0.423126
M3	Concentration, adjusted for activity	4,297	0.25 (−3.22 to 3.73)	0.879	0.423126

Panel B. Added in-sample model fit (descriptive comparisons)

Nested comparison	Δ weighted R^2	Relative reduction in weighted MSE
Activity added after concentration (M2 → M3)	0.005782	0.99%
Concentration added after activity (M1 → M3)	0.000005	0.0009%

Panel C. Joint-model sensitivity and variance-estimation checks

Check	Exposure in M3	N	CAP difference, dB/m (95% CI)	P value
Stratified JKn inference	Activity	4,297	8.70 (4.36 to 13.04)	0.00066
Stratified JKn inference	Concentration	4,297	0.25 (−3.27 to 3.78)	0.880
Weighted 1st–99th percentiles	Activity	4,178	9.64 (5.82 to 13.46)	<0.001
Weighted 1st–99th percentiles	Concentration	4,178	0.77 (−3.05 to 4.60)	0.672

Notes. M0 included age (natural spline, 3 df), sex, race/ethnicity, BMI, diabetes, hypertension, smoking, and past-year alcohol use; M1 = M0 + BChE activity; M2 = M0 + BChE concentration; M3 = M0 + both BChE measures. Exposure standard deviations were obtained from the original eligible survey-weighted sample and held fixed in all models. Estimates are covariate-adjusted CAP differences per 1 original weighted SD of the indicated measure. The survey-weighted activity–concentration correlation was 0.836, and the conditional two-exposure VIF was 3.02. Weighted R^2 and changes in weighted MSE were calculated within the same analytic sample and should not be interpreted as cross-validated discrimination, added diagnostic utility, or causal independence. Single-coefficient 95% CIs and P values used design-based SEs with 15 parent survey degrees of freedom; the jackknife comparison used 30 stratified replicate weights. The M3 design matrix was full rank (18 parameters), but the Taylor-linearized covariance matrix had rank 15. Taylor and JKn SEs for the M3 activity coefficient were 1.993 and 2.035 dB/m, respectively. Omission of individual survey strata yielded positive activity coefficients throughout (8.05–9.46 dB/m), but these are leverage diagnostics, not independent validation. Because joint-model inference may be sensitive to limited survey degrees of freedom, findings should be regarded as post-hoc and hypothesis-generating. BChE, butyrylcholinesterase; CAP, controlled attenuation parameter; CI, confidence interval; MSE, mean squared error; JKn, stratified jackknife.

Supplementary Table S11. Subgroup-Specific Associations of BChE Activity With Hepatic Steatosis and Tests for Interaction on Additive and Multiplicative Scales

Subgroup	N	Weighted mean CAP, dB/m	CAP difference, dB/m (95% CI)	P-int	Weighted prevalence of CAP ≥274, %	PR (95% CI)	P-int	Prevalence difference, % points (95% CI)	P-int
All participants	**4,298**	**262.2**	**8.90 (6.74–11.07)**		**40.4**	**1.19 (1.14–1.23)**		**6.1 (4.9–7.3)**	
Sex				**0.825**			**0.124**		**0.859**
Men	1,990	268.7	7.56 (4.77–10.35)		45.2	1.15 (1.08–1.23)		5.2 (2.6–7.8)	
Women	2,308	255.5	9.97 (7.10–12.85)		35.5	1.22 (1.17–1.27)		6.6 (4.4–8.8)	
Age, years				**0.274**			**0.479**		**0.012**
20–39	1,113	248.1	7.32 (4.11–10.53)		31.1	1.08 (0.99–1.19)		2.4 (−0.3 to 5.0)	
40–59	1,257	272.1	10.71 (8.09–13.32)		47.7	1.23 (1.17–1.29)		8.7 (6.2–11.3)	
≥60	1,928	267.9	8.18 (4.85–11.51)		43.3	1.20 (1.15–1.26)		6.9 (4.3–9.6)	
Obesity status				**0.640**			**<0.001**		**0.830**
BMI <30 kg/m²	2,613	238.0	8.87 (6.35–11.39)		23.2	1.26 (1.18–1.34)		5.5 (3.7–7.3)	
BMI ≥30 kg/m²	1,685	300.0	7.21 (3.76–10.67)		67.3	1.08 (1.04–1.12)		5.3 (2.6–8.0)	
BMI category, kg/m²				**0.295**			**<0.001**		**0.003**
<25	1,167	216.0	8.77 (4.35–13.20)		9.0	1.42 (1.10–1.83)		2.8 (0.2–5.5)	
25–29.9	1,446	256.1	9.28 (6.70–11.86)		34.9	1.24 (1.15–1.33)		7.5 (4.6–10.4)	
≥30	1,685	300.0	7.21 (3.76–10.67)		67.3	1.08 (1.04–1.12)		5.3 (2.6–8.0)	
Diabetes				**0.653**			**0.164**		**0.413**
No	3,763	257.8	8.68 (6.67–10.69)		37.3	1.19 (1.14–1.24)		5.9 (4.5–7.2)	
Yes	535	300.9	8.88 (2.65–15.10)		67.9	1.12 (1.05–1.19)		7.2 (2.9–11.6)	
Hypertension				**0.390**			**0.046**		**0.172**
No	2,791	254.0	9.08 (6.83–11.34)		33.9	1.19 (1.12–1.26)		5.2 (3.5–6.8)	
Yes	1,507	282.2	8.31 (3.76–12.87)		56.3	1.16 (1.11–1.21)		7.8 (5.6–10.0)	
Race/ethnicity				**0.062**			**0.663**		**0.999**
Non-Hispanic White	2,662	264.2	9.99 (7.59–12.39)		40.9	1.18 (1.12–1.24)		5.9 (4.0–7.7)	
Mexican American	278	267.9	2.14 (−1.97 to 6.26)		48.3	1.22 (1.07–1.39)		7.0 (1.5–12.5)	
Other Hispanic	428	262.0	8.78 (1.84–15.72)		42.5	1.19 (1.01–1.41)		6.6 (−1.6 to 14.7)	
Non-Hispanic Black	458	252.7	10.46 (5.53–15.38)		33.9	1.24 (1.09–1.41)		7.1 (1.6–12.5)	
Non-Hispanic Asian	201	258.4	0.70 (−9.03 to 10.43)		39.0	1.13 (0.86–1.50)		3.3 (−7.1 to 13.6)	

Subgroup	N	Weighted mean CAP, dB/m	CAP difference, dB/m (95% CI)	P-int	Weighted prevalence of CAP ≥274, %	PR (95% CI)	P-int	Prevalence difference, % points (95% CI)	P-int
Other/multiracial	271	254.8	8.25 (−5.39 to 21.89)		35.2	1.35 (1.16–1.56)		7.7 (−1.3 to 16.7)	
Continuous effect modifiers (interaction terms)									
BChE × BMI, per 5 kg/m²	4,298	—	−0.02 (−1.13 to 1.08)	0.965	—	0.929 (0.909–0.950)	<0.001	−0.01 (−1.24 to 1.23)	0.989
BChE × age, per 10 years	4,298	—	0.15 (−0.83 to 1.13)	0.751	—	1.004 (0.981–1.028)	0.709	0.99 (0.14–1.83)	0.025

Estimates are per 1 survey-weighted SD (0.8988 U/mL) higher BChE activity from survey-weighted models fitted within each subgroup as a domain of the full survey design, adjusted for age, sex, race/ethnicity, BMI, diabetes, hypertension, smoking, and alcohol use, excluding the stratifying variable (age modeled linearly within age strata; continuous BMI retained within BMI strata). CAP differences are from linear regression, prevalence ratios (PRs) from Poisson regression with a log link, and prevalence differences from linear probability models. P values for interaction are design-based Wald tests of BChE × modifier product terms added to the primary model in the full primary analytic sample (F tests with 15 denominator degrees of freedom for multi-category modifiers). For continuous modifiers, the CAP and prevalence-difference columns give the change in the BChE coefficient per unit of the modifier and the PR column gives the ratio of PRs. Interaction analyses were exploratory and not adjusted for multiple comparisons. P-int, P for interaction; N are unweighted counts. BChE, butyrylcholinesterase; BMI, body mass index; CAP, controlled attenuation parameter.

Supplementary Table S12. Sensitivity Analyses of the Incremental Predictive Value of BChE Activity

Analysis	N	Cases	AUC, reference model	AUC, + added marker	ΔAUC (95% CI)	P value	IDI (95% CI)	Category-free NRI (95% CI)
Clinical model vs clinical model + BChE								
Primary analysis (CAP ≥274 dB/m)	4,298	1,745	0.820	0.829	0.009 (0.005–0.013)	<0.001	0.018 (0.011–0.025)	0.313 (0.209–0.418)
Linear age and BMI terms	4,298	1,745	0.816	0.826	0.010 (0.006–0.015)	<0.001	0.022 (0.013–0.030)	0.323 (0.227–0.418)
Extended clinical model (+ race/ethnicity, smoking, alcohol use)	4,298	1,745	0.831	0.838	0.007 (0.004–0.010)	<0.001	0.013 (0.007–0.020)	0.267 (0.177–0.357)
All participants with complete clinical-model data	4,883	1,990	0.825	0.833	0.009 (0.006–0.012)	<0.001	0.018 (0.012–0.024)	0.303 (0.245–0.361)
CAP ≥248 dB/m	4,298	2,401	0.811	0.822	0.011 (0.005–0.017)	0.001	0.021 (0.011–0.032)	0.317 (0.214–0.420)
CAP ≥285 dB/m	4,298	1,485	0.823	0.833	0.010 (0.006–0.014)	<0.001	0.019 (0.012–0.027)	0.294 (0.229–0.359)
CAP ≥302 dB/m	4,298	1,154	0.823	0.831	0.009 (0.002–0.015)	0.012	0.017 (0.004–0.029)	0.276 (0.162–0.391)
CAP ≥331 dB/m	4,298	654	0.831	0.837	0.006 (0.000 to 0.012)	0.062	0.008 (−0.002 to 0.019)	0.225 (0.094–0.356)
MASLD (CAP ≥274 dB/m) vs no steatosis	4,121	1,568	0.830	0.840	0.009 (0.005–0.013)	<0.001	0.019 (0.010–0.027)	0.311 (0.212–0.409)
Unweighted logistic models (DeLong test)	4,298	1,745	0.816	0.826	0.010 (0.006–0.014)	<0.001	—	—
Reference comparisons with alanine aminotransferase (ALT)								
Clinical model vs clinical model + ALT	4,178	1,697	0.819	0.831	0.011 (0.005–0.017)	0.001	0.023 (0.013–0.033)	0.327 (0.189–0.466)
Clinical model + ALT vs clinical model + ALT + BChE	4,178	1,697	0.831	0.836	0.005 (0.003–0.008)	<0.001	0.011 (0.006–0.016)	0.245 (0.165–0.325)

The clinical model included age and BMI (natural cubic splines, 3 degrees of freedom), sex, diabetes, and hypertension; BChE activity was added per 1 survey-weighted SD. Unless stated otherwise, models were fitted with survey-weighted logistic regression in the primary analytic sample, and AUC, ΔAUC, integrated discrimination improvement (IDI), and category-free net reclassification improvement (NRI) were survey weighted, with 95% CIs and P values from 30 stratified jackknife replicate weights (15 degrees of freedom). The extended model additionally included race/ethnicity, smoking, and past-year alcohol use. The broader sample comprised all participants with valid CAP, BChE activity, and complete clinical-model predictors, regardless of the remaining primary covariates. The unweighted analysis used conventional logistic regression and DeLong's test, which can be conservative for nested models. ALT was log-transformed; ALT analyses were restricted to participants with available ALT. Cases are participants meeting the outcome definition. MASLD, metabolic dysfunction-associated steatotic liver disease.

Supplementary Table S13. Construction of the MASLD Phenotype, Distribution of Steatotic Liver Disease Categories, and Additional MASLD Analyses

Panel A. Cardiometabolic criteria among participants with CAP ≥274 dB/m (N=1,745)

Criterion	Consensus definition (adults)	Operationalization in this analysis (NHANES variables)	Met, n (weighted %)
1. Adiposity	BMI ≥25 kg/m² (≥23 kg/m² in Asian individuals) or waist circumference >94 cm (men) / >80 cm (women), or ethnicity-adjusted equivalents	BMI ≥25 kg/m² (≥23 kg/m² if non-Hispanic Asian) or waist >94 cm in men (>90 cm if non-Hispanic Asian) or >80 cm in women (BMXBMI, BMXWAIST)	1,711 (98.0)
2. Glycemia	Fasting glucose ≥100 mg/dL, 2-hour glucose ≥140 mg/dL, HbA1c ≥5.7%, type 2 diabetes, or treatment for type 2 diabetes	Self-reported diabetes (DIQ010) or prediabetes (DIQ160), insulin (DIQ050) or oral glucose-lowering medication (DIQ070), or serum glucose ≥200 mg/dL (LBXSGL)	647 (31.8)

Criterion	Consensus definition (adults)	Operationalization in this analysis (NHANES variables)	Met, n (weighted %)
3. Blood pressure	Blood pressure ≥130/85 mmHg or specific antihypertensive treatment	Self-reported hypertension (BPQ020) or current antihypertensive medication (BPQ150); measured blood pressure not incorporated	800 (40.5)
4. Triglycerides	Plasma triglycerides ≥150 mg/dL or lipid-lowering treatment	Serum triglycerides ≥150 mg/dL (LBXSTR, standard biochemistry) or current lipid-lowering medication (BPQ101D)	1,065 (57.6)
5. HDL-C	HDL-C ≤40 mg/dL (men) / ≤50 mg/dL (women) or lipid-lowering treatment	HDL-C not incorporated; captured only through lipid-lowering medication (criterion 4)	—
At least one criterion			**1,726 (98.8)**

Panel B. Steatotic liver disease categories by CAP threshold in the primary analytic sample (N=4,298), n (weighted %)

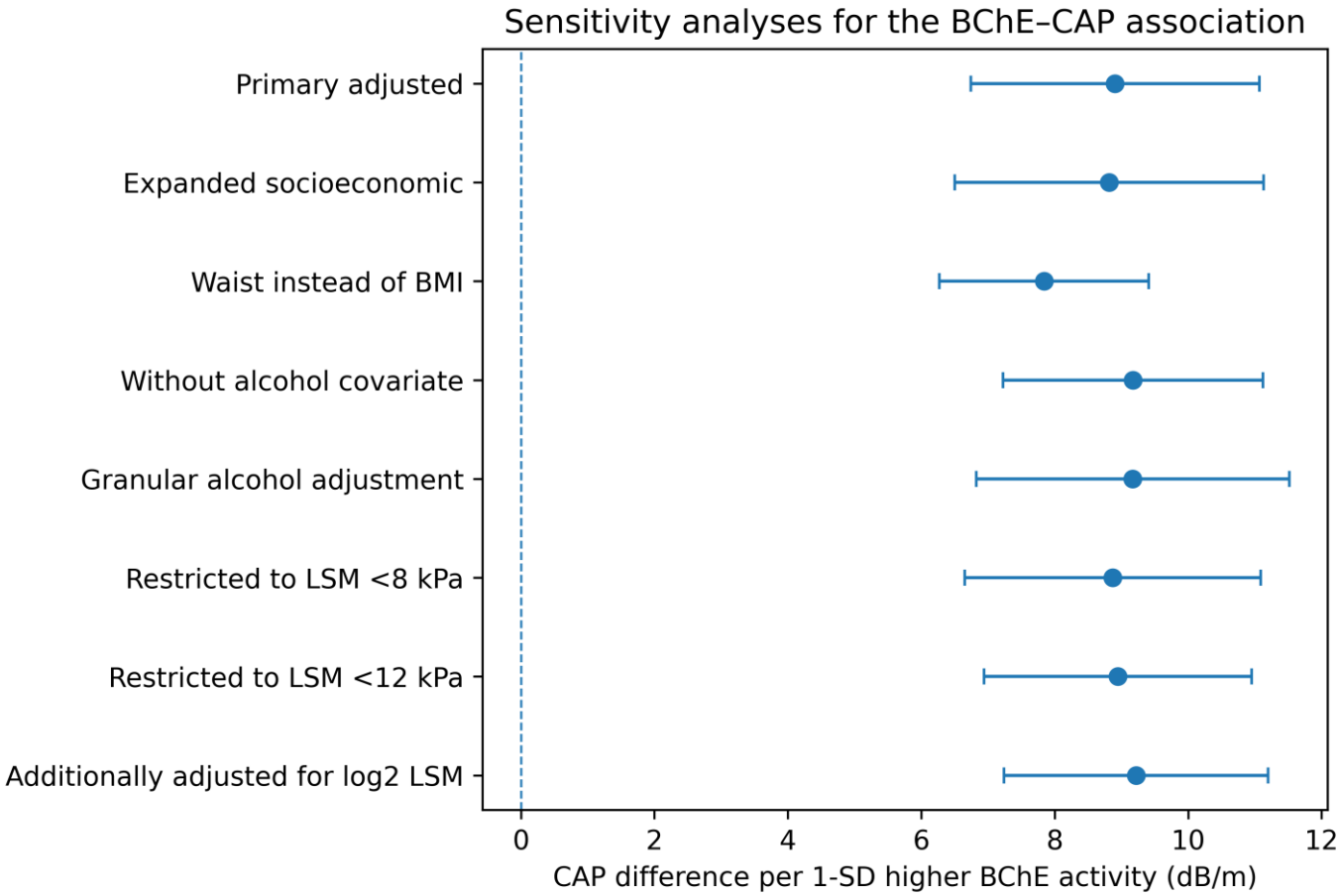
Category	CAP ≥248 dB/m	CAP ≥274 dB/m	CAP ≥285 dB/m	CAP ≥302 dB/m
No steatosis	1,897 (44.1)	2,553 (59.6)	2,813 (65.7)	3,144 (73.3)
MASLD	2,126 (49.0)	1,568 (35.9)	1,338 (30.4)	1,032 (23.5)
MetALD	165 (3.9)	105 (2.6)	94 (2.3)	80 (1.9)
Alcohol intake above MetALD range	47 (1.4)	42 (1.2)	35 (1.1)	32 (1.0)
Steatosis without a cardiometabolic criterion	50 (1.4)	19 (0.5)	11 (0.3)	7 (0.2)
Steatosis with unclassifiable alcohol intake	13 (0.3)	11 (0.2)	7 (0.2)	3 (0.1)

Panel C. Additional MASLD and alcohol-restricted analyses (per 1 survey-weighted SD higher BChE activity)

Analysis	N	Cases	Estimate (95% CI)	P value
MASLD vs no steatosis, adjusted for age, sex, and race/ethnicity	4,133	1,578	PR 1.35 (1.28–1.43)	<0.001
MASLD vs no steatosis, primary model + education and poverty-income ratio	3,618	1,370	PR 1.21 (1.15–1.27)	<0.001
MASLD vs no steatosis, waist circumference replacing BMI	4,048	1,537	PR 1.18 (1.13–1.23)	<0.001
Continuous CAP, participants with alcohol intake below the MetALD range	3,907	—	9.54 dB/m (7.12–11.95)	<0.001
Continuous CAP, alcohol below the MetALD range and ≥1 cardiometabolic criterion	3,484	—	9.58 dB/m (6.99–12.16)	<0.001

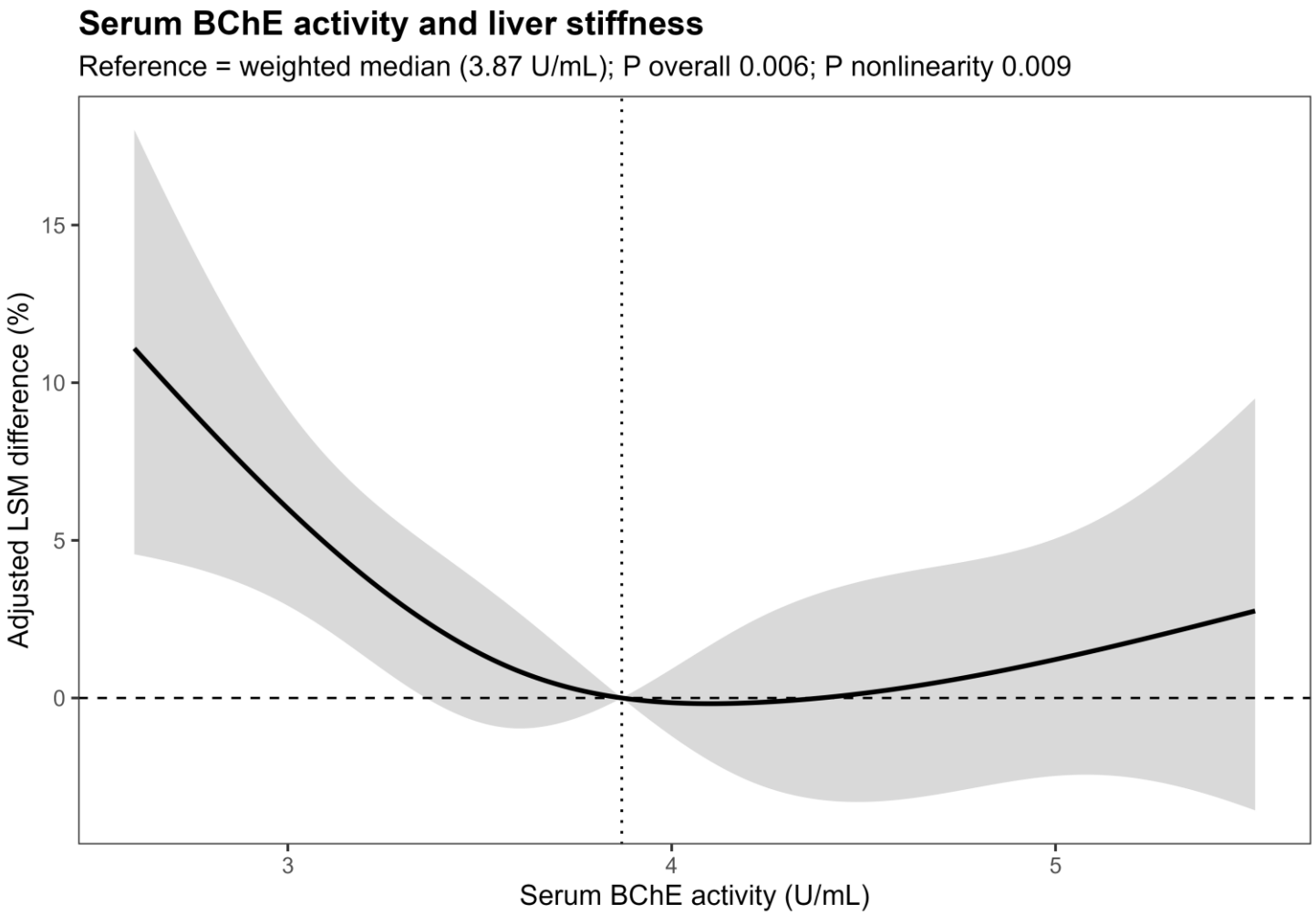
Consensus definitions follow the 2023 multisociety Delphi statement; MASLD required CAP-defined steatosis, at least one cardiometabolic criterion, and alcohol intake below 140 g/week (women) or 210 g/week (men); MetALD corresponded to 140–350 and 210–420 g/week, respectively. Weighted percentages in Panel A refer to participants with CAP ≥274 dB/m. Glucose and triglycerides came from the standard biochemistry profile, which was not restricted to fasting samples. Panel C models used the primary covariates unless stated otherwise; PRs are from survey-weighted Poisson regression and CAP differences from survey-weighted linear regression. BChE, butyrylcholinesterase; BMI, body mass index; CAP, controlled attenuation parameter; HDL-C, high-density lipoprotein cholesterol; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, MASLD with increased alcohol intake; PR, prevalence ratio.

Supplementary Figure S1. Sensitivity Analyses for the BChE–CAP Association



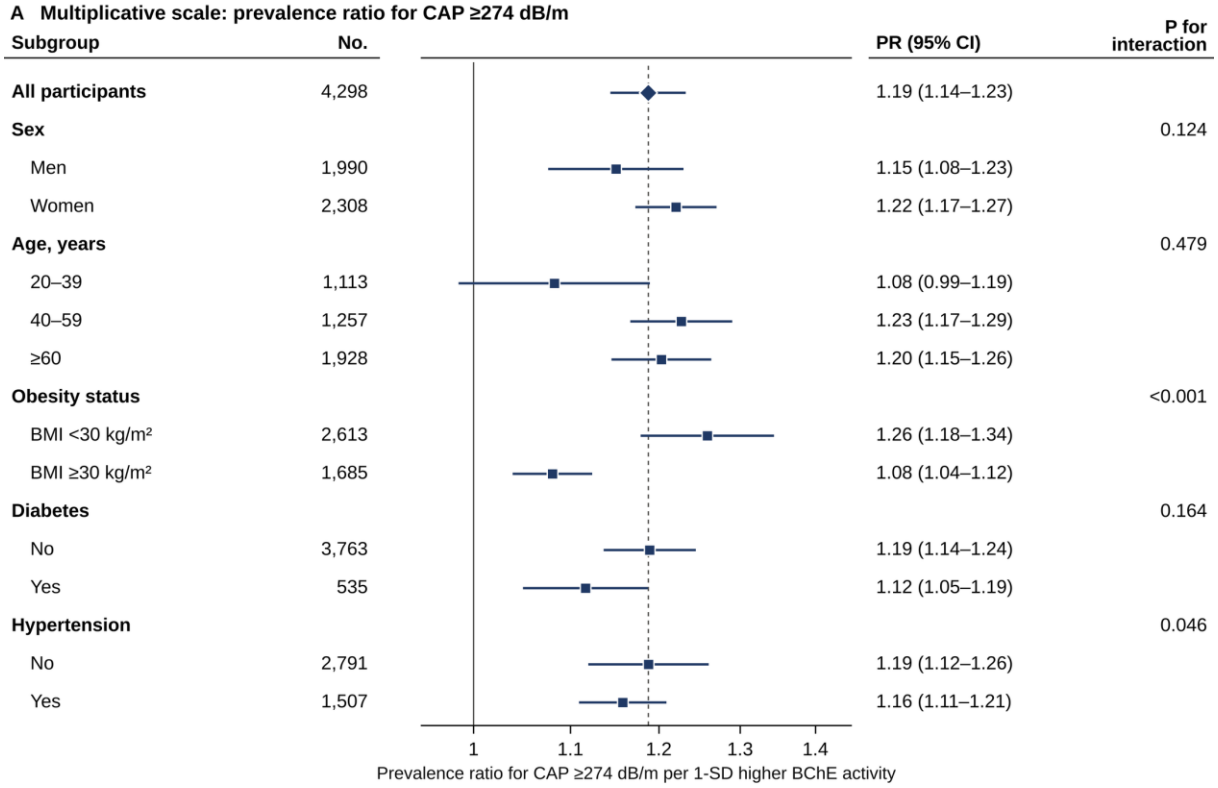
Points represent adjusted mean differences in CAP per 1 survey-weighted SD higher BChE activity; horizontal bars represent 95% confidence intervals. The dashed vertical line denotes a null CAP difference of 0 dB/m. The phenotypic-specificity model additionally adjusted CAP for log2-transformed LSM.

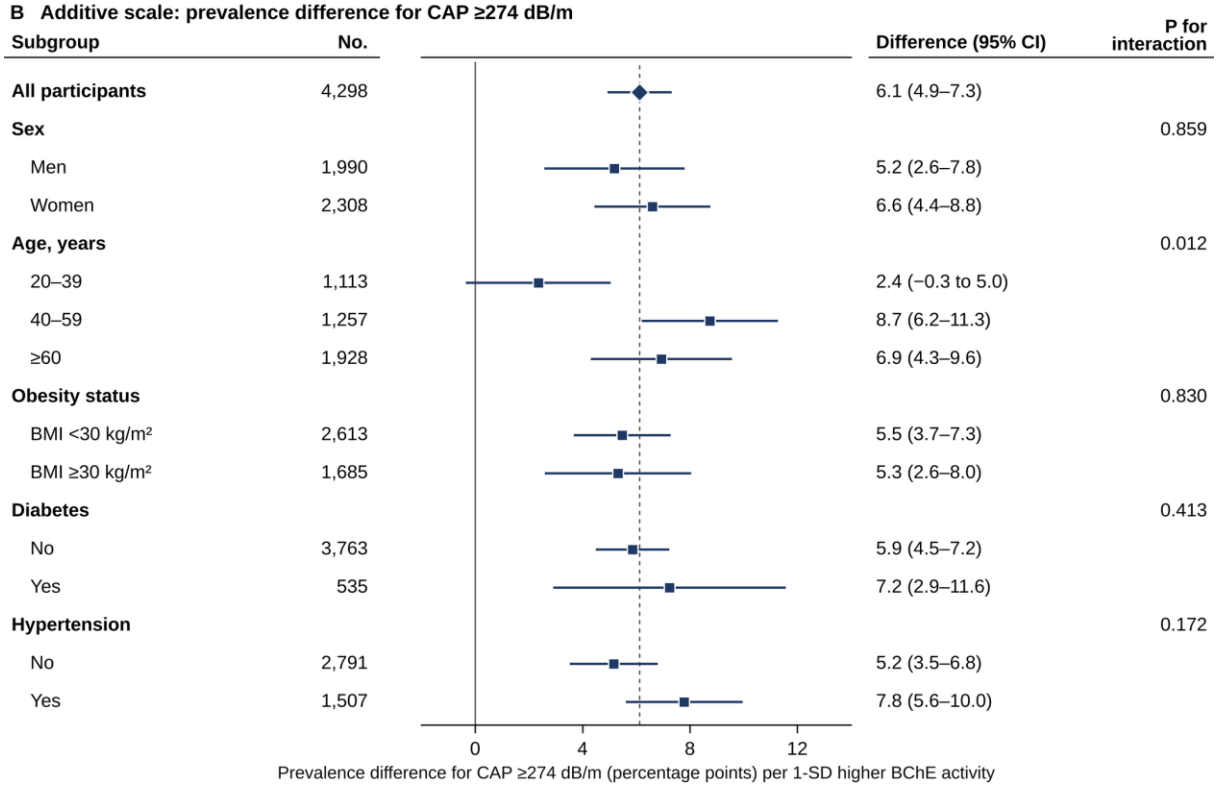
Supplementary Figure S2. Restricted Cubic Spline Association Between Serum Butyrylcholinesterase Activity and Liver Stiffness.



The solid line represents the adjusted percentage difference in liver stiffness measurement relative to the survey-weighted median serum BChE activity, and the shaded region represents the 95% confidence interval. The model was based on survey-weighted regression of log2-transformed liver stiffness measurement using a four-knot restricted cubic spline with knots placed at the survey-weighted 5th, 35th, 65th, and 95th percentiles. The survey-weighted median BChE activity of 3.87 U/mL was used as the reference. The model adjusted for age, sex, race/ethnicity, body mass index, diabetes, hypertension, smoking status, and alcohol use. P for overall association = 0.006; P for nonlinearity = 0.009.

Supplementary Figure S3. Subgroup-Specific Associations of BChE Activity With CAP ≥274 dB/m on Multiplicative and Additive Scales





(A) Prevalence ratios (PRs) and (B) prevalence differences for CAP ≥274 dB/m per 1 survey-weighted SD higher BChE activity, from survey-weighted Poisson and linear probability models fitted within each subgroup and adjusted as in Figure 3. P values for interaction refer to product terms on the corresponding scale. The smaller PR among participants with obesity accompanies a nearly identical prevalence difference, reflecting their higher baseline prevalence; the smaller prevalence difference among adults aged 20–39 years reflects their lower CAP distribution relative to the threshold. The dashed vertical line marks the overall estimate.