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Relationship of Tea Consumption and its Circulating Biomarkers with Longitudinal Bone Mineral Changes: A Prospective Cohort Study With Repeated Measurements

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Declaration of Interests

The authors report no conflicts of interest

Abstract

Background: Tea is a dietary source of flavan-3-ols, which may influence bone health, but prospective evidence linking tea intake and its circulating biomarkers with longitudinal bone mineral status remains limited.

Objective: To examine associations of tea consumption and its serum biomarkers with bone mineral status in Guangzhou Nutrition and Health Study (GNHS).

Methods: In 2,133 participants, whole-body, total hip, lumbar spine, and femoral neck bone mineral density (BMD) and bone mineral content (BMC) were measured using dual-energy X-ray absorptiometry at four repeated visits during a 13-year follow-up period. Baseline serum flavan-3-ols, including Epicatechin [EC], Epigallocatechin gallate [EGCG], Epicatechin gallate [ECG], Epigallocatechin [EGC], and Catechin, were quantified using UHPLC–MS/MS. Tea consumption was categorized as non-, low-, moderate-, or high-frequency. Multivariable linear mixed-effects models, including exposure × visit interactions, were used to evaluate visit-averaged bone mineral measures and longitudinal trajectories.

Results: Among 1,708 participants, significant tea consumption group × visit interactions were observed for whole-body, lumbar spine, and femoral neck BMD and whole-body BMC (P-interaction ranged from < 0.001 to 0.022). Moderate-frequency tea drinkers generally had the highest visit-averaged adjusted marginal mean Z-scores, including 0.107 (95% CI: 0.025, 0.190) for whole-body BMD, 0.090 (0.012, 0.168) for lumbar spine BMD, and 0.108 (0.029, 0.187) for femoral neck BMD (P-diff ranged from < 0.001 to 0.017). In analyses of serum tea biomarkers, higher biomarker categories were generally associated with more favorable bone mineral measures; however, the associations were not consistently linear, and several biomarkers showed non-monotonic patterns. Visit-averaged differences were most extensive across ECG and EGCG categories.

Conclusions: Moderate-frequency tea consumption and higher categories of selected circulating tea biomarkers were associated with more favorable bone mineral status

Keywords: tea consumption; flavan-3-ols; bone mineral density; bone mineral content; longitudinal study

1. Introduction

Osteoporosis is a systemic metabolic bone disorder characterized by reduced bone mass and deterioration of bone microarchitecture [1]. It increases fracture risk and contributes substantially to disability and mortality [2]. Globally, the prevalence of osteoporosis is approximately 23.1% in females and 11.7% in males [3]. In China, the disease burden continues to rise with population aging. Projections suggest that by 2035, osteoporotic fractures will reach nearly 4.83 million cases, imposing an economic burden of close to $20 billion [4]. Therefore, identifying safe and scalable interventions to delay bone loss is a public health priority.

Beyond non-modifiable determinants such as genetics and endocrine factors, modifiable behaviors—including medication use, dietary intake, and lifestyle patterns—play vital roles in shaping bone health [5, 6]. Tea consumption has attracted increasing interest because of its widespread consumption, favorable safety profile, and high content of bioactive compounds. Tea is a major dietary source of flavan-3-ols, particularly catechins such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and catechin. In green tea, catechins account for approximately 30–42% of dry leaf weight, with EGCG comprising 50–70% of total catechins[7] Experimental evidence suggests that these compounds may influence bone metabolism by suppressing osteoclast activity, promoting osteoblast differentiation, and modulating signaling pathways involved in bone remodeling [8, 9].

A recent meta-analysis based on 40 observational studies indicated that tea consumption is inversely correlated with the risk of osteoporosis and fracture, while positively correlated with bone mineral density (BMD). However, only 14 cohort studies were included, most of which had limited follow-up duration and relied solely on self-reported tea intake assessed through questionnaires rather than objective biomarkers, potentially weakening causal inference[10]. These methodological differences in study design and exposure assessment may partly explain the inconsistent findings across previous studies, with some reporting protective associations of tea consumption with BMD or fracture risk and others showing null associations .[11-14]. Although tea consumption has been examined in relation to BMD and fracture risk, existing evidence remains incomplete. Reliance on questionnaires may fail to capture internal exposure to tea-derived flavan-3-ols, and nonlinear associations remain poorly studied. Prospective evidence based on repeated bone measurements remains limited. For example, a 10-year cohort study reported positive associations between tea consumption and femoral neck and total hip BMD in older females, but did not assess BMC or circulating tea biomarkers [15].

The present study aimed to examine the longitudinal associations of tea consumption and circulating tea biomarkers with repeated site-specific BMD and BMC Z-scores at the whole body, total hip, lumbar spine, and femoral neck over a

13-year follow-up among middle-aged and older Chinese adults. Repeated site-specific BMD and BMC Z-scores were the primary outcomes.

2. Materials and Methods

2.1 Design and participants

The study population was drawn from the Guangzhou Nutrition and Health Study (GNHS), a community-based prospective cohort. The original GNHS recruited approximately 4,048 community-dwelling Chinese adults aged 40–80 years who had lived in Guangzhou for more than 5 years. Participants were recruited from multiple communities covering most areas of Guangzhou through local advertisements, health talks, and referrals between 2008 and 2013 [16].

Of these, 3,169 participants were recruited between 2008 and 2010, and an additional 879 participants were recruited between 2012 and 2013. For participants recruited between 2008 and 2010, F0 represented the baseline assessment, F1 – F3 represented subsequent questionnaire follow-up visits, and bone measurements were obtained from F1 through F4. Participants recruited between 2012 and 2013 did not undergo the F0 assessment and therefore contributed tea consumption information from F1 through F3 and bone measurements from the corresponding follow-up visits.

Participants were eligible for the primary tea consumption analysis if they had sufficient repeated tea consumption data to be classified into one of the predefined long-term tea consumption groups, at least one valid bone mineral density (BMD) or

bone mineral content (BMC) measurement during F1–F4, and complete covariate information after cross-visit supplementation according to predefined visit-priority rules. Participants were excluded if tea consumption information required for longitudinal classification was unavailable, or if their observed tea consumption pattern did not meet the predefined classification criteria. The final primary analysis included 1,708 participants, comprising 537 non-tea drinkers, 543 low-frequency tea drinkers, 522 moderate-frequency tea drinkers, and 106 high-frequency tea drinkers. Among these participants, 247 had no available baseline serum tea biomarker measurements, leaving 1,461 participants for the serum biomarker analyses (Supplementary Figure S1).

Prevalent osteoporosis and use of anti-osteoporosis treatment were not used as exclusion criteria in the primary analyses because corresponding information was not uniformly available at the participant-specific baseline across the two recruitment batches. A sensitivity analysis was subsequently performed for the tea consumption analysis after excluding participants with osteoporosis identified at F1, defined as a femoral neck T-score ≤ -2.5.

The study protocol was approved by the Ethics Committee of the School of Public Health, Sun Yat-sen University, and all participants provided written informed consent.

2.2 Bone Mineral Density and Bone Mineral Content

The primary outcomes of this study were BMD (g/cm^2) and bone BMC (g).

According to the GNHS study protocol[16], Repeated measurements of BMD and BMC were subsequently obtained at four follow-up visits using dual-energy X-ray absorptiometry (DXA; Hologic QDR-1000, version 6.10; Hologic Inc., Waltham, MA, USA). DXA equipment was not available at the original baseline examination (F0); therefore, BMD and BMC were not measured at F0.. The assessed skeletal sites included the whole body, lumbar spine (L1–L4), total hip, and femoral neck. All scans were analyzed using Hologic Discovery software (version 3.2). The precision of BMD measurements was assessed in a subset of 30 participants who underwent duplicate scans with repositioning. BMC measurements were obtained concurrently from the same DXA scans using standardized manufacturer algorithms and were subject to the same quality control procedures.

To account for sex-related differences in absolute bone measurements, BMD and BMC values were standardized separately within females and males and expressed as sex-specific Z-scores. The resulting Z-scores were used in the primary regression and longitudinal trajectory analyses.

2.3 Assessment of tea consumption and serum tea biomarkers

2.3.1 Long-term tea consumption groups

Tea consumption frequency was assessed using structured questionnaires administered at F0–F3 for participants in the first recruitment batch and at F1–F3 for participants in the second recruitment batch. Participants reported their weekly number of tea-brewing occasions. Tea consumption at each visit was categorized as non-tea

drinking (0 times/week), low-frequency consumption (1–6 times/week), moderate-frequency consumption (7 times/week), or high-frequency consumption (>7 times/week).

Long-term tea consumption groups were defined according to predefined longitudinal criteria using all available questionnaire assessments. Non-tea drinkers reported no tea consumption at all available visits. Long-term tea consumption groups were defined according to predefined longitudinal criteria using all available questionnaire assessments. Non-tea drinkers reported no tea consumption at all available visits. Low-frequency tea drinkers reported low-frequency tea consumption at one or more visits and non-tea drinking at all remaining visits. Moderate-frequency tea drinkers reported moderate-frequency tea consumption at one or more visits and low-frequency tea consumption at all remaining visits. High-frequency tea drinkers reported high-frequency tea consumption at one or more visits and moderate-frequency tea consumption at all remaining visits. Participants whose longitudinal tea consumption patterns did not meet these predefined criteria were not assigned to a long-term tea consumption group (Supplementary Table S1)..

In a sensitivity analysis, tea consumption was modeled as a time-varying exposure. Tea consumption reported at each questionnaire visit was paired with the bone measurement obtained at the same visit: F1 tea consumption with F1 bone outcomes, F2 tea consumption with F2 bone outcomes, and F3 tea consumption with F3 bone outcomes. F4 was not included in this analysis because corresponding tea consumption

154 data were unavailable. A cumulative TeaScore was also constructed using five
155 tea-related characteristics assessed at each questionnaire visit: tea-drinking duration,
156 weekly tea-drinking frequency, annual tea consumption amount, tea infusion strength,
157 and tea type, and evaluated in sensitivity analyses, as described in the Supplemental
158 Methods.

159 **2.3.2 Serum tea biomarkers**

160 Fasting venous blood samples were collected from participants in the morning
161 after a 10-hour overnight fast for the determination of tea-derived polyphenols. Serum
162 was separated within 2–4 hours of collection and stored at −80 °C until analysis.
163 Serum tea biomarkers, including Catechin, Epicatechin (EC), epigallocatechin (EGC),
164 epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), were quantified
165 using an Agilent 1290 ultra-high-performance liquid chromatography (UHPLC)
166 system coupled with an Agilent 6495 triple quadrupole mass spectrometer. Prior to
167 analysis, serum samples were incubated with ascorbic acid, Tris-HCl buffer (pH 8.0),
168 and recombinant β-glucuronidase/sulfatase (IMCSzyme) at 37 °C for 2 hours to
169 hydrolyze conjugated flavan-3-ols. Samples were subsequently extracted with ethyl
170 acetate, evaporated under nitrogen, and reconstituted in aqueous methanol for
171 UHPLC–MS/MS analysis.

172 Similar numbers of samples from different exposure groups were included within
173 each analytical batch to minimize potential batch effects. The limits of detection were
174 0.01 ng/mL for Catechin, 0.005 ng/mL for EC, 0.05 ng/mL for EGC, 0.005 ng/mL for

ECG, and 0.001 ng/mL for EGCG. Inter-day coefficients of variation were 7.57% for Catechin, 5.50% for EC, 7.25% for EGC, 6.22% for ECG, and 7.40% for EGCG [17]. Concentrations below the corresponding limits of detection were recorded as zero in the analytical dataset and were not statistically imputed.

To facilitate the evaluation of potential dose–response relationships, serum tea biomarkers were categorized into four groups based on sex-specific distributions (Supplementary Table S2). For serum EGC and Epicatechin, samples with zero concentrations were assigned to Group 1, and non-zero concentrations were divided into sex-specific tertiles (Groups 2–4). For serum Catechin, EGCG, and ECG, concentrations were categorized into sex-specific quartiles (Groups 1–4). This mixed categorization strategy was adopted to account for marked differences in concentration distributions across metabolites, as concentrations of zero were common for serum EGC (56.5%) and Epicatechin (27.5%), whereas zero values were relatively infrequent for the other metabolites. These group variables were subsequently used in sex-standardized linear mixed-effects models, as well as in adjusted mean plots and forest plots, to evaluate associations with bone mass parameters.

2.4 Covariates

Information on socio-demographic characteristics, lifestyle factors, physical activity, dietary intake, and menopausal status (females only) was collected through face-to-face interviews conducted by trained interviewers using structured

196 questionnaires at each follow-up visit.

197 Age, sex, education level, smoking status, alcohol consumption, vitamin and
198 calcium supplement use, physical activity, energy intake, years since menopause, and
199 total body fat mass were selected a priori as potential covariates based on
200 epidemiologic evidence regarding determinants of bone health and tea-drinking
201 behavior, biological plausibility, and their potential roles as confounders [18, 19].
202 Physical activity was assessed using a validated 19-item questionnaire and expressed as
203 total metabolic equivalent of task hours per day (MET-h/day), excluding time spent
204 sleeping and in sedentary activities [20]. Dietary energy intake was estimated from food
205 frequency data and portion sizes using the 2009 Chinese Food Composition Table and
206 expressed as kcal/day[21]. Total body fat mass was measured by dual-energy X-ray
207 absorptiometry and expressed as grams (g). It was selected instead of BMI as the
208 adiposity-related covariate because it provides a more direct DXA-derived measure of
209 body composition, distinguishes fat mass from lean and bone mass, and can be
210 modeled as a time-varying covariate during follow-up.

211 Participants were classified as smokers or non-smokers based on self-reported
212 lifetime smoking history. Individuals who reported having smoked more than five
213 packs of cigarettes over their lifetime were categorized as smokers, whereas those who
214 had not were classified as non-smokers. Similarly, participants were classified as
215 drinkers or non-drinkers according to self-reported alcohol consumption history.
216 Individuals who reported regular alcohol consumption, defined as drinking at least

once per week for a continuous period of six months or longer, were categorized as drinkers; all others were classified as non-drinkers. Regular users of vitamin or calcium supplements were defined as participants reporting more than 30 doses during the previous year.

Menopausal status was assessed among female participants, and years since menopause were calculated based on self-reported age at natural menopause. Education level was categorized into three groups: junior high school or below, high school or technical secondary school, and college or above. Sex, age, education level, smoking status, alcohol consumption, use of complex vitamin supplements, calcium supplementation, years since menopause, energy intake, and physical activity were treated as baseline covariates, whereas total body fat mass and follow-up interval were treated as time-varying covariates when repeated measurements were available. Missing baseline covariate information was supplemented using available information from other questionnaire visits according to prespecified visit-priority rules. Participants with unresolved missing covariate information were excluded from fully adjusted analyses.

2.5 Statistical Analysis

Continuous variables were presented as means (SDs) or medians (IQRs), as appropriate, and categorical variables were presented as numbers and percentages. Baseline serum tea biomarker concentrations across long-term tea consumption groups were visualized using box-and-jitter plots. Overall differences among tea consumption groups were assessed using Kruskal–Wallis tests, and linear trends across ordered tea consumption categories were evaluated using unadjusted linear regression models with tea consumption groups assigned ordinal scores.

To assess whether longitudinal patterns of BMD and BMC differed across long-term tea consumption and serum biomarker groups, linear mixed-effects models were constructed including categorical visit, tea consumption group, and tea consumption group × visit interaction terms. F1 and non-tea drinkers were specified as the reference visit and reference group, respectively. Visit-specific and overall adjusted marginal means and 95% CIs using proportional weights were estimated. Two models were applied. Model 1 was adjusted for sex, age, and follow-up interval. Model 2 was additionally adjusted for education level, smoking status, alcohol consumption, use of complex vitamin supplements, calcium supplementation, years since menopause, energy intake, physical activity expressed as total metabolic equivalent of task, and time-varying total body fat mass. Linear mixed-effects model assumptions were evaluated using residual-versus-fitted plots and scale − location

plots to assess linearity and homoscedasticity, and normal Q – Q plots and residual histograms to assess the approximate normality of residuals. Model convergence and singularity were also examined. Missing repeated BMD and BMC measurements were not imputed; linear mixed-effects models were fitted using all available repeated outcome measurements, and previous methodological research found no clear benefit of performing multiple imputation before longitudinal mixed-model analysis [22].

Sensitivity analyses based on linear mixed models were also conducted after excluding participants with osteoporosis at F1, defined as a femoral neck T-score ≤ -2.5. Tea consumption was also modeled as a time-varying exposure by pairing tea consumption status with bone measurements obtained at the same visit (F1, F2, and F3). The associations of tea consumption with bone status were also evaluated using the constructed cumulative TeaScore. Potential nonlinear associations between continuous serum tea biomarker concentrations and BMD or BMC Z-scores were examined using restricted cubic spline models within the linear mixed-effects modeling framework. Biomarker concentrations were natural log-transformed as $\ln(x + 1 \times 10^{-6})$ and subsequently standardized within sex. Restricted cubic spline functions were fitted using four knots located at the 5th, 35th, 65th, and 95th percentiles.

As this study was based on an existing prospective cohort, no a priori sample size calculation was performed. To assess the adequacy of the available sample size, a post

hoc power analysis was conducted using the pooled standardized mean difference for hip BMD between tea consumers and non-consumers reported in a previous meta-analysis [23]. Using an effect size of 0.19, the actual sample sizes of 1,171 tea consumers and 537 non-consumers, and a two-sided significance level of 0.05, the available sample provided approximately 95.4% statistical power to detect the reported effect under an independent two-sample comparison.

All statistical analyses were conducted using R version 4.4.1. All tests were two-sided, and P values <0.05 were considered statistically significant. No formal adjustment for multiple comparisons was applied because the analyses involved prespecified, biologically related exposures and highly correlated bone outcomes. The primary analyses focused on the associations of long-term tea-drinking frequency with BMD and BMC, whereas serum tea biomarker analyses and sensitivity analyses were considered supportive. Results were interpreted based on the overall exposure-group and exposure-by-visit interaction tests, together with the consistency, magnitude, and precision of site- and visit-specific estimates.

3. Results

3.1 Characteristics of the participants

A total of 1,708 participants were included in the analysis, comprising 537 non–tea drinkers, 543 low-frequency tea drinkers, 522 moderate-frequency tea drinkers, and 106 high-frequency tea drinkers. Baseline characteristics according to tea consumption

293 categories are presented in Table 1. The median age was similar across the four tea
294 consumption groups, ranging from 57 to 58 years. The proportion of male participants
295 increased with tea consumption frequency, from 15.1% among non-tea drinkers to 66.0%
296 among high-frequency tea drinkers.

297 Serum tea biomarker concentrations differed across long-term tea consumption
298 groups (Supplementary Figure S2), and descriptive longitudinal trajectories of
299 site-specific BMD and BMC by sex are shown in Supplementary Figure S3. The
300 intra-individual coefficients of variation (CVs) for repeated BMD measurements were
301 1.18% for whole-body BMD, 0.87% for lumbar spine BMD, 1.02% for total hip BMD,
302 and 1.92% for femoral neck BMD [24].

303 **3.2 Associations of Tea Consumption with BMD and BMC**

304 Figure 1 and Supplementary Table S3 show the longitudinal trajectories of BMD
305 and BMC across long-term tea consumption groups in Model 2, with findings generally
306 consistent with those from Model 1 presented in Supplementary Figure S4. After fully
307 adjustment, Significant tea consumption group × visit interactions were observed for
308 whole-body, lumbar spine, and femoral neck BMD and whole-body BMC (P for
309 interaction ranged from < 0.001 to 0.022), indicating that their longitudinal changes
310 differed across tea consumption groups.

311 Adjusted marginal means of sex-specific BMD and BMC Z-scores across
312 long-term tea consumption groups are presented in Figure 2 and Supplementary Table
313 S4. Significant overall differences were observed for whole-body, lumbar spine, and

femoral neck BMD and BMC (P-overall ranged from < 0.001 to P ≤ 0.017), but not for total hip measures. Moderate-frequency tea drinkers generally had the highest adjusted marginal means.

Sensitivity analyses broadly supported the primary findings with the esclusion of participants with osteoporosis at the beginning of bone follow-up (Supplementary Figure S5). When tea consumption was treated as a time-varying exposure, associations differed across follow-up visits. Low-frequency tea drinkers showed more favorable changes in whole-body BMD and total hip BMC, particularly at F2, compared with non-drinkers. Moderate- and high-frequency tea drinkers showed greater increases in whole-body BMC at F2 and more favorable femoral neck BMD and BMC changes during follow-up. Total hip BMC also showed positive associations, particularly at later visits (Supplementary Figure S6). Analyses based on cumulative TeaScore categories showed significant overall interactions for most BMD and BMC outcomes, with more favorable changes generally observed in the high TeaScore group (Supplementary Figure S7). .

3.3 Associations of Serum tea biomarkers with BMD and BMC

Longitudinal trajectories of BMD and BMC across baseline serum tea biomarker categories after fully adjustment are shown in Figures 3, 4, Supplementary Table S5 and Table S6. The findings were generally consistent with those from Model 1, presented in Supplementary Figures S8–S9.

After adjustment for all potential confounders, significant overall interactions

335 were observed mainly for lumbar spine outcomes. Catechin categories were associated
336 with lumbar spine BMD and BMC trajectories (both P for interaction <0.001).
337 Compared with Group 1, the F1-to-F4 changes in lumbar spine BMD and BMC were
338 0.282 and 0.279 sex-specific SD units greater, respectively, in Group 3 (BMD: β =
339 0.282; 95% CI: 0.184, 0.380; BMC: β = 0.279; 95% CI: 0.181, 0.378; both P < 0.001).
340 ECG categories were associated with lumbar spine BMC trajectories (P for overall
341 < 0.001; P for interaction = 0.042). Compared with Group 1, Group 4 showed a greater
342 F1-to-F4 increase in lumbar spine BMC (β = 0.165; 95% CI: 0.066, 0.264; P = 0.001).
343 EGC categories were associated with whole-body and lumbar spine BMD and BMC
344 trajectories (all P-overall ≤ 0.009; all P-interaction ≤ 0.007). Compared with Group 1,
345 the largest F1-to-F4 increases were observed in Group 3 for whole-body BMD and
346 BMC (β = 0.164 and 0.118, respectively), in Groups 2 and 3 for lumbar spine BMD (β
347 = 0.169 and 0.166, respectively), and in Group 4 for lumbar spine BMC (β = 0.186;
348 all P ≤ 0.001). Epicatechin categories were associated with whole-body and lumbar
349 spine BMD and lumbar spine BMC trajectories (P-interaction = 0.022, 0.002, and
350 0.003, respectively), with the largest F1-to-F4 increases observed in Group 3 for
351 lumbar spine BMD and Group 4 for lumbar spine BMC (β = 0.194 and 0.185,
352 respectively; both P < 0.001).
353 No significant overall interactions were observed for EGCG. Several isolated
354 visit-specific coefficients were also observed for outcomes without significant overall
355 interactions and were interpreted cautiously. Overall, the longitudinal associations were

concentrated mainly at the lumbar spine and varied across biomarkers, categories, and visits.

Visit-averaged adjusted marginal means are shown in Figures 5 and 6 and Supplementary Table S7. Significant differences across ECG categories were observed for whole-body, lumbar spine, and femoral neck BMD and for whole-body and lumbar spine BMC (P-diff ranged from <0.001 to 0.010). EGCG categories also differed in lumbar spine and femoral neck BMD and in whole-body and lumbar spine BMC (P-diff ranged from <0.001 to 0.028). Participants with higher catechin and epicatechin also tended to have higher BMD and/or BMC at lumbar spine and femoral neck (P-trend ranged from 0.020 to 0.034). No significant overall differences or linear trend were observed for EGC.

Supplementary Figures S10–S14 show the sensitivity analyses treating serum biomarker concentrations as continuous exposures using restricted cubic spline models. Significant overall associations were observed for selected outcomes, with the most extensive findings for ECG and EGCG (all P-overall $\leq$ 0.001), whereas catechin, EGC, and epicatechin showed more site-specific associations (P-overall ranged from < 0.001 to 0.042). Significant biomarker × visit interactions were also observed for several outcomes, indicating that the associations varied across follow-up visits. Nonlinear associations were most evident for EGCG (P-nonlinearity ranged from < 0.001 to 0.004), particularly across multiple BMC sites, while more limited and outcome-specific nonlinearity was observed for catechin, ECG, EGC, and epicatechin

(P-nonlinearity ranged from 0.003 to 0.043).

4. Discussion

This prospective cohort study systematically evaluated the associations of long-term tea consumption and baseline serum tea biomarker concentrations with repeated BMD and BMC measurements at multiple skeletal sites in middle-aged and older adults. Moderate- frequency tea drinkers generally exhibited better bone mineral status and more favorable longitudinal changes. Higher categories of several baseline serum tea biomarkers were generally associated with better bone mineral status.

To date, several meta-analyses have evaluated the association between tea consumption—assessed using dietary intake estimates—and bone health. A recent meta-analysis of 18 studies including 48,615 postmenopausal women reported higher BMD at several skeletal sites and lower risks of osteoporosis and fractures among tea consumers[25]. However, other meta-analyses have failed to find a significant association between tea consumption and the risk of osteoporotic fractures[26, 27]. A recent prospective cohort study with repeated measurements of tea drinking and bone mineral status over 10 years of follow-up showed that tea consumption was positively associated with total hip BMD[15]. In our study, moderate-frequency tea drinkers generally had the highest adjusted bone mineral measures. These findings are broadly consistent with the meta-analysis by Sheng et al., which suggested a nonlinear relationship between tea intake and hip fracture risk[28]. A one-stage dose–response analysis showed that individuals who consumed fewer than 4.5 cups of tea per day had

a statistically significant lower risk of bone health–related outcomes than non-tea drinkers [10]. Together, these findings suggest that the association between tea consumption and bone health may be nonlinear and that higher intake does not necessarily confer proportionally greater skeletal benefits.

We also found that higher categories of several serum tea biomarkers were generally associated with better bone mineral status, with the most consistent visit-averaged associations observed for ECG and EGCG. Participants with higher circulating levels of most biomarkers also exhibited more favorable changes in bone measures across most skeletal sites. Human intervention evidence provides some biological support for our findings, although results have not been entirely consistent. In a 6-month randomized trial among postmenopausal women with osteopenia [29], supplementation with 500 mg/day of green tea polyphenols increased bone-specific alkaline phosphatase (BAP) and the ratio of BAP to tartrate-resistant acid phosphatase (TRAP). A secondary analysis of the same trial showed that supplementation increased circulating EGCG and ECG concentrations and reduced urinary oxidative DNA damage [30]. In contrast, a 12-month trial providing decaffeinated green tea extract containing 843 mg/day of EGCG did not significantly improve BMD in overweight or obese postmenopausal women [31]. Short-term changes in bone turnover markers may not necessarily translate into measurable improvements in BMD within 6–12 months. Moreover, these trials support biological plausibility but do not directly establish associations between naturally occurring circulating catechin concentrations

and bone mineral outcomes.

Experimental studies suggest that major Catechins, including EGCG, ECG, and Epicatechin, may influence bone remodeling through distinct anti-resorptive and pro-osteogenic mechanisms. EGCG has been shown to suppress RANKL-induced osteoclastogenesis by inhibiting NF-κB and MAPK/AP-1 signaling and downregulating osteoclastogenic genes such as NFATc1 and cathepsin K[32, 33], while Epicatechin, ECG, and EGCG have been reported to promote osteoblast differentiation through upregulation of Runx2 and osterix and enhancement of mineralized matrix formation[34-36]. These dual anti-resorptive and pro-formative actions are closely linked to structural features: gallated catechins (EGCG, ECG) exhibit stronger protein-binding affinity and redox activity than non-gallated forms[37-41], whereas differences in hydroxylation patterns influence reactive oxygen species scavenging and modulation of redox-sensitive signaling pathways[42]. Nevertheless, these biological effects may vary according to exposure level and metabolic context. At physiologically relevant concentrations, Catechins attenuate TNF-α–mediated inflammatory signaling and oxidative stress, thereby favoring balanced remodeling[43-46]. Furthermore, extensive phase II metabolism and gut microbial biotransformation generate circulating metabolites with altered bioactivity profiles, which may contribute to variation in the biological effects associated with different circulating biomarkers [47, 48]. These structural and metabolic differences may partly explain the biomarker-specific patterns observed in our study, although the

440 underlying mechanisms remain uncertain.

441 **Advantages and Limitations**

442 This study has several notable strengths. Using a large community-based cohort
443 with a long follow-up and repeated bone assessments, we comprehensively evaluated
444 associations between tea consumption and both BMD and BMC across multiple
445 skeletal sites. This design provides a more complete characterization of bone mineral
446 status and longitudinal changes than studies limited to a single site or to fracture
447 outcomes alone. In addition, integrating self-reported tea intake with objective serum
448 tea biomarkers (flavan-3-ol metabolites) complemented the questionnaire-based
449 exposure assessment and provided additional information on internal exposure.
450 Nonetheless, several limitations merit consideration: First, residual confounding and
451 reverse causality cannot be excluded. Second, tea consumption was self-reported and
452 may have been misclassified; serum flavan-3-ol biomarkers were measured only once
453 and could not capture changes during follow-up. Third, the high-frequency tea group
454 was small, resulting in less precise estimates. Although the exposures and outcomes
455 were prespecified, the number of biomarker-, skeletal-site-, and visit-specific
456 comparisons increased the possibility of chance findings. Because no formal
457 adjustment for multiple comparisons was applied, isolated associations, particularly
458 those without significant overall tests, should be interpreted cautiously and require
459 confirmation in independent studies. Finally, participants were recruited from
460 Guangzhou, and the results may not be generalizable to populations with different

461 tea-drinking habits, tea types, or preparation methods.

462 **5. Conclusion**

463 In this prospective cohort, moderate-frequency tea consumption was associated
464 with better bone mineral status and more favorable longitudinal changes. These
465 findings suggest that habitual tea consumption and circulating flavan-3-ol
466 concentrations may be related to bone health; however, they do not establish an optimal
467 level of tea intake or serum biomarker concentration. Further studies incorporating
468 repeated biomarker measurements and more detailed assessments of tea intake, dose,
469 and preparation methods are warranted.

470 **Author contributions**

471 Silei He: Methodology, Formal analysis, Writing – original draft, Writing – review &
472 editing, Visualization. Yan Yan: Investigation, Data curation. Sijia Zhou: Methodology,
473 Writing – original draft, Writing – review & editing, Visualization. Zhen Hong: Data
474 curation. Shuyang Huang: Data curation. Yi Liu: Data curation. Enhui Tang: Data
475 curation. Yue Xi: Investigation. Yuming Chen: Conceptualization, Supervision &
476 manuscript revision, Project administration, Funding acquisition. Zheqing Zhang:
477 Conceptualization, Supervision & manuscript revision, Project administration, Funding
478 acquisition.

479 **Data sharing statement**

480 The clinical and dietary datasets used and analysed in the current study are available
481 from the corresponding author upon reasonable request. The bone mineral density and

bone mineral content data are not publicly available due to participant privacy restrictions.

AI usage statement

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language editing and improvement of readability. After using this tool, the authors carefully reviewed and revised the content and take full responsibility for the content of the manuscript.

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Appendix A. Supplementary data

Supplementary tables and figures for this article are provided in the Supplementary File submitted with this manuscript.

References

[1]. NIH Consensus Development Panel on Osteoporosis Prevention D, and Therapy. Osteoporosis prevention, diagnosis, and therapy [J]. JAMA. 2001;285:785-95.

[2]. Morin SN, Leslie WD, Schousboe JT. Osteoporosis: A Review [J]. JAMA. 2025;334:894-907.

[3]. Salari N, Ghasemi H, Mohammadi L, Behzadi MH, Rabieenia E, Shohaimi S, et al. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis [J]. J Orthop Surg Res. 2021;16:609.

[4]. Si L, Winzenberg TM, Jiang Q, Chen M, Palmer AJ. Projection of osteoporosis-related fractures and costs in China: 2010-2050 [J]. Osteoporos Int. 2015;26:1929-37.

[5]. Ong AM, Kang K, Weiler HA, Morin SN. Fermented Milk Products and Bone Health in Postmenopausal Women: A Systematic Review of Randomized Controlled Trials, Prospective Cohorts, and Case-Control Studies [J]. Adv Nutr. 2020;11:251-65.
[6]. Zhou C, Fang L, Chen Y, Zhong J, Wang H, Xie P. Effect of selective serotonin reuptake inhibitors on bone mineral density: a systematic review and meta-analysis [J]. Osteoporos Int. 2018;29:1243-51.
[7]. Radeva-Ilieva M, Stoeva S, Hvarchanova N, Georgiev KD. Green Tea: Current Knowledge and Issues [J]. Foods. 2025;14(5).
[8]. McAlpine MD, Gittings W, MacNeil AJ, Ward WE. Black and Green Tea as Well as Specialty Teas Increase Osteoblast Mineralization with Varying Effectiveness [J]. J Med Food. 2021;24:866-72.
[9]. Yamamoto N, Tokuda H, Kuroyanagi G, Kainuma S, Ohguchi R, Fujita K, et al. Amplification by (-)-epigallocatechin gallate and chlorogenic acid of TNF-alpha-stimulated interleukin-6 synthesis in osteoblasts [J]. Int J Mol Med. 2015;36:1707-12.
[10]. Zhou F, Wang T, Li L, Yu J, Liu Z, Zhang J, et al. Tea consumption and risk of bone health: an updated systematic review and meta-analysis [J]. J Bone Miner Metab. 2024;42:99-114.
[11]. Chen CC, Shen YM, Li SB, Huang SW, Kuo YJ, Chen YP. Association of Coffee and Tea Intake with Bone Mineral Density and Hip Fracture: A Meta-Analysis [J]. Medicina (Kaunas). 2023;59(6).
[12]. Xia SL, Ma ZY, Wang B, Guo SY, Zhou XX, Gao F. The Association between Tea Consumption and the Risk of Fracture: A Dose-Response Meta-Analysis of Prospective Cohort Studies [J]. J Nutr Health Aging. 2021;25(9):1046-52.
[13]. Xiang W, Gu K, Wang W, Jiang X. Tea consumption and risk of fractures: an updated meta-analysis [J]. Osteoporos Int. 2019;30(10):1941-51.
[14]. Chen B, Shi HF, Wu SC. Tea consumption didn't modify the risk of fracture: a dose-response meta-analysis of observational studies [J]. Diagn Pathol. 2014;9:44.
[15]. Liu RY, Liu E. Longitudinal Association of Coffee and Tea Consumption with Bone Mineral Density in Older Women: A 10-Year Repeated-Measures Analysis in the Study of Osteoporotic Fractures [J]. Nutrients. 2025;17(23).
[16]. Ling CW, Zhong H, Zeng FF, Chen G, Fu Y, Wang C, et al. Cohort Profile: Guangzhou Nutrition and Health Study (GNHS): A Population-based Multi-omics Study [J]. J Epidemiol. 2024;34(6):301-6.
[17]. Feng XL, Zhan XX, Zuo LS, Mo XF, Zhang X, Liu KY, et al. Associations between serum concentration of flavonoids and breast cancer risk among Chinese women [J]. Eur J Nutr. 2021;60(3):1347-62.
[18]. Xi G, Jiang-fan Y, Xiang-ying XIE, Li-qiong LIN. Research on The Status of Chinese Tea Consumption and Factors of Tea Consumer’s Behavior [J]. J Tea Sci. 2011;31(6):546-51.
[19]. Thulkar J, Singh S, Sharma S, Thulkar T. Preventable risk factors for osteoporosis in postmenopausal women: Systematic review and meta-analysis [J]. J

Midlife Health. 2016;7(3):108-13.
[20]. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values [J]. Med Sci Sports Exerc. 2011;43(8):1575-81.
[21]. Yang Y, Wang G-y, Pan X-c. China food composition [J]. Peking University Medical Press: Beijing, China. 2009;42:795-9.
[22]. Twisk J, de Boer M, de Vente W, Heymans M. Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis [J]. J Clin Epidemiol. 2013;66(9):1022-8.
[23]. Zhang ZF, Yang JL, Jiang HC, Lai Z, Wu F, Liu ZX. Updated association of tea consumption and bone mineral density: A meta-analysis [J]. Medicine (Baltimore). 2017;96(12):e6437.
[24]. Liu ZM, Huang Q, Long HH, Li SY, Wu Y, Zhang SJ, et al. Increased Dietary Intakes of Total Protein, Animal Protein and White Meat Protein Were Associated with Reduced Bone Loss-A Prospective Analysis Based on Guangzhou Health and Nutrition Cohort, South China [J]. Nutrients. 2023;15(6).
[25]. Zhang M, Li S, Wu S, Zhou D, Lu M, Lin C, et al. Tea consumption and bone health in postmenopausal women: a systematic review and meta-analysis [J]. Arch Osteoporos. 2025;20(1):20.
[26]. Guo M, Qu H, Xu L, Shi D-Z. Tea consumption may decrease the risk of osteoporosis: an updated meta-analysis of observational studies [J]. Nutr Res. 2017;42:1-10.
[27]. Chen B, Shi H-F, Wu S-C. Tea consumption didn't modify the risk of fracture: a dose-response meta-analysis of observational studies [J]. Diagn Pathol. 2014;9:44-.
[28]. Sheng J, Qu X, Zhang X, Zhai Z, Li H, Liu X, et al. Coffee, tea, and the risk of hip fracture: a meta-analysis [J]. Osteoporos Int. 2014;25(1):141-50.
[29]. Shen CL, Chyu MC, Yeh JK, Zhang Y, Pence BC, Felton CK, et al. Effect of green tea and Tai Chi on bone health in postmenopausal osteopenic women: a 6-month randomized placebo-controlled trial [J]. Osteoporos Int. 2012;23(5):1541-52.
[30]. Qian G, Xue K, Tang L, Wang F, Song X, Chyu MC, et al. Mitigation of oxidative damage by green tea polyphenols and Tai Chi exercise in postmenopausal women with osteopenia [J]. PLoS One. 2012;7(10):e48090.
[31]. Dostal AM, Arikawa A, Espejo L, Kurzer MS. Long-Term Supplementation of Green Tea Extract Does Not Modify Adiposity or Bone Mineral Density in a Randomized Trial of Overweight and Obese Postmenopausal Women [J]. J Nutr. 2016;146(2):256-64.
[32]. Xu H, Liu T, Jia Y, Li J, Jiang L, Hu C, et al. (-)-Epigallocatechin-3-gallate inhibits osteoclastogenesis by blocking RANKL-RANK interaction and suppressing NF-κB and MAPK signaling pathways [J]. Int Immunopharmacol. 2021;95:107464.
[33]. Lee J-H, Jin H, Shim H-E, Kim H-N, Ha H, Lee ZH. Epigallocatechin-3-gallate inhibits osteoclastogenesis by down-regulating c-Fos expression and suppressing the nuclear factor-kappaB signal [J]. Mol Pharmacol.

2010;77:17-25.
[34]. Palma-Lara I, Calzada-Mendoza CC, Mera-Jiménez E, Romero López E, Amaya-Espinoza JL, Parra-Barrera A, et al. Phytochemical Properties of (-)-Epicatechin Promotes Bone Regeneration Inducing Osteogenic Markers Expression BMP2, SPARC, and RUNX2 in Mesenchymal Stem Cells In Vitro [J]. J Med Food. 2025;28:757-67.
[35]. Byun MR, Sung MK, Kim AR, Lee CH, Jang EJ, Jeong MG, et al. (-)-Epicatechin gallate (ECG) stimulates osteoblast differentiation via Runt-related transcription factor 2 (RUNX2) and transcriptional coactivator with PDZ-binding motif (TAZ)-mediated transcriptional activation [J]. J Biol Chem. 2014;289:9926-35.
[36]. Chen C-H, Ho M-L, Chang J-K, Hung S-H, Wang G-J. Green tea catechin enhances osteogenesis in a bone marrow mesenchymal stem cell line [J]. Osteoporos Int. 2005;16:2039-45.
[37]. Nag A, Dhull N, Gupta A. Evaluation of tea (Camellia sinensis L.) phytochemicals as multi-disease modulators, a multidimensional in silico strategy with the combinations of network pharmacology, pharmacophore analysis, statistics and molecular docking [J]. Mol Divers. 2023;27:487-509.
[38]. Pal S, Dey SK, Saha C. Inhibition of catalase by tea catechins in free and cellular state: a biophysical approach [J]. PloS one. 2014;9:e102460.
[39]. Xiao J, Kai G. A review of dietary polyphenol-plasma protein interactions: characterization, influence on the bioactivity, and structure-affinity relationship [J]. Crit Rev Food Sci Nutr. 2012;52:85-101.
[40]. Speisky H, Shahidi F, Costa de Camargo A, Fuentes J. Revisiting the Oxidation of Flavonoids: Loss, Conservation or Enhancement of Their Antioxidant Properties [J]. Antioxidants (Basel). 2022;11(1).
[41]. Vo QV, Nam PC, Thong NM, Trung NT, Phan CD, Mechler A. Antioxidant Motifs in Flavonoids: O-H versus C-H Bond Dissociation [J]. ACS Omega. 2019;4(5):8935-42.
[42]. Katiyar SK, Agarwal R, Mukhtar H. Inhibition of spontaneous and photo-enhanced lipid peroxidation in mouse epidermal microsomes by epicatechin derivatives from green tea [J]. Cancer letters. 1994;79:61-6.
[43]. de Oliveira AFS, Vasconcellos GL, Lopes DJP, de Macedo LR, Silva M. Effect of Green Tea Supplementation on Inflammatory Markers among Patients with Metabolic Syndrome and Related Disorders: A Systematic Review and Meta-Analysis [J]. Prev Nutr Food Sci. 2024;29:106-17.
[44]. Bettaieb A, Cremonini E, Kang H, Kang J, Haj FG, Oteiza PI. Anti-inflammatory actions of (-)-epicatechin in the adipose tissue of obese mice [J]. Int J Biochem Cell Biol. 2016;81:383-92.
[45]. Peluso I, Raguzzini A, Serafini M. Effect of flavonoids on circulating levels of TNF-alpha and IL-6 in humans: a systematic review and meta-analysis [J]. Mol Nutr Food Res. 2013;57:784-801.
[46]. Vazquez-Prieto MA, Bettaieb A, Haj FG, Fraga CG, Oteiza PI.

(-)-Epicatechin prevents TNFα-induced activation of signaling cascades involved in inflammation and insulin sensitivity in 3T3-L1 adipocytes [J]. Arch Biochem Biophys. 2012;527:113-8.

[47]. Cai Z-Y, Li X-M, Liang J-P, Xiang L-P, Wang K-R, Shi Y-L, et al. Bioavailability of Tea Catechins and Its Improvement [J]. Molecules. 2018;23:2346.

[48]. Wein S, Beyer B, Gohlke A, Blank R, Metges CC, Wolffram S. Systemic Absorption of Catechins after Intraruminal or Intraduodenal Application of a Green Tea Extract in Cows [J]. PloS one. 2016;11:e0159428.

643 Table 1. Participant characteristics by tea consumption frequency (n =1,708)

Variable	Non-tea drinkers (n=537)	Low-frequency tea drinkers (n=543)	Moderate-frequency tea drinkers (n=522)	High-frequency tea drinkers (n=106)
Age (years)	58 (54.7-62)	57 (54-61.2)	58 (54-63)	57 (54-60.8)
Sex (n,%)				
female	456 (84.9%)	403 (74.2%)	289 (55.4%)	36 (34%)
male	81 (15.1%)	140 (25.8%)	233 (44.6%)	70 (66%)
BMI (kg/m^2)	22.3 (20.5-24)	23.2 (21.4-25.2)	23.6 (21.5-25.5)	23.8 (22.1-26)
MET (MET-h/day)	36 (30.6-56.1)	35.5 (30.6-44.2)	36 (31.1-45.2)	35.2 (31.4-50.7)
Energy intake (kcal/d)	1636.4 (1346.9-1988.9)	1697.5 (1450-2010.1)	1669.8 (1426.7-2070.6)	2053 (1675.6-2426.3)
Years Since Menopause (years)	6 (1-11)	4 (0-9)	1 (0-7)	0 (0-1.9)
Serum tea biomarkers (ng/mL)				
Catechin	0.2 (0-0.6)	0.3 (0.1-0.8)	0.6 (0.2-1.3)	0.9 (0.2-1.8)
Epicatechin	0 (0-0.2)	0.1 (0-0.2)	0.3 (0.1-1.1)	0.8 (0.3-3.1)
EGC	0 (0-0)	0 (0-0)	0.1 (0-0.4)	0.2 (0-1.3)
EGCG	0.1 (0-0.7)	0.6 (0.1-2)	3.9 (0.8-11.7)	8.7 (1.5-29.2)
ECG	0.4 (0.1-1.6)	1.6 (0.3-4.6)	8 (2.4-20.4)	16.6 (4.9-36.7)
Education Level (n,%)				
Junior high or below	161 (30%)	125 (23%)	123 (23.6%)	32 (30.2%)
High school or technical secondary	244 (45.4%)	259 (47.7%)	245 (46.9%)	45 (42.5%)
College or above	132 (24.6%)	159 (29.3%)	154 (29.5%)	29 (27.4%)
Smoking Status (n,%)				
no	516 (96.1%)	483 (89%)	406 (77.8%)	53 (50%)
yes	21 (3.9%)	60 (11%)	116 (22.2%)	53 (50%)

Alcohol Consumption (n,%)				
no	525 (97.8%)	520 (95.8%)	461 (88.3%)	93 (87.7%)
yes	12 (2.2%)	23 (4.2%)	61 (11.7%)	13 (12.3%)
Complex vitamin users (n,%)				
no	404 (75.2%)	416 (76.6%)	430 (82.4%)	85 (80.2%)
yes	133 (24.8%)	127 (23.4%)	92 (17.6%)	21 (19.8%)
Calcium supplement users (n,%)				
no	359 (66.9%)	367 (67.6%)	385 (73.8%)	71 (67%)
yes	178 (33.1%)	176 (32.4%)	137 (26.2%)	35 (33%)

Note: Continuous variables are presented as median (interquartile range, IQR) due to non-normal distribution, and categorical variables are presented as number (percentage). Smoking status and alcohol consumption were categorized as yes/no based on self-reported history. Education level was categorized as junior high school or below, high school or technical secondary school, and college or above. Complex vitamin users and calcium supplement users were defined as participants reporting more than 30 doses during the previous year.

Abbreviations: BMI, body mass index; MET, metabolic equivalent of task.

Figure legends

Figure 1. Longitudinal trajectories of BMD and BMC according to long-term tea consumption groups

Note: Analyses included 1,708 participants. The y-axis displays adjusted marginal means of sex-specific bone mineral density (BMD) and bone mineral content (BMC) Z-scores. Points and error bars represent adjusted marginal means and 95% confidence intervals (CIs) of sex-specific bone mineral density (BMD) and bone mineral content (BMC) Z-scores at each visit, estimated using linear mixed-effects models with tea consumption group, visit, and tea consumption group × visit interaction terms and a participant-specific random intercept. Non-tea drinkers and F1 were the reference categories. P-overall and P-interaction were obtained using likelihood-ratio tests; individual interaction coefficients were tested using t tests with Satterthwaite-approximated degrees of freedom. Models were adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and calcium supplement use, years since menopause, energy intake, total physical activity, time-varying total body fat mass, and follow-up interval. Only interaction coefficients with $P < 0.05$ are displayed. WB, whole body; LS, lumbar spine; FN, femoral neck; TH, total hip.

Figure 2. Adjusted mean BMD and BMC across tea consumption groups after accounting for tea group × visit interactions

Note: Analyses included 1,708 participants. Bars and error bars represent visit-averaged adjusted marginal means and 95% confidence intervals (CIs) of

674 sex-specific bone mineral density (BMD) and bone mineral content (BMC) Z-scores.
675 Estimates were obtained from linear mixed-effects models including tea consumption
676 group, visit, and tea consumption group × visit interaction terms, with a
677 participant-specific random intercept, and were averaged across visits using
678 proportional weighting. P-diff was obtained using a joint Wald test of equality across
679 the four visit-averaged means; P-trend was obtained using a Wald t test of a
680 prespecified linear contrast across ordered tea consumption groups. Models were
681 adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and
682 calcium supplement use, years since menopause, energy intake, total physical activity,
683 time-varying total body fat mass, and follow-up interval. WB, whole body; LS, lumbar
684 spine; FN, femoral neck; TH, total hip.

685 **Figure 3. Longitudinal trajectories of BMD according to baseline serum tea**
686 **biomarker categories**

687 Note: Panels A–E represent the following serum tea biomarkers: A, catechin; B,
688 epicatechin gallate (ECG); C, epigallocatechin (EGC); D, epigallocatechin gallate
689 (EGCG); and E, epicatechin. Analyses included 1,461 participants with baseline serum
690 tea biomarker measurements and bone follow-up data. The y-axis displays adjusted
691 marginal means of sex-specific bone mineral density (BMD) and bone mineral
692 content (BMC) Z-scores. Points and error bars represent adjusted marginal means and
693 95% confidence intervals (CIs) of sex-specific bone mineral density (BMD) Z-scores at
694 each visit, estimated using linear mixed-effects models including biomarker category,

visit, and biomarker category × visit interaction terms, with a participant-specific random intercept. Group 1 and F1 were the reference categories. P-overall and P-interaction were obtained using likelihood-ratio tests; individual interaction coefficients were tested using t tests with Satterthwaite-approximated degrees of freedom. Models were adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and calcium supplement use, years since menopause, energy intake, total physical activity, time-varying total body fat mass, and follow-up interval. Only interaction coefficients with $P < 0.05$ are displayed. WB, whole body; LS, lumbar spine; FN, femoral neck; TH, total hip.

Figure 4. Longitudinal trajectories of BMC according to baseline serum tea biomarker categories

Note: Panels A–E represent the following serum tea biomarkers: A, catechin; B, epicatechin gallate (ECG); C, epigallocatechin (EGC); D, epigallocatechin gallate (EGCG); and E, epicatechin. Analyses included 1,461 participants with baseline serum tea biomarker measurements and bone follow-up data. The y-axis displays adjusted marginal means of sex-specific bone mineral density (BMD) and bone mineral content (BMC) Z-scores. Points and error bars represent adjusted marginal means and 95% confidence intervals (CIs) of sex-specific bone mineral content (BMC) Z-scores at each visit, estimated using linear mixed-effects models including biomarker category, visit, and biomarker category × visit interaction terms, with a participant-specific random intercept. Group 1 and F1 were the reference categories. P-overall and P-interaction

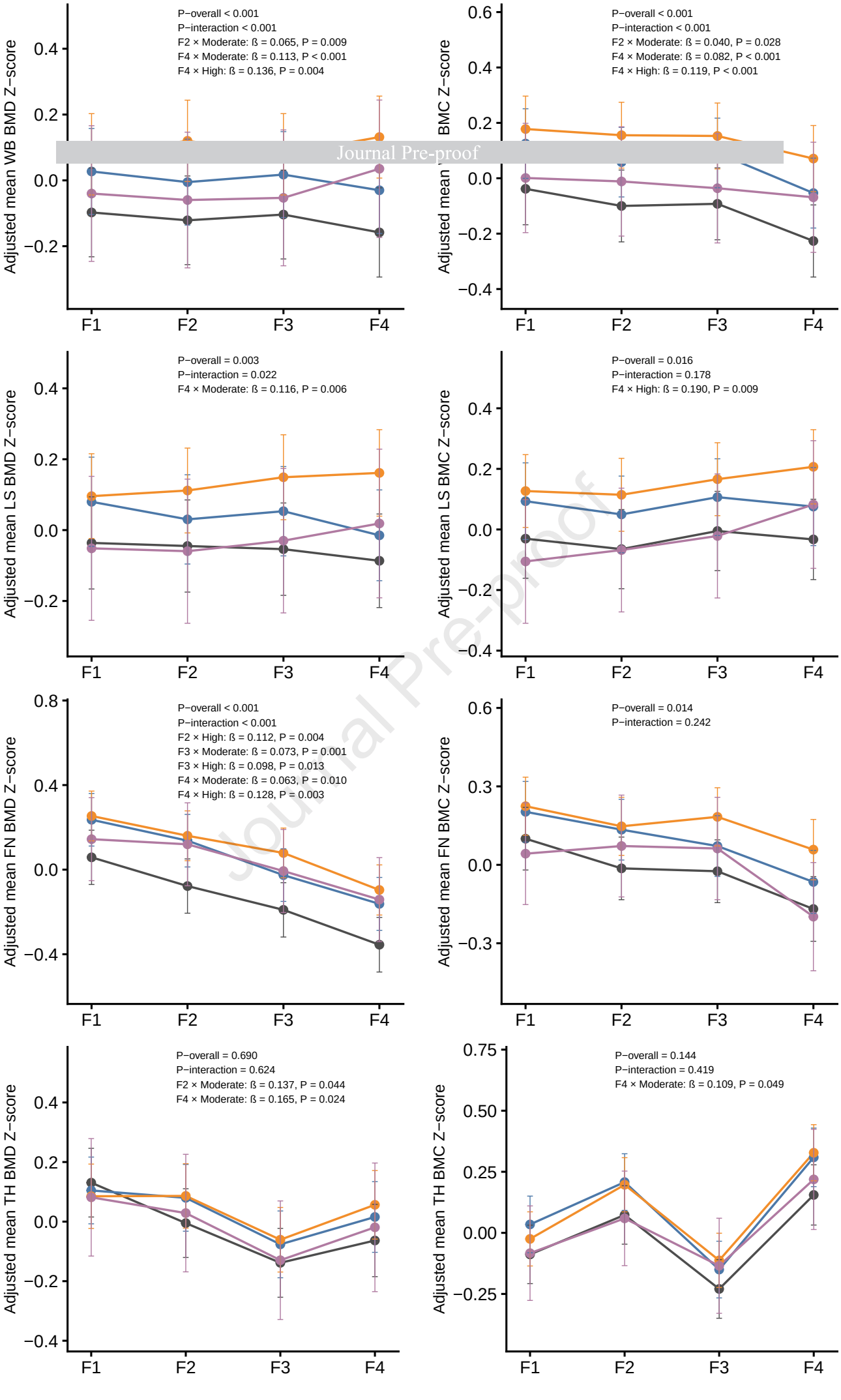
were obtained using likelihood-ratio tests; individual interaction coefficients were tested using t tests with Satterthwaite-approximated degrees of freedom. Models were adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and calcium supplement use, years since menopause, energy intake, total physical activity, time-varying total body fat mass, and follow-up interval. Only interaction coefficients with $P < 0.05$ are displayed. WB, whole body; LS, lumbar spine; FN, femoral neck; TH, total hip.

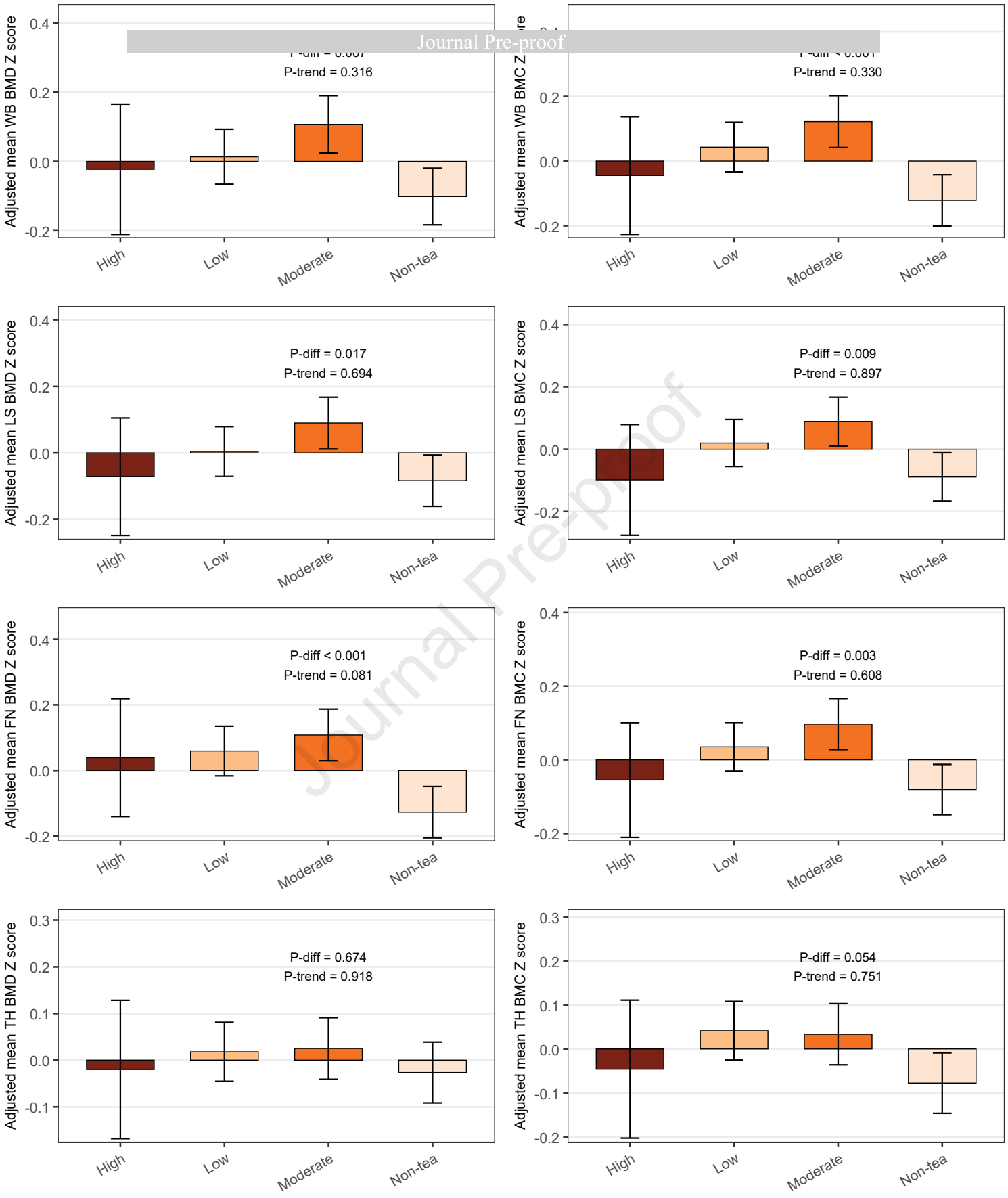
Figure 5. Adjusted mean BMD across serum tea biomarker categories after accounting for biomarker category × visit interactions

Note: Analyses included 1,461 participants with baseline serum tea biomarker measurements; panel-specific sample sizes are shown in the figure. Points and error bars represent visit-averaged adjusted marginal means and 95% confidence intervals (CIs) of sex-specific bone mineral density (BMD) Z-scores. Estimates were obtained from linear mixed-effects models including biomarker category, visit, and biomarker category × visit interaction terms, with a participant-specific random intercept, and were averaged across visits using proportional weighting. P-diff was obtained using a joint Wald test of equality across the four visit-averaged means; P-trend was obtained using a Wald t test of a prespecified linear contrast across ordered biomarker categories. Models were adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and calcium supplement use, years since menopause, energy intake, total physical activity, time-varying total body fat mass, and follow-up interval.

Figure 6. Adjusted mean BMC across serum tea biomarker categories after accounting for biomarker category × visit interactions

Note: Analyses included 1,461 participants with baseline serum tea biomarker measurements; panel-specific sample sizes are shown in the figure. Points and error bars represent visit-averaged adjusted marginal means and 95% confidence intervals (CIs) of sex-specific bone mineral content (BMC) Z-scores. Estimates were obtained from linear mixed-effects models including biomarker category, visit, and biomarker category × visit interaction terms, with a participant-specific random intercept, and were averaged across visits using proportional weighting. P-diff was obtained using a joint Wald test of equality across the four visit-averaged means; P-trend was obtained using a Wald t test of a prespecified linear contrast across ordered biomarker categories. Models were adjusted for age, sex, education, smoking, alcohol consumption, complex vitamin and calcium supplement use, years since menopause, energy intake, total physical activity, time-varying total body fat mass, and follow-up interval.





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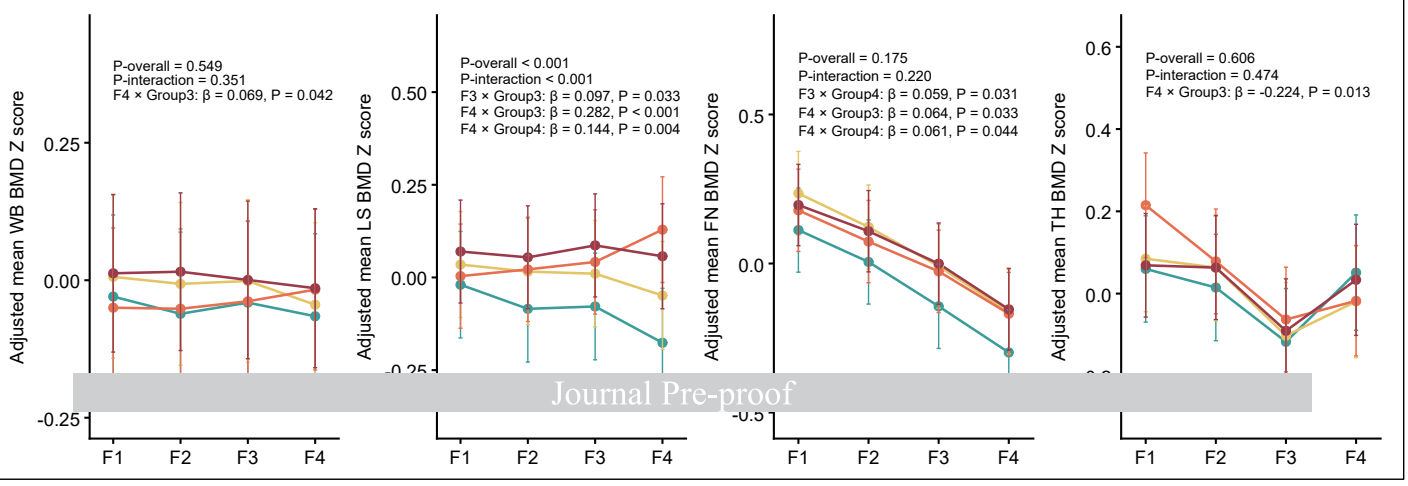

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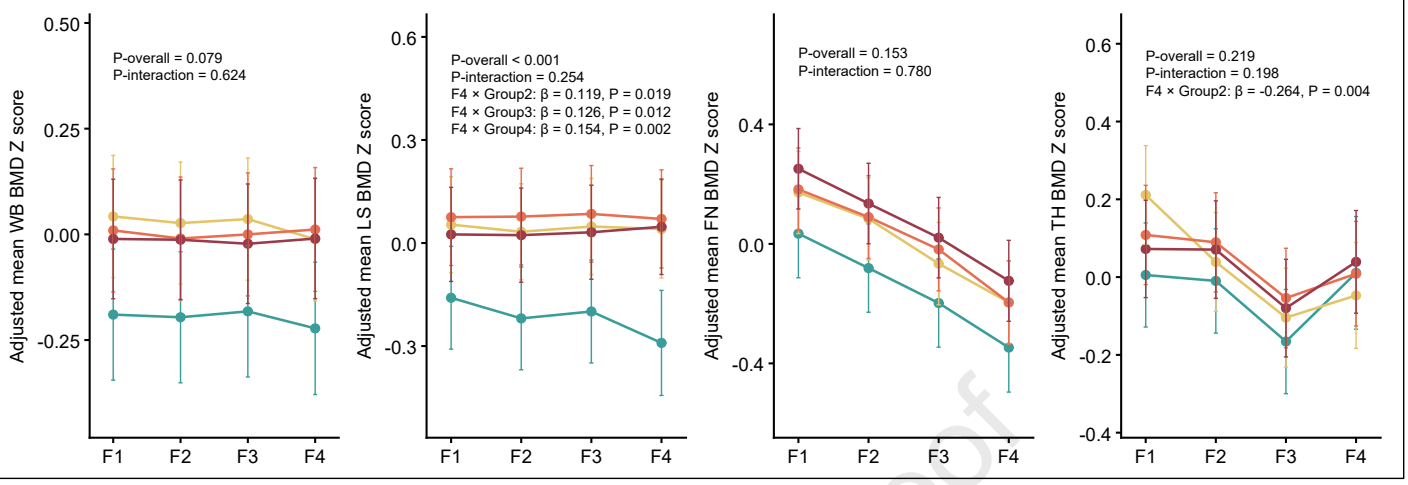

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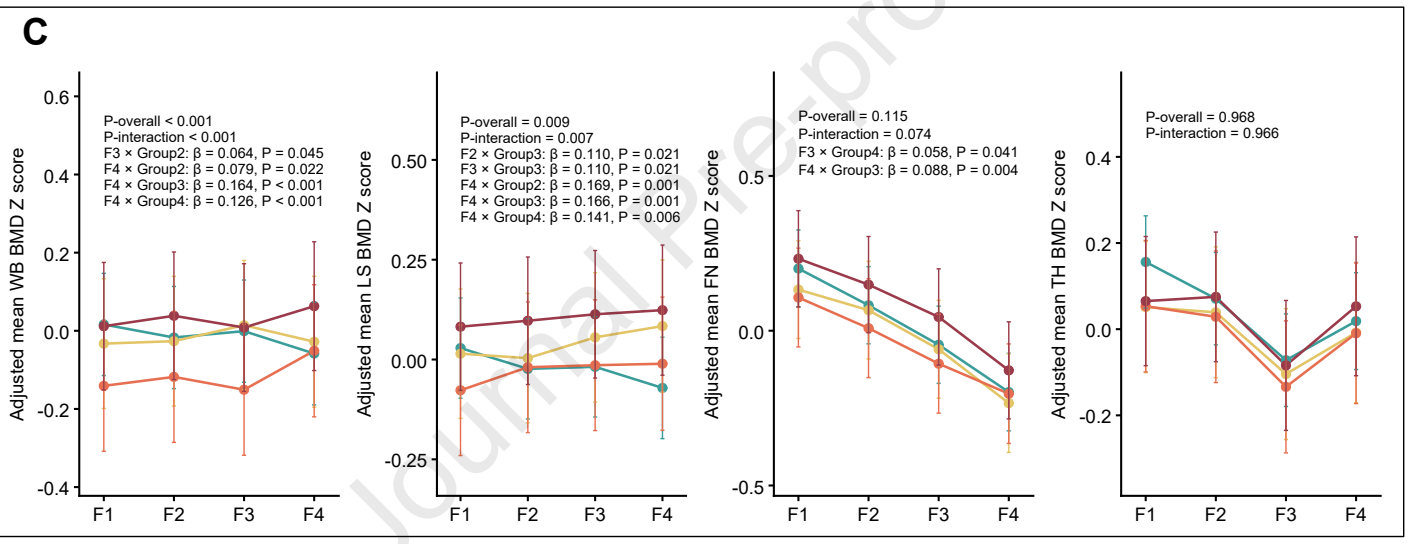

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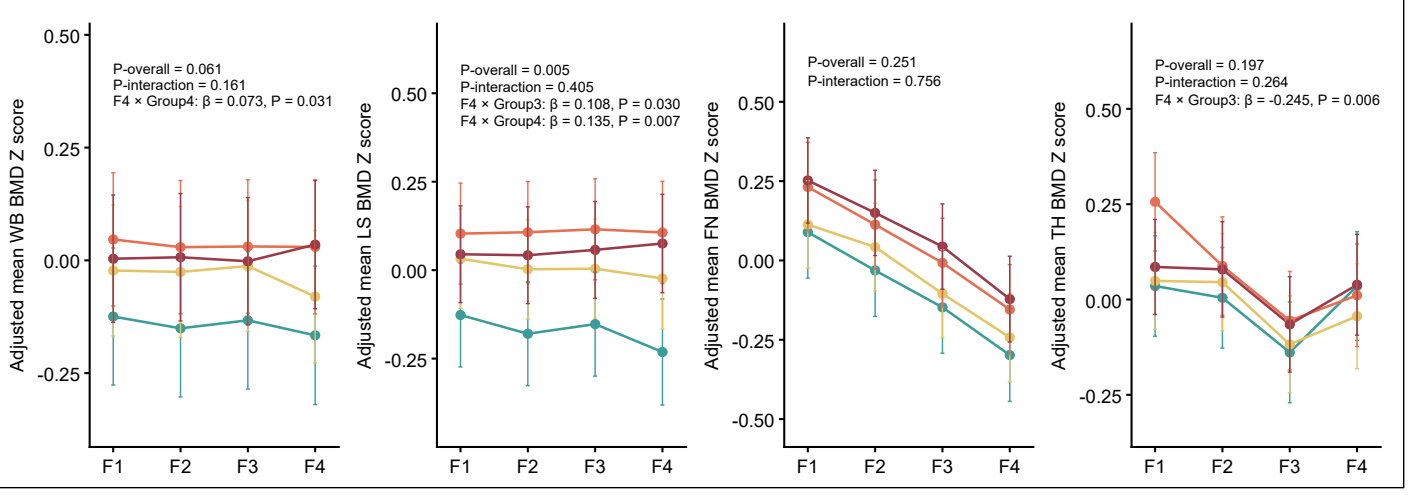

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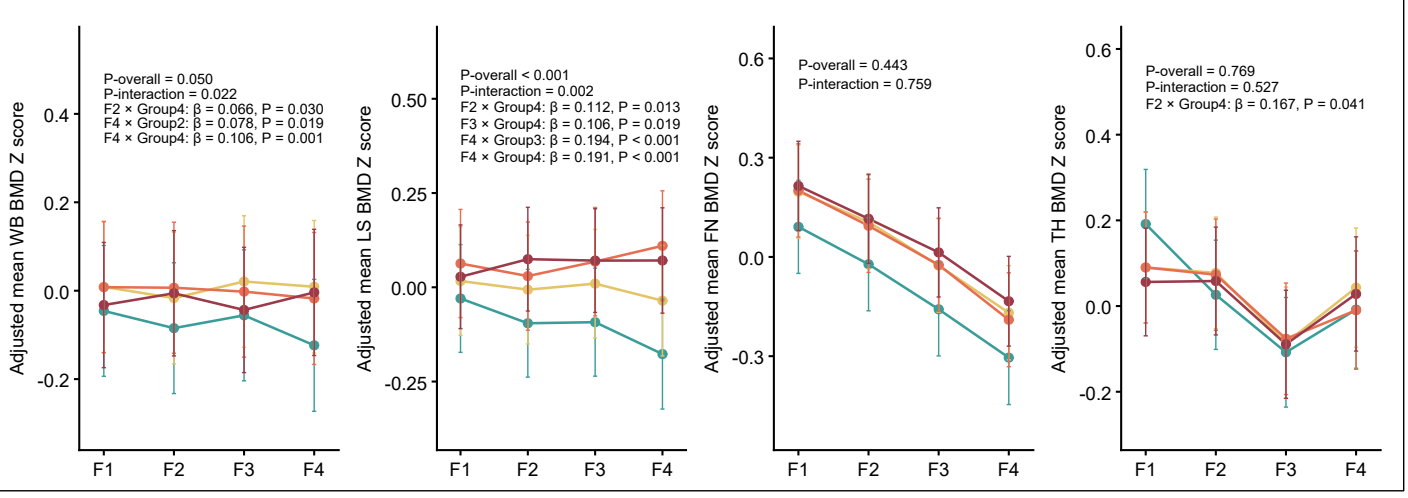
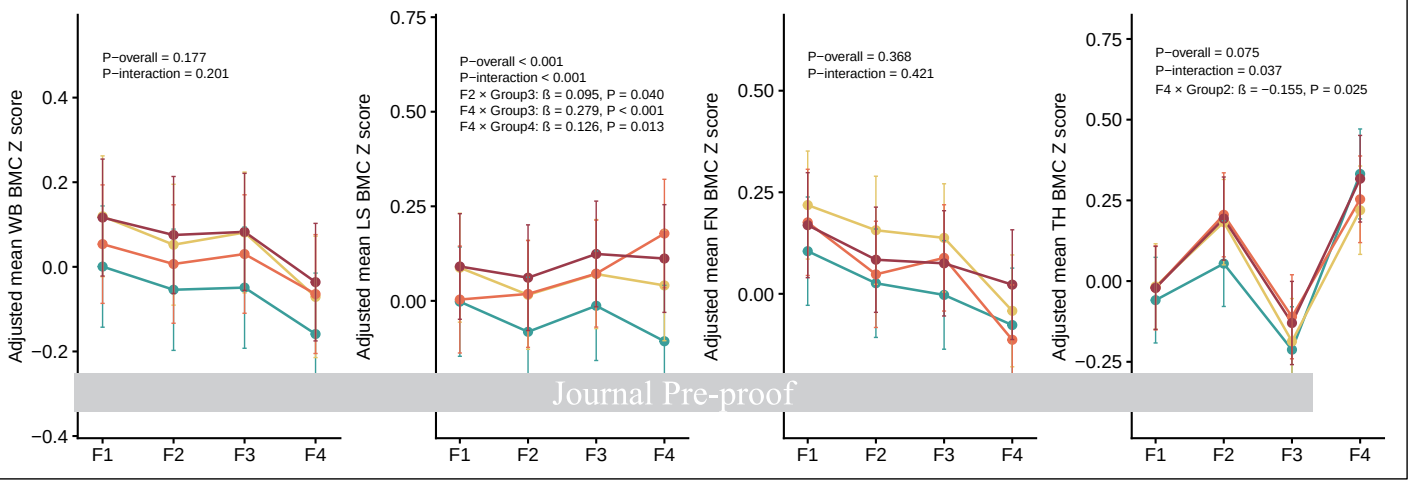


Group 1 Group 2 Group 3 Group 4

A



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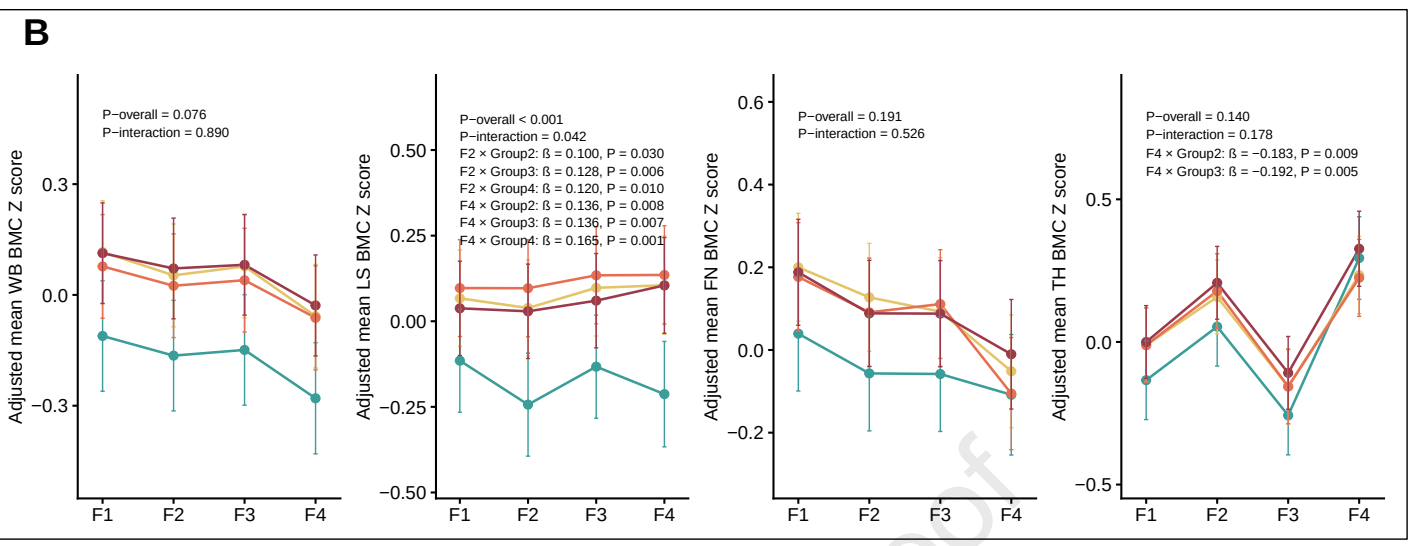

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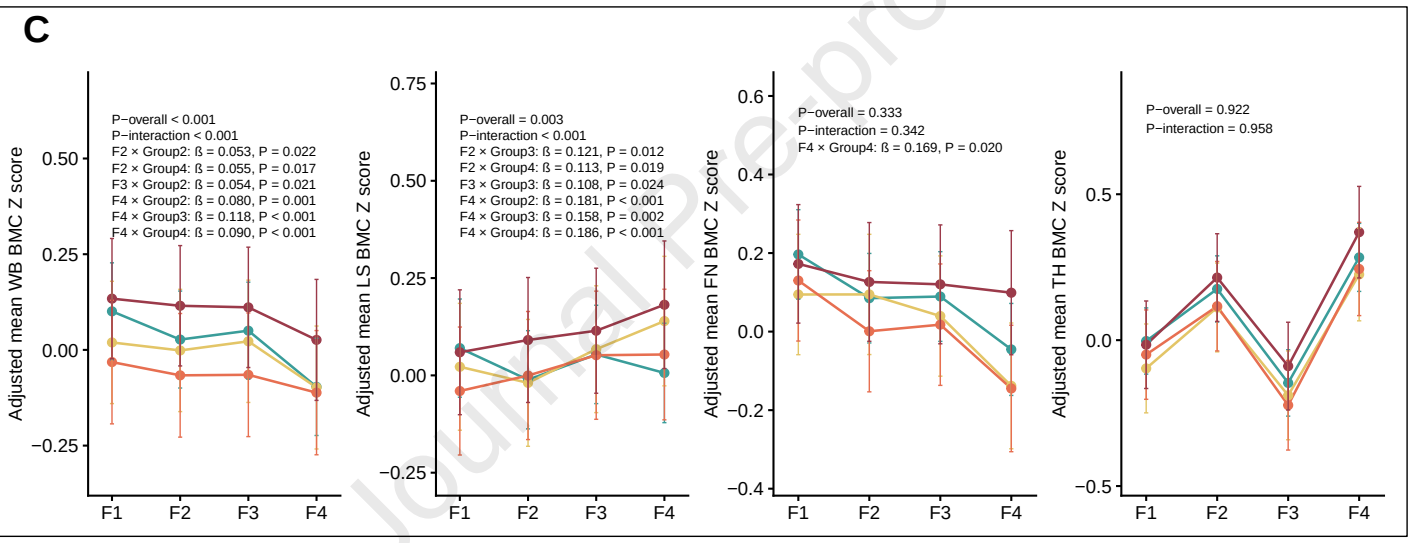

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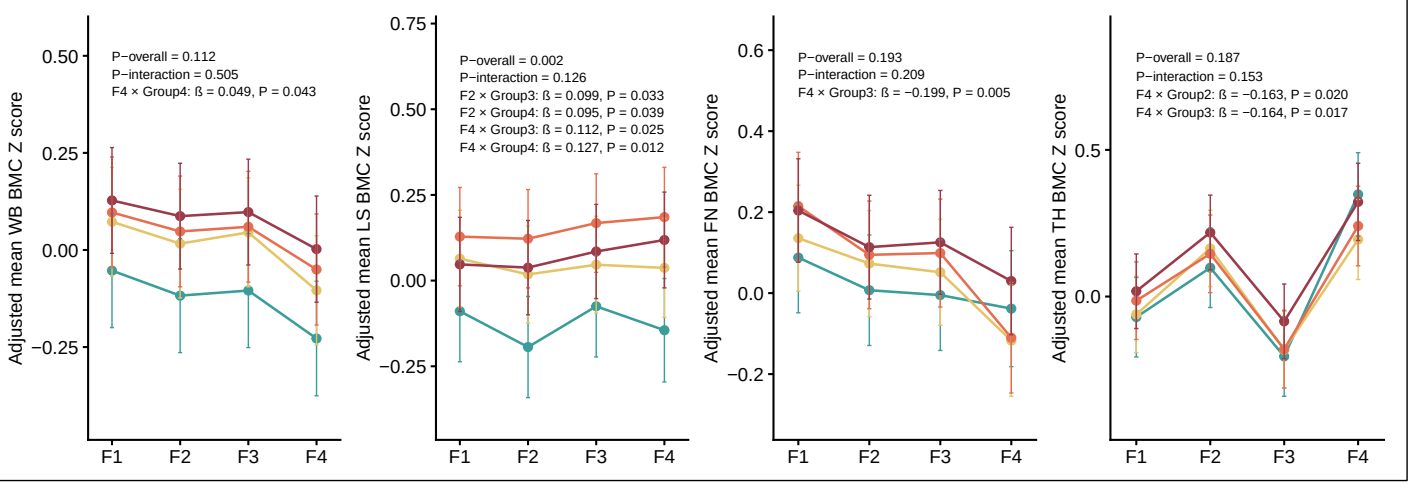

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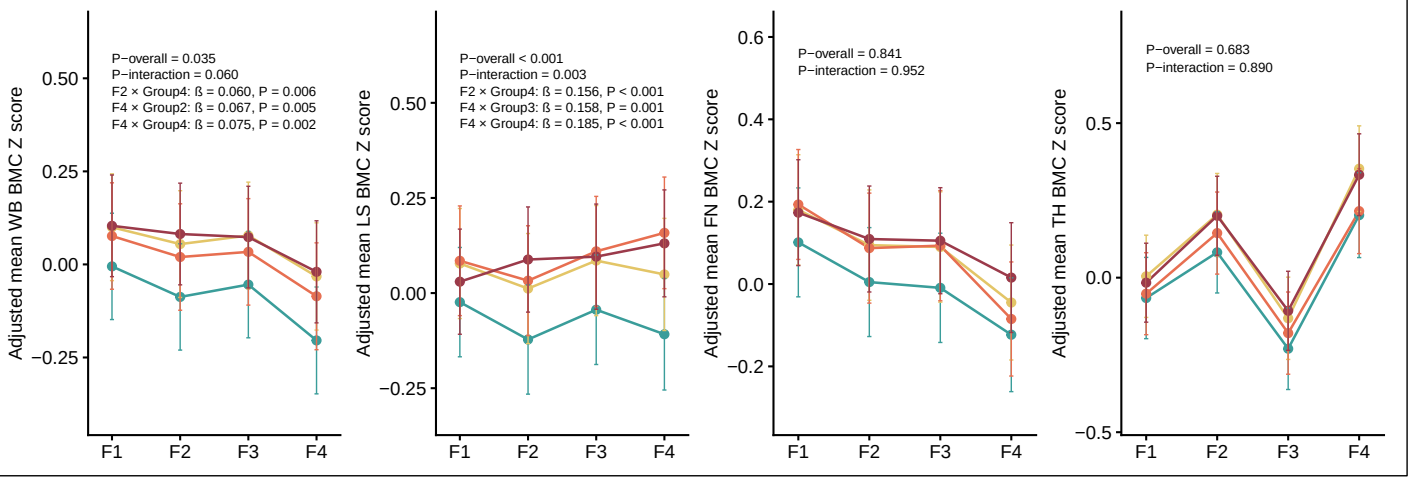

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