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Bone mass, depressive and anxiety symptoms in adolescent girls: Variation by smoking and alcohol use

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Abstract

PURPOSE—The purpose of the study was to examine (a) the association between depressive and anxiety symptoms with bone health, (b) the association of smoking or alcohol use with bone health, and, in turn, (c) whether the association between depressive and anxiety symptoms with bone health varied by smoking or alcohol use individually or by combined use. Bone health included total body bone mineral content (TB BMC) and bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck. Previous literature has not examined these issues in adolescence, a time when more than 50% of bone mass is accrued.

METHODS—An observational study enrolled 262 healthy adolescent girls by age cohort (11, 13, 15, and 17 years). Participants completed questionnaires and interviews on substance use, depressive symptoms, and anxiety. BMC and BMD were measured by dual energy x-ray absorptiometry.

RESULTS—Higher depressive symptoms were associated with lower TB BMC and BMD (total hip, femoral neck). Those with the lowest level of smoking had higher BMD of the hip and femoral neck whereas no differences were noted by alcohol use. Regular users of both cigarettes and alcohol demonstrated a stronger negative association between depressive symptoms and TB BMC compared with non-users/experimental users and regular alcohol users. Findings were parallel for anxiety symptoms.

CONCLUSION—Depressive and anxiety symptoms may negatively influence bone health in adolescent girls. Consideration of multiple substances, rather than cigarettes or alcohol separately, may be particularly informative with respect to the association of depression with bone health.

Keywords

bone density; bone mass; depression; anxiety; smoking; alcohol; adolescence; female

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INTRODUCTION

Osteoporosis is a significant health problem incurring high economic costs. Although osteoporosis occurs primarily in the elderly, its prevention should be considered in adolescence (1–3) when nearly 50% of bone mineral content (BMC) is accrued(1).

Among adults, depression is related to lower bone mineral density (BMD) (4, 5). Only one study examining such issues in healthy adolescents was conducted (6) and showed a similar negative association. In addition, adult smokers have lower BMD compared with nonsmokers (7, 8) and, among postmenopausal smokers, an increase in fracture risk was evident (9, 10). Similarly, chronic alcohol use had detrimental effects on adult BMD (11), whereas moderate use was associated with higher BMD in some (12–14) but not in all studies (15). Little is known about the effects of smoking and/or alcohol intake on bone health among adolescents, however. In animal models, chronic alcohol exposure in adolescence had negative effects on BMD (16), even following later abstinence (17, 18). Nicotine exposure showed similar negative effects in young animals (19, 20); thus, examining these issues in human adolescents may be crucial.

Initiation of substance use in adolescence and the coincident escalation of depression, (21) may have detrimental bone consequences. Studies are lacking regarding the impact of substance use and depression or anxiety on bone health during this critical time of bone mineral accrual. Therefore, this study examined (a) the association of depressive and anxiety symptoms with total body (TB) BMC and regional BMD in adolescent girls, (b) the association of smoking or alcohol use with bone health, and, in turn, (c) whether the association of depressive and anxiety symptoms with bone health varied by smoking or alcohol use individually or by combined use.

MATERIALS AND METHODS

Design and sample

This study used baseline data from a prospective study focused on the impact of mood and smoking behavior on reproductive and bone health in community adolescent girls. Girls (N = 262) were enrolled into a cross sequential design by age cohort (11, 13, 15, and 17 years) and lifetime smoking history. Additionally, girls were enrolled with the goal of encompassing a range of depressive symptoms (e.g., minimal symptoms to meeting diagnostic criteria). Baseline data were collected from December 2003 through October 2007.

Exclusionary criteria were (1) pregnancy/breastfeeding within 6 months, (2) primary amenorrhea (menarche >16 years), (3) secondary amenorrhea (<6 cycles/year), (4) body mass index (BMI) $\leq$ 1st percentile or weight >300 pounds, (5) medication/illness influencing bone, and (6) psychological disabilities impairing comprehension/compliance. Hormone contraceptives were not an exclusion criterion as it was desired to have the sample more representative of a wider range of community adolescents. Recruitment was from a large Midwestern children's hospital and its community. The study received Institutional Review Board approval. Parents provided informed consent, and girls provided assent. Parents and adolescents completed their measures in separate rooms at a General Clinical Research Center. Girls had a physical, followed by a blood draw, questionnaires, interviews, and measurement of BMC and BMD by dual energy x-ray absorptiometry.

Measures

BMC and BMD were examined as indicators of bone health. Total body, lumbar spine, and proximal femur scans were performed using a Hologic QDR4500 bone densitometer (Hologic, Inc., Bedford, MA) and analyzed using software release 12.4. As BMC has been advocated as the appropriate measure to use during growth, total body BMC was the primary outcome owing to interest in factors that affect peak bone mass (22). Lumbar spine, total hip, and femoral neck BMD were obtained because they are common sites of osteoporotic fracture.

Depressive symptoms were measured with the self-report Children's Depression Inventory (CDI) (23). The 27-item CDI demonstrated high reliability ($\alpha = 0.89$). T-scores were used in the analyses (mean = 50; SD = 10), with scores ≥ 65 considered clinically significant.

Anxiety symptoms were measured by the State Trait Anxiety Inventory for Children (STAIC). Two versions were used: the STAIC (24) for ages <12 years and the STAI (25) for ages ≥ 12 years. Reliability was high in this sample ($\alpha = .85-.89$). Trait anxiety (T-score) was used in the analyses because it is stable and more likely to be related to bone health.

Smoking behavior was determined by questionnaire asking whether participants had ever smoked tobacco in their life. Categories included never ($n = 104$), one puff to two cigarettes ($n = 54$), three to 99 cigarettes ($n = 53$), or more than 100 cigarettes ($n = 51$). These categories are referred to as "graded smoking."

Alcohol use was determined by the Diagnostic Interview Schedule for Children (DISC) (26) using the adolescent report. The DISC is a standardized computer interview focusing on diagnostic symptoms of psychopathology using *DSM* criteria (27). Participants were categorized as no drinks ($n = 135$), one to five drinks ($n = 59$), and six drinks or more ($n = 67$) within the past year (28). These categories are referred to as "graded alcohol."

Other participant characteristics were measured and examined as potential covariates. *Race* was determined by parental report. *Socioeconomic status (SES)* was estimated by parent report of occupation and education, (29) with possible scores ranging from 8 (lower) to 66 (higher). Height was obtained by wall-mounted stadiometer (Holtain Ltd., Crosswell, United Kingdom), and weight was measured by digital scale (Scaletronix, Carol Stream, IL). Measures were obtained in triplicate by trained nursing personnel, and the mean was used. *Pubertal maturation* was determined by physical examination by trained clinicians using visualization and breast palpation and inspection of pubic hair (30) and was categorized by Tanner criteria. A nonrandom sample ($n = 27$) examined by two raters showed 100% agreement on both stages. *Gynecological age* was obtained by clinician interview asking each girl whether she ever had a menstrual period and, if yes, how old (year and month) she was at that time. Methodology to enhance accuracy was utilized (31). The date of menarche was subtracted from the date of the visit to determine gynecological age. *Hormone contraceptive history* was obtained by clinician interview including oral contraceptive pills (OCPs), depot medroxyprogesterone acetate (DMPA), transdermal patch, and intravaginal ring. Duration of use was categorized as 1 = 1–3 months, 2 = 4–6 months, 3 = 7–9 months, 4 = 10–12 months, 5 = >1 year but < 2 years, 6 = ≥ 2 years but < 3 years, and 7 ≥ 3 years. Due to differential effects of DMPA versus other contraceptive methods containing estradiol and a progestin on bone health, DMPA duration was examined separately. The duration of use for estradiol-containing methods (e.g., OCPs, transdermal patch, intravaginal ring) was summed to represent a combined duration of exposure. Summed scores were capped at 7 representing more than 3 years of combined use. *Calcium intake* was measured by food frequency questionnaire reflecting intake of 19 calcium-rich foods (32). Calcium supplement use was collected also. *Physical activity* was estimated using the Physical Activity

Questionnaire for Older Children (PAQ-C) (33). Participants recalled their performance of moderate to vigorous physical activities within the last 7 days, and mean scores were calculated (1 [low] to 5 [high]). *Serum 25-hydroxy-vitamin D [25(OH)D] concentration* was analyzed using radioimmunoassay. Inter- and intra-assay coefficients of variation ranged from 3.5%–4.4% and 11.1%–16.9%, respectively; sensitivity was 1.5 ng/mL.

Data Analysis

Participant characteristics were compared among groups using ANOVA and Chi-square tests. Primary aims were examined using series of multiple regression analysis. Specifically, the effects of anxiety, depression, smoking, and alcohol intake on BMC and BMD were examined with separate regression models for each dependent variable: TB BMC and BMD of the lumbar spine, total hip, and femoral neck. Independent variables were smoking and alcohol use status and depressive and anxiety symptoms. Interactions between substance use and symptoms (e.g., smoking status by depressive symptoms) also were tested; all interaction terms were created from centered variables. As age is an important confounder of substance use and bone health, all analyses adjusted for chronological age. Additionally potential covariates (defined in the measures section) were tested. After accounting for age, only significant covariates ($p < .05$, 2-sided) were retained in subsequent models (age, race, height, weight, Tanner breast stage).

RESULTS

Descriptives

On average, girls were aged 14.9 years ($SD=2.2$), and the majority were Caucasian (61.8%) or African American (32.8%) with some mixed race/other (5.4%). Race was dichotomized as Caucasian versus non-Caucasian. Most participants were in later puberty (Tanner breast stage I = 1.5%, II = 1.9%, III = 10.3%, IV = 14.9%, and V = 71.4%), and 79.8% were postmenarcheal. Age differences were observed for many of our demographic and health measures (Table 1). Older girls demonstrated lower SES and as expected, older girls also demonstrated greater physical and pubertal maturation, longer duration of contraceptive use, and lower physical activity. Additionally, age was positively correlated ($p < .01$) with greater alcohol use, cigarette use, depressive symptoms, anxiety symptoms, and all measures of bone health (data not shown).

Associations of Depressive and Anxiety Symptoms and Bone Health

Multiple regression analyses controlling for age, race, height, weight, and Tanner breast stage demonstrated a significant main effect between depressive symptoms and TB BMC ($b = -2.21$, $p < .05$) such that a 1-SD increase (10 points) in depressive symptoms was associated with a 22.1-g decrease in TB BMC. This decrease is equivalent to 1.2% of the sample mean. Additionally, results demonstrated a significant main effect between depressive symptoms and femoral neck BMD ($b = -.001$, $p < .01$) such that a 1-SD increase in depressive symptoms was associated with a .01 g decrease in femoral neck BMD (equivalent to 1.1% of sample mean). There was no main effect between depressive symptoms and lumbar spine and hip BMD ($p > .10$), and no main effect between anxiety symptoms and all bone measures ($p > .10$).

Cigarette and Alcohol Use and Differences in Bone Health

To determine the optimal way to categorize substance use, we examined the dose-response for each substance with measures of bone health. After controlling for stated covariates, no significant main effect emerged for graded lifetime smoking on TB BMC or lumbar spine BMD. There were main effects of graded smoking on total hip BMD ($p = .02$; effect size η^2

= .04) and femoral neck BMD ($p = .02$; effect size $\eta^2 = .04$). Post hoc comparisons using Bonferroni correction indicated that participants in the one puff to two cigarettes group had significantly greater total hip BMD ($M\Delta = .062 \text{ g/cm}^2$, $p < .05$; 6.5% of sample mean) and greater BMD of the femoral neck ($M\Delta = .053 \text{ g/cm}^2$, $p < .05$; 6.0% of sample mean) than participants who smoked more than 100 cigarettes ($M\Delta = .06 \text{ g/cm}^2$; 6.8% of sample mean). Additionally, participants in the one puff to two cigarettes group had significantly greater BMD of the femoral neck than participants who ever smoked three or more cigarettes ($M\Delta = .048 \text{ g/cm}^2$; 5.4% of sample mean). No significant main effects emerged for graded alcohol on TB BMC or any BMD regions ($p > .10$).

Generally, findings did not suggest a dose-response of cigarettes or alcohol on TB BMC or BMD regions. Rather, results suggested a clustering of effects around infrequent use (never or one puff to two cigarettes ever) versus more regular use for cigarettes ($\geq$ three cigarettes ever), indicating that infrequent substance users may have indicators of bone health that more closely resemble those who have never used cigarettes or alcohol rather than regular users of cigarettes ($\geq$ three cigarettes ever) and alcohol ($\geq$ six drinks, past year). Hence, subsequent analyses examined smoking as a dichotomy of no use/experimental use ($\leq$ two cigarettes ever) versus regular use ($\geq$ three cigarettes ever) and alcohol use as a dichotomy of no use/experimental use ($\leq$ five drinks, past year) versus regular use ($\geq$ six drinks, past year).

Does the Association between Depressive and Anxiety Symptoms and Bone Health Differ by Cigarette and Alcohol Use?

Regression models controlling for stated covariates included the main effects of symptoms (depressive or anxiety) and substance use (cigarettes or alcohol) and the interactions between symptoms and substance use for each bone measure. Two significant interactions ($p < .05$) emerged; the depression and anxiety effects on TB BMC were found only in the regular alcohol user group, for whom higher depressive ($b = -5.29$, $p < .01$) and anxiety symptoms ($b = -5.14$, $p < .01$) were associated with lower TB BMC. That is, for regular alcohol users a 1-SD increase (10 points) in depressive or anxious symptoms was associated with a 52.9-g or 51.4-g lower TB BMC, respectively. These differences are equivalent to approximately 3% of the sample mean. The association between depressive and anxiety symptoms and spine and hip BMD regions did not differ by alcohol use (no use/experimental use versus regular use) nor were differences observed by smoking use (no use/experimental use versus regular use) ($p > .10$).

Given that 50% of participants categorized as regular smokers were also categorized as regular drinkers, it is possible that potential interaction effects between substance use and depressive and anxiety symptoms were masked by differences between adolescents who use only one substance versus those who use both cigarettes and alcohol. Hence, subsequent analyses examined non-user/experimental user versus regular user of both cigarettes and alcohol to form a multicategory combined substance use variable resulting in the following categories: non-user/experimental user of cigarettes and alcohol (54%), regular cigarette-only user (20%), regular alcohol-only user (6%), or regular combined substance user (20%).

Does the Association between Depressive and Anxiety Symptoms and Bone Health Differ by Combined Cigarette and Alcohol Use?

For descriptive purposes, mean depressive and anxiety symptoms and bone health by combined substance groups use are illustrated in Table 2. Controlling for age, race, height, weight and Tanner breast, ANOVAs demonstrated that, on average, girls in the regular cigarette-only group had significant lower ($p < .05$) BMD of the hip and femoral neck

regions compared to girls in the non-user/experimental user group. No other main effects of combined substance use were observed.

After controlling for stated covariates, results indicated a significant interaction between combined substance use status and depressive symptoms on TB BMC ($p < .05$). Girls in the combined user group (regular user of cigarettes and alcohol) demonstrated a stronger negative association between depressive symptoms and TB BMC compared with girls in the non-user/experimental user and regular alcohol-only user groups (Figure 1). Specifically, for the combined user group, a 1-SD increase (10 points) in depressive symptoms was associated with a 62.9-g lower TB BMC. This difference is equivalent to 3.2% of the sample mean. A similar effect emerged for trait anxiety ($p \leq .01$) such that the combined user group demonstrated a stronger negative association between trait anxiety and TB BMC compared with the non-user/experimental user and regular alcohol-only user groups. For girls in the combined user group, a 1-SD increase (10 points) in trait anxiety was associated with a 68.19-g lower TB BMC (equivalent to 3.6% of sample mean). Contrary to expectation and in contrast to the regular alcohol user and combined substance user groups, adolescent girls in the regular cigarette user group demonstrated a positive association between depressive symptoms and TB BMC ($p = .10$, trend) and trait anxiety and TB BMC ($p = .05$). Specifically, for the regular cigarette user group, a 1-SD increase in depressive or anxiety symptoms was associated with a 45.8-g (2.4% of sample mean) and 56.2-g increase in TB BMC (2.9% of sample mean), respectively (Figure 1). No other interaction effects for TB BMC emerged.

The interaction effect between combined substance use categories and symptoms of depression and anxiety were also examined for spine and hip BMD. Analyses controlled for age, race, height, weight, and Tanner breast stage. Significant main effects occurred, but only one trend effect ($p < .10$) emerged for the interaction between combined substance use categories and depressive symptoms when examining spine BMD. Specifically, only girls in the combined user group demonstrated a negative association between depressive symptoms and lumbar spine BMD ($p < .05$) such that a 1-SD increase in depressive symptoms was associated with a .03-g decrease in spine BMD (Figure 2). This difference is equivalent to 3.1% of the sample mean. No other interaction effects for spine and hip BMD emerged.

DISCUSSION

To our knowledge, this is one of the first studies to report a negative association between depressive symptoms and bone health in a normative sample of adolescent girls. Congruent with many adult studies (4, 5) and confirming our preliminary analyses [6], girls with higher numbers of depressive symptoms had poorer bone health. These findings provide preliminary evidence that adolescence is a developmental period ripe for consideration in osteoporosis prevention efforts. In particular, depressive symptoms or depression may be a risk factor for poor bone health and considering this knowledge in a pubertal age cohort may be important. Further, the findings lend preliminary support that depression may be considered a systemic illness affecting the skeleton as well as other body systems. Longitudinal studies will need to confirm causality.

This study also examined whether substance use was associated with bone health. It is unique in that effects of smoking and alcohol were examined separately as well as in combination, with the latter offering unanticipated findings. No differences emerged in TB BMC or BMD of the lumbar spine by graded smoking categories. However, knowing that BMD of the hip and femoral neck—two common fracture sites in the elderly—was higher in those with the lowest level of smoking may have import for future research and public health intervention and prevention efforts. Knowing that smoking status is related to bone

health, even at this young age, indicates the need for further research. With respect to graded alcohol use, no differences were noted in measures of bone health. Lack of difference may reflect the lower level of alcohol use in our young cohort. Only one study has examined these issues where lower BMD was evident in 9th graders 4 years later in those with self-reported alcohol and smoking (34); however, significant methodological flaws were noted (e.g., small N, attrition > 50%, limited smoking variable), hampering its utility. Additionally, research in a rat model demonstrates a link between lower BMD and chronic alcohol exposure and acute or chronic binge alcohol consumption during adolescence and abstinence did not reverse negative effects (17–19). Similarly, acute and chronic exposure had detrimental effects on bone in adolescent rats (35). With this in mind, future studies in adolescence should continue examining alcohol as its experimentation begins in adolescence and can escalate (36).

Contrary to expectations, smoking alone did not appear to modify the association of depressive and anxiety symptoms with bone. Preliminary findings showed a negative association, but our complete sample was not enrolled at that time; nor were there alcohol measures included (6). Additionally, because 50% of participants categorized as regular smokers were also regular drinkers, it is possible that effects on bone health were masked by differences between adolescents who used only cigarettes versus those who used both cigarettes and alcohol. Subsequent analyses with a combined substance use variable of cigarettes and alcohol revealed those who used both substances and were higher on depressive and anxiety symptoms also had lower TB BMC and had a trend for lower BMD of the spine. As these effects emerged most strongly for girls who used both cigarettes and alcohol regularly, it suggests that polysubstance use may be particularly detrimental for bone health in adolescence. Notably, the alcohol-only group demonstrated a similar association between depressive and anxiety symptoms and bone health compared to combined users suggesting that there may be something unique about alcohol consumption and bone health. However, the fact that the alcohol only group was small warrants further study. An alternative explanation for the negative impact of combined substance use is that alcohol and smoking at this age may be a marker of multiple other (or yet unknown) risk factors that have a negative influence on bone.

Contrary to our hypothesis, using the combined substance use variable, regular cigarette only users had a stronger positive association between TB BMC and depressive and anxiety symptoms. That is, regular cigarette-only users with lower symptoms had the poorest bone health. Of consideration, the regular cigarette only group also had the lowest BMD of the hip and femoral neck, on average, which could put them at higher risk for poorer bone health. They also had the highest weight and BMI-Z score as well as the highest gynecological age (indicative of estrogen exposure), both of which are associated with higher TB BMC and BMD; however, we did control for these factors.

Our cross-sectional findings are novel in the adolescent population. At first glance the difference in bone health seems small (3–6%) when considering measures of substance use or depressive or anxiety symptoms. However, concern should be noted as these percentages are in line with annual loss experienced by postmenopausal women (2 – 5%) or with women on depot medroxyprogesterone acetate (2–3%). Subsequent longitudinal analyses will shed light on the impact that increasing depressive symptoms and substance use may have on bone health in developing girls. In turn, if the findings hold longitudinally, efforts to maximize bone accrual during adolescence could profitably consider substance use, depression, and anxiety as negative influences on bone health. In adults, others have suggested that depression should be considered a risk factor for osteoporosis [4] and thus be marked as a target of prevention and intervention efforts. Its importance remains

understudied in adolescence but maximizing peak mass or the “bone bank” at this time is crucial across the entire lifespan. (37)

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Figure 1. Association between Depressive and Anxiety Symptoms and Total Body Bone Mineral Content by Combined Substance Use Groups in Adolescent Girls

Note. Figure 1 illustrates the combined substance use by depressive symptoms interaction ($p < .05$) and the combined substance use by anxiety symptoms interaction ($p < .01$).

Regression lines for group were fit at the sample mean for each covariate (age, race, height, weight, and Tanner breast stage). The p values are given for each regression line.

Figure 2. Association between Depressive Symptoms and Spine Bone Mineral Density by Combined Substance Use Groups in Adolescent Girls

Note. Figure 2 illustrates the combined substance use by depressive symptoms interaction ($p < .10$). Regression lines for each group were fit at the sample mean for each covariate (age, race, height, weight, and Tanner breast stage). The p values are given for each regression line.

Table 1

Comparison of demographics and health measures by age cohort

Variable	Total Sample (N = 261)	Age 11 Cohort (n = 52)	Age 13 Cohort (n = 52)	Age 15 Cohort (n = 87)	Age 17 Cohort (n = 71)	Group Comparison
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	p value
Socioeconomic status (SES)	37.3 (13.7)	41.5 (14.2) ^a	39.3 (13.3) ^{a,b}	33.4 (13.7) ^b	37.6 (12.4) ^{a,b}	<.01
Height (m)	1.6 (0.1)	1.5 (0.1) ^a	1.6 (0.1) ^b	1.6 (0.1) ^b	1.6 (0.1) ^b	<.01
Weight (kg)	62.2 (18.2)	43.4 (15.7) ^a	59.0 (12.1) ^b	67.1 (17.9) ^c	68.5 (18.3) ^c	<.01
BMI-Z score	0.7 (1.01)	0.6 (1.1) ^a	0.8 (0.9) ^a	0.8 (1.0) ^a	0.7 (1.0) ^a	ns
Tanner breast stage	4.5 (0.9)	3.3 (1.1) ^a	4.5 (0.7) ^b	4.9 (0.3) ^c	5.0 (0.2) ^c	<.01
Gynecological age (y)	3.4 (1.9)	0.1 (0.3) ^a	1.2 (1.1) ^a	3.3 (1.3) ^b	5.02 (1.6) ^c	<.01
DMPA duration	0.6 (1.5)	0.0 (0.0) ^a	0.3 (1.0) ^a	0.6 (1.3) ^{a,b}	1.1 (2.2) ^b	<.01
OCPs + patch + ring duration	0.9 (1.8)	0.0 (0.0) ^a	0.2 (0.6) ^{a,b}	0.8 (1.7) ^b	2.1 (2.6) ^c	<.01
Physical Activity(PAQ)	2.1 (0.6)	2.4 (0.6) ^a	2.4 (0.5) ^a	2.1 (0.6) ^b	1.9 (0.6) ^b	<.01
Calcium intake (mg/day)	1,062 (829)	1,189 (1041) ^a	1,103 (930) ^a	1,004 (762) ^a	899 (627) ^a	ns
Serum 25(OH)D (ng/mL)	20.4 (9.2)	19.2 (6.7) ^a	19.9 (7.5) ^a	21.3 (10.8) ^a	20.7 (10.0) ^a	ns

Note. Depo Provera (DMPA), oral contraceptive pills (OCPs), transdermal patch, and intravaginal ring numbers for duration indicate average duration of use (1 = 1–3 months, 2 = 4–6 months, 3 = 7–9 months, 4 = 10–12 months, 5 = >1 year but <2 years, 6 = ≥2 years but <3 years, and 7 = ≥3 years); PAQ (range 1–5), higher number indicates greater activity; means in same row not sharing superscripts differ at $p < .05$ in the Bonferroni significant difference comparison; ns = nonsignificant ($p > .05$).

Table 2

Estimated marginal means for depressive and anxiety symptoms and bone health by combined substance use groups

	Non-User/ Experimental User (n = 142)	Regular Cigarette-Only User (n = 52)	Regular Alcohol-Only User (n = 16)	Regular Combined User (n = 51)	Group Comparison
	Mean (SE)	M (SE)	M (SE)	M (SE)	p value
Symptomatology					
Depression (CDI)	44.4 (1.0)	47.6 (1.6)	49.4 (2.8)	49.3 (1.7)	ns
Trait anxiety (STAI)	45.1 (1.0)	46.4 (1.5)	50.5 (2.7)	48.6 (1.6)	ns
Bone Measures					
TB BMC (g)	1,925 (16.9)	1,906 (26.0)	1,867 (257)	1,889 (27.9)	ns
BMD hip (g/cm ²)	0.98 (0.01) ^a	0.92 (0.01) ^b	0.94 (0.03) ^{a,b}	0.94 (0.02) ^{a,b}	<.01
BMD lumbar spine (g/cm ²)	0.98 (0.01)	0.97 (0.01)	0.96 (0.03)	0.97 (0.02)	ns
BMD femoral neck (g/cm ²)	0.91 (0.01) ^a	0.84 (0.01) ^b	0.85 (0.02) ^{a,b}	0.87 (0.02) ^{a,b}	<.001

Note. Estimated marginal means adjust for mean of age, race (white vs. other), height (m), weight (kg), and Tanner breast stage. Means in same row not sharing superscripts differ at $p < .05$ in the Bonferroni significant difference comparison. Combined substance use groups are mutually exclusive. Children's Depression Inventory (CDI) and State Trait Anxiety Inventory (STAI) are T-scores (mean = 50, SD = 10); total body bone mineral content = TB BMC; bone mineral density = BMD