Osteocalcin: A novel biomarker of adolescent psychopathology

Katerina Rnic, Ellen Jopling, Alison Tracy, Ashley Battaglini, Bronwen Grocott, Raymond W. Lam, Joelle LeMoult

PII: S0306-4530(24)00180-X

DOI: https://doi.org/10.1016/j.psyneuen.2024.107136

Reference: PNEC107136

To appear in: *Psychoneuroendocrinology*

Received date: 5 February 2024
Revised date: 12 July 2024
Accepted date: 13 July 2024

Please cite this article as: Katerina Rnic, Ellen Jopling, Alison Tracy, Ashley Battaglini, Bronwen Grocott, Raymond W. Lam and Joelle LeMoult, Osteocalcin: A novel biomarker of adolescent psychopathology, *Psychoneuroendocrinology,* (2024) doi:https://doi.org/10.1016/j.psyneuen.2024.107136

Osteocalcin: A novel biomarker of adolescent psychopathology

Katerina Rnic, PhD[1*]; Ellen Jopling, MA[1]; Alison Tracy, PhD[1]; Ashley Battaglini, MA[1]; Bronwen Grocott, MA[1]; Raymond W. Lam, MD[2]; Joelle LeMoult, PhD[1]

[1]Department of Psychology, University of British Columbia, Vancouver, Canada, V6T 1Z4
[2]Department of Psychiatry, University of British Columbia, Vancouver, Canada, V6T 2A1

*Correspondence concerning this article should be addressed to Katerina Rnic, Department of Psychology, University of British Columbia, 2136 West Mall, Vancouver, British Columbia, Canada V6T 1Z4. Email: krnic@psych.ubc.ca Phone: 1-226-456-0599

Declarations of interest: none

The preregistration for this study is available at: https://aspredicted.org/VNG_FD9. Data and code are available on the Open Science Framework at: https://osf.io/ygjc6/?view_only=abcad4b27a614969b7a593926396e5ab.

Abstract

Osteocalcin is a bone-derived hormone implicated in the acute stress response and recently linked to adult depression. Yet it is unclear whether osteocalcin is a biomarker of other forms of psychopathology and whether osteocalcin-psychopathology associations emerge during developmentally sensitive periods earlier in life. Thus, in the current pilot study we examined salivary osteocalcin and psychiatric symptoms and disorders among 48 early adolescents during a period of stress. A logistic regression indicated lower osteocalcin was associated with meeting criteria for a psychiatric disorder, OR = 0.43, 95% CI [.002, .924], and showed

moderate-to-large cross-sectional associations with a range of elevated psychopathology symptoms, *B*s ≥ |-3.44|, *p*s ≤ .034. Multilevel linear growth models indicated that low osteocalcin prospectively predicted an even greater range of psychopathology symptoms at one-year follow-up as well as increases in some symptoms over time, *B*s ≥ |-1.83|, *p*s ≤ .021. Findings introduce osteocalcin as a biomarker of diverse forms of psychopathology in youth. Osteocalcin is a potential transdiagnostic mechanism through which dysregulated responses to stress could cause or exacerbate various types of psychopathology, highlighting a promising target for clinical assessment and early intervention.

Keywords: Osteocalcin, Psychopathology, Mental Disorders, Psychiatric Disorders, Adolescence

Rapidly increasing rates of psychopathology among young people in recent years have been attributed to high levels of stress (Hossain et al., 2022). Understanding how stress becomes biologically embedded to influence psychiatric symptoms and disorders is therefore crucial. One promising candidate mechanism involves osteocalcin, a bone-derived hormone recently shown to drive the acute stress response in humans and other boney vertebrates. In contrast to the more commonly studied glucocorticoids, osteocalcin is both *necessary,* and in some contexts, *sufficient* to mount a stress response (Berger et al., 2019; Berger & Karsenty, 2022). Indeed, amygdalar signaling during stress triggers the release of the neurotransmitter glutamate, which enters osteoblasts in the skeleton, resulting in the release of osteocalcin into circulation within minutes. Osteocalcin in turn inhibits parasympathetic tone, allowing the sympathetic nervous system to initiate an unopposed fight-or-flight response. Osteocalcin is therefore an integral component of the stress response system – a system with well-documented links to the onset and maintenance of psychopathology (Doom & Gunnar, 2013). Consistent with this, evidence from bone mineral density studies in adults has linked low

osteocalcin with major depressive disorder (Michelson et al., 1996). Surprisingly, however, the field has not extended beyond depression to examine associations with other types of psychopathology.

Several converging lines of research suggest that low osteocalcin may have ramifications for a broad range of psychiatric symptoms and disorders. Specifically, animal models have shown that osteocalcin crosses the blood-brain barrier, where it promotes learning and memory, neurogenesis, synthesis of monoamine neurotransmitters, and inhibition of GABA synthesis (Oury et al., 2013) – all factors inversely linked to the pathogenesis of diverse psychiatric problems. Additionally, genetically manipulated osteocalcin-deficient mice evince not only greater depressive, but also anxious, behaviors. These effects are reversed with the infusion of osteocalcin (Oury et al., 2013). Given these compelling findings, longitudinal research is needed to determine whether osteocalcin represents a biomarker for varied psychiatric symptoms and disorders in humans.

Understanding when the putative link between osteocalcin and psychopathology emerges is also crucial for investigating its utility as a biomarker and potential risk factor. Early adolescence is a developmental period in which patterns of biological stress responses become embedded and corresponding risk for psychiatric disorders increases (Costello et al., 2011; Ganzel et al., 2013). However, the few studies that have examined osteocalcin in adolescents have primarily focused on metabolic rather than psychiatric outcomes (Garanty-Bogacka et al., 2013).

In sum, it is critical to investigate osteocalcin as a biomarker of diverse psychiatric symptoms and disorders during a developmental period of elevated risk. Thus, in the current pilot study we examined, for the first time, cross-sectional and prospective associations of salivary osteocalcin with various types of psychopathology in a community sample of early adolescents. Data were collected during the COVID-19 pandemic, a period of elevated stress.

We hypothesized that low salivary osteocalcin would be associated with psychiatric disorders at baseline and with a range of elevated psychopathology symptoms at baseline and follow-up.

Materials and Methods

Participants were recruited to the UBC Study on Adolescents from the Lower Mainland of British Columbia, Canada. Youth and their caregivers provided assent and consent at each wave of data collection. In May/June 2020, a few months into the COVID-19 pandemic, participants collected saliva samples and completed the Youth Self Report (YSR; Achenbach, 1991), a self-report measure of psychopathology symptoms, and the Kiddie Schedule for Affective Disorders and Schizophrenia for DSM-5 (K-SADS; Kaufman et al., 2016), a diagnostic interview. Of the 79 individuals enrolled, 50 opted to provide saliva samples, and 48 provided sufficient quantities of saliva to assay osteocalcin. An a priori power analysis indicated that with $\alpha = 0.05$, and power = 0.80, this sample size was adequate to detect a large effect ($r = .50$).

Participants were invited to complete an additional wave of data collection one year later in May/June 2021, in which 23 youth re-completed the YSR. Individuals lost to attrition did not differ from those who remained in the study in terms of sex, gender, age, race, pubertal status, BMI, medical disorder status, medication use, psychiatric diagnostic status, or baseline symptoms of psychopathology, all *p*s > .081. We refer here to the wave of data collection in May/June 2020 as baseline, and May/June 2021 as follow-up. See Table 1 for participant characteristics.

Salivary Osteocalcin. Saliva samples were collected using the passive drool method. Participants were instructed to collect the sample just before dinner to reduce the influence of diurnal variation, and not to eat, drink, smoke, vape, chew gum, or brush their teeth one hour prior to collecting the sample. Furthermore, participants were instructed not to take an antihistamine or anti-inflammatory medication 24 hours prior to sample collection and not to

engage in vigorous exercise the day of sample collection. Participants reported on any deviations from the collection protocol.

Samples were immediately stored by participants in their household freezers (typical temperature = -20°C) before being transported on ice and stored at -80°C at our laboratory within approximately one week of collection. Samples were shipped on dry ice to Salimetrics SalivaLab (Carlsbad, CA) for assaying. Samples were assayed for undercarboxylated osteocalcin using an electrochemiluminescence method developed and validated for saliva by Salimetrics. This assay has been validated against serum measures. The average intra- and inter-assay coefficients of variation for all samples tested were 7.07% and 8.65% respectively. Sample test volume was 25 µL of saliva per determination. The assay has a lower limit of sensitivity of 9.77 pg/mL, and dynamic range from 9.77 pg/mL-10,000 pg/mL. Due to its characteristic positive skew, raw osteocalcin values were log-transformed.

Statistical Analysis

We conducted a logistic regression to test whether osteocalcin is associated with current and recently remitted psychiatric diagnostic status, as assessed with the K-SADS, at baseline. We next conducted a single hierarchical multivariate linear regression testing the cross-sectional association of osteocalcin with baseline symptoms of psychopathology as assessed via symptom scales of the YSR. Given that multivariate regression allows for the inclusion of multiple dependent variables, only one analysis was necessary. Effect sizes were reported as Partial η^2, whereby .01 corresponds to a small effect, .06 a medium effect, and .14 a large effect.

To investigate associations of osteocalcin with symptoms at T2 follow-up and with T1-T2 symptom change over time, we conducted a random intercept and slope multilevel linear growth model for each symptom type assessed with the YSR. Measurement occasions (i.e., time:

baseline and follow-up) were nested within participants. Time was centred at follow-up and included at Level 1, such that the intercept of each model represented psychopathology symptoms at Time 2 and slopes represented change in symptoms over time. The effect of osteocalcin on change in symptoms over time was modeled by a cross-level osteocalcin by time interaction. Osteocalcin was grand-mean centered. Linear growth models include the full sample and are robust to missing data. Models were estimated using restricted maximum likelihood (REML) and robust standard errors.

Effect sizes (Cohen's f^2) for associations of osteocalcin with symptoms at follow-up and with symptom change over time were manually computed from R^2 values based on the variances of the intercepts and slopes of (1) a model that included the effect of time at Level 1 and sex at Level 2, and (2) a model that included the same effects with the addition of the effect of osteocalcin on the intercept or slope, as relevant, at Level 2 (see Scientific Software International, 2023). Cohen's f^2 of .02 corresponds to a small effect, .15 is a medium effect, and .35 is a large effect.

Logistic and multivariate linear regressions were conducted in IBM SPSS Statistics version 27 and linear growth models were conducted in HLM 8.

Results

Potential Covariates

Data were missing completely at random, Little's MCAR test: $\chi^2(65) = 78.85$, $p = .116$. We tested a series of variables as potential covariates: sex, gender, age, race, pubertal status, BMI, medical disorder status, medication use, and time of day that saliva was collected. All participants self-reported as being cis-gender, thus, we refer to sex/gender as a single variable. Sex/gender was significantly associated with osteocalcin, $t(46) = -2.54$, $p = .015$, consistent with

prior research (Vanderschueren et al.). Thus, all analyses controlled for sex/gender (0=female/girl, 1=male/boy). No other potential covariates were significant, *p*s ≥ .050.

Cross-sectional Associations of Osteocalcin with Diagnostic Status

We tested whether osteocalcin was associated with baseline diagnostic status by conducting a logistic regression. As expected, $\chi^2(2, N = 48) = 6.09$, $p = .048$, lower osteocalcin predicted greater odds of meeting criteria for a psychiatric disorder, $B = -3.14$, Wald $\chi^2(1) = 4.04$, $p = .044$, OR = 0.43, 95% CI [.002, .924].

Cross-sectional Associations of Osteocalcin with Baseline Psychopathology Symptoms

We investigated cross-sectional associations between osteocalcin and psychopathology symptoms via one multivariate linear regression. Interestingly, osteocalcin predicted some forms of psychopathology but not others, Wilk's $\lambda = .69$, $F(7, 39) = 2.55$, $p = .029$, Partial $\eta^2 = .314$. Specifically, lower osteocalcin showed moderate-large to large associations with greater withdrawn-depressed symptoms, $B = -3.44$, $p = .006$, Partial $\eta^2 = .154$, anxious-depressed symptoms, $B = -7.54$, $p = .034$, Partial $\eta^2 = .096$, and attention problems, $B = -4.14$, $p = .004$, Partial $\eta^2 = .171$. However, osteocalcin was not associated with social problems, thought problems, delinquent behavior, or aggressive behavior, *B*s ≤ |-.3.09|, *p*s ≥ .068.

Prospective Associations of Osteocalcin with Follow-up Psychopathology Symptoms and Symptom Change

Multilevel linear growth models indicated that osteocalcin predicted many forms of later psychopathology. Specifically, lower baseline osteocalcin significantly predicted greater withdrawn-depressed symptoms, $B = -4.02$, $p = .003$, $f^2 = .11$, anxious-depressed symptoms, $B = -10.85$, $p = .019$, $f^2 = .19$, social problems, $B = -1.83$, $p = .021$, $f^2 = .04$, thought problems, $B = -4.49$, $p = .011$, $f^2 = .16$, and attention problems, $B = -6.11$, $p = .011$, $f^2 = .11$, at follow-up, and it predicted increases in delinquent behavior over time, $B = 1.99$, $p = .001$, $f^2 = .07$, see Figure 1.

In contrast, osteocalcin did not predict aggressive behavior at follow-up or changes for any other types of symptoms, *B*s ≤ |5.49|, *p*s ≥ .058.[1] Given the number of multilevel linear growth models conducted, we used the Benjamini-Hochberg approach to account for the false discovery rate from conducting multiple tests. Notably, all significant effects remained.

Discussion

Despite the skeleton having long been viewed as solely a structural organ, this study adds to an emerging body of research implicating bone as an endocrine organ with important downstream effects. The current pilot study is the first to document that salivary osteocalcin is strongly associated, both concurrently and prospectively, with a broad array of psychiatric symptoms and disorders. In doing so, we introduce osteocalcin as a biomarker of diverse forms of psychopathology in youth. Early adolescence is a developmentally sensitive period during which patterns of stress responses become embedded and psychopathology begins to emerge (Costello et al., 2011; Ganzel et al., 2013). By demonstrating cross-sectional and longitudinal links between osteocalcin (a vital driver of the acute stress response) and several types of psychopathology (well-documented consequences of stress), the current findings suggest that osteocalcin may be implicated in a novel pathway through which stress influences youth's wellbeing. Thus, findings present osteocalcin as a candidate mechanism for an array of psychiatric disorders.

[1] Because time was centered at follow-up, positive *B* values for slope indicate that low osteocalcin predicted a decrease in symptoms. Additional model information is available in the online supplement.

An examination of the distributions for the YSR symptom scales (see online supplement) indicated that there was an outlier for T2 aggressive behavior and T2 delinquent behavior. We thus winsorized this individuals' T2 aggressive and delinquent behavior scores. Importantly, a reanalysis using the winsorized scores evinced the same pattern and direction of findings, indicating that the outlier did not substantively influence findings.

We conducted additional post hoc analyses for internalizing and externalizing summary scales of the YSR. Given that these analyses were not planned a priori, they are available in the online supplement.

Consistent with hypotheses, osteocalcin was associated with not only depression, but also anxious-depressed symptoms, attention problems, social anxiety disorder, generalized anxiety disorder, and eating disorders. Osteocalcin also predicted thought problems, social problems, and delinquent behavior at follow-up, paralleling the emergence of externalizing and interpersonal problems that occur later in adolescence (Kjeldsen et al., 2021), and reflecting the centrality of the biological stress response system to youth mental health (Buss et al., 2018). Considering that osteocalcin was not associated with aggressive behavior and was associated with forms of psychopathology that can be subsumed under internalizing psychopathology, externalizing psychopathology, or both, it may be premature to draw conclusions regarding the degree of osteocalcin's specificity versus generalizability in predicting types of psychopathology. Furthermore, given high levels of comorbidity among mental disorders, an important future direction is to determine whether specific psychiatric symptoms or disorders particularly drive effects.

The majority of effect sizes were moderate to large, underscoring the potential impact of osteocalcin-psychopathology links. The magnitude of effect sizes is particularly remarkable given that the sample was a nonclinical, community sample that was not selected for elevated risk. That osteocalcin predicts an array of psychiatric symptoms in youth indicates this association emerges early in life and likely plays a mechanistic role in the onset or maintenance of various forms of psychopathology. As such, better understanding this novel biomarker will have broad implications for informing disorder pathogenesis as well as clinical assessment and intervention.

Limitations

Findings should be interpreted in the context of this pilot study's small sample and loss of participants to attrition at follow-up. Moreover, given that osteocalcin predicted symptom change only for delinquent behavior, it is possible that for other types of symptoms, osteocalcin

may only be associated with the time-stable component of psychopathology. Future studies with larger samples are needed to investigate longitudinal links between osteocalcin and psychopathology to inform the pattern and direction of effects.

Conclusions

In sum, the current study showed, for the first time, that low osteocalcin is a biomarker and promising risk factor for a broad range of psychiatric symptoms and disorders in early adolescents. This is intriguing as osteocalcin represents a fundamental yet understudied component of the biological stress response system. Results point to osteocalcin as a compelling mechanism through which dysregulated responses to life stress might be associated with some forms of psychopathology, highlighting a potential target for early intervention. As such, results motivate future studies examining the osteocalcin-psychopathology link among clinical samples or individuals at elevated risk for the development of mental disorders. Doing so would cement the novel link between the skeleton and diverse forms of mental illness, thereby forming a more integrative understanding of numerous psychiatric disorders and their likely determinants.

References

Achenbach, T.M. 1991. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. Department of Psychiatry, University of Vermont.

Berger, J.M., & Karsenty, G., 2022. Osteocalcin and the physiology of danger. FEBS Lett. 596, 665–680. https://doi.org/10.1002/1873-3468.14259

Berger, J.M., Singh, P., Khrimian, L., Morgan, D.A., Chowdhury, S., Arteaga-Solis, E., Horvath, T.L., Domingos, A.I., Marsland, A.L., Yadav, V.K., Rahmouni, K., Gao, X.-B., & Karsenty, G., 2019. Mediation of the acute stress response by the skeleton. Cell Metab. 30, 890-902. https://doi.org/10.1016/j.cmet.2019.08.012

Buss, K.A., Jaffee, S., Wadsworth, M.E., & Kliewer, W., 2018. Impact of psychophysiological stress-response systems on psychological development: Moving beyond the single biomarker approach. Dev. Psychol. 54, 1601-1605. https://doi.org/10.1037/dev0000596

Costello, E.J., Copeland, W., & Angold, A., 2011. Trends in psychopathology across the adolescent years: What changes when children become adolescents, and when adolescents become adults? J. Child Psychol. Psychiatry. 52, 1015–1025. https://doi.org/10.1111/j.1469-7610.2011.02446.x

Doom, J.R., & Gunnar, M.R., 2013. Stress physiology and developmental psychopathology: Past, present, and future. Dev. Psychopathol. 25, 1359–1373. https://doi.org/10.1017/S0954579413000667

Ganzel, B.L., Kim, P., Gilmore, H., Tottenham, N., & Temple, E., 2013. Stress and the healthy adolescent brain: Evidence for the neural embedding of life events. Dev. Psychopathol. 25, 879–889. https://doi.org/10.1017/S0954579413000242

Garanty-Bogacka, B., Syrenicz, M., Rać, M., Krupa, B., Czaja-Bulsa, G., Walczak, M., Sowińska-Przepiera, E., & Syrenicz, A., 2013. Association between serum osteocalcin, adiposity and metabolic risk in obese children and adolescents. Endokrynol. Pol. 64, 346–352. https://doi.org/10.5603/EP.2013.0016

Hossain, M.M., Nesa, F., Das, J., Aggad, R., Tasnim, S., Bairwa, M., Ma, P., & Ramirez, G., 2022. Global burden of mental health problems among children and adolescents during COVID-19 pandemic: An umbrella review. Psychiatry Res. 317, 114814. https://doi.org/10.1016/j.psychres.2022.114814

Kaufman, J., Birmaher, B., Axelson, D., Perepletchikova, F., Brent, D., & Ryan, N., 2016. Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL DSM-5). Western Psychiatric Institute.

Kjeldsen, A., Nes, R.B., Sanson, A., Ystrom, E., & Karevold, E.B., 2021. Understanding trajectories of externalizing problems: Stability and emergence of risk factors from infancy to middle adolescence. Dev. Psychopathol. 33, 264-283. https://doi.org/10.1017/S0954579419001755

Michelson, D., Stratakis, C., Hill, L., Reynolds, J., Galliven, E., Chrousos, G., & Gold, P., 1996. Bone mineral density in women with depression. N. Engl. J. Med. 335, 1176–1181. https://doi.org/10.1056/NEJM199610173351602

Oury, F., Khrimian, L., Denny, C.A., Gardin, A., Chamouni, A., Goeden, N., Huang, Y., Lee, H., Srinivas, P., Gao, X.-B., Suyama, S., Langer, T., Mann, John. J., Horvath, T. L., Bonnin, A., & Karsenty, G., 2013. Maternal and offspring pools of osteocalcin influence brain development and functions. Cell. 155, 228–241. https://doi.org/10.1016/j.cell.2013.08.042

Scientific Software International. (2023). Effect size and standardized coefficients. HLM Support. https://ssicentral.com/wp-content/uploads/2020/07/FAQ_Effect_sizes.pdf

Vanderschueren, D., Gevers, G., Raymaekers, G., Devos, P., & Dequeker, J. (1990). Sex- and age-related changes in bone and serum osteocalcin. Calcif. Tissue Int. 46, 179-182. https://doi.org/10.1007/BF02555041

Table 1

Participant Characteristics

Characteristics	Descriptive Statistics
Age, *M* (*SD*)	13.97 (0.31)
Sex/Gender: Female; Girl *n* (%)	21 (43.8); 21 (43.8)
Race[a,b], *n* (%)	
Asian	15 (31.3)
Indigenous	2 (4.2)
Latinx/Hispanic	2 (4.2)
White	39 (81.3)
Household Income[a], *n* (%)	
$20,000 to $39,999	2 (4.2)
$40,000 to $59,999	3 (6.3)
$60,000 to $79,999	1 (2.1)
$80,000 to $99,999	6 (12.5)
$100,000 to $139,999	13 (27.0)
$140,000 to $179,999	11 (22.9)
$180,000 and over	10 (20.8)
Don’t Know or Missing	2 (4.2)
Osteocalcin pg/mL, *M* (*SD*)	665.75 (406.92)
T1 Psychiatric Disorders, *n* (%)	10 (20.8)
Generalized Anxiety Disorder	2 (4.2)
Major Depressive Disorder	1 (2.1)
Recently Remitted Unspecified Eating Disorder	1 (2.1)
Social Anxiety Disorder	4 (8.3)
Specific Phobia	2 (4.2)
Unspecified Anxiety or Depressive Disorder	3 (6.3)
Psychopathology Symptoms, *M* (*SD*)	
T1 Withdrawn-Depressed	3.15 (2.37)
T1 Anxious-Depressed	6.59 (6.68)
T1 Social Problems	2.69 (2.20)
T1 Thought Problems	2.42 (2.03)

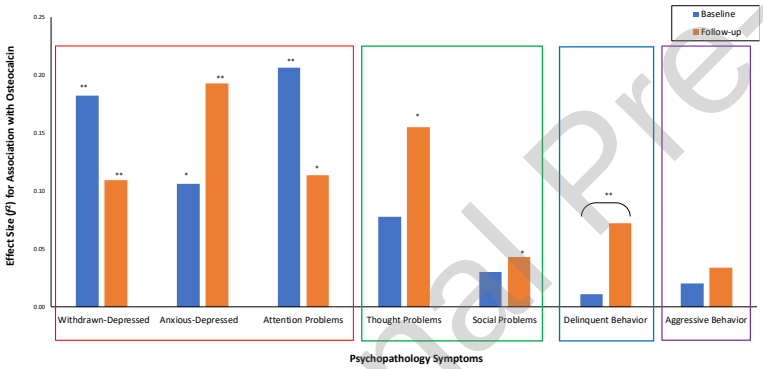
T1 Attention Problems	5.39 (2.66)
T1 Delinquent Behavior	1.73 (1.70)
T1 Aggressive Behavior	7.66 (5.79)
T2 Withdrawn-Depressed	4.70 (2.89)
T2 Anxious-Depressed	8.09 (6.52)
T2 Social Problems	2.36 (1.52)
T2 Thought Problems	3.48 (2.64)
T2 Attention Problems	6.61 (3.79)
T2 Delinquent Behavior	1.74 (1.91)
T2 Aggressive Behavior	8.57 (6.25)

[a]Parent-reported.

[b]Percentages add up to more than 100 as parents could report more than one race.

Figure 1

Effect Sizes for Associations of Osteocalcin with Psychopathology Symptoms at Baseline and Follow-Up



Note. Effects sizes in Cohen's f^2 for direct comparison and visualization. Red box: osteocalcin associated with symptoms at both time points. Green box: osteocalcin associated with symptoms at follow-up only. Blue box: osteocalcin associated with change in symptoms. Purple box: osteocalcin not associated with symptoms at any time point. $^{**}p<.01$. $^{*}p<.05$.

Declaration of Competing Interest:

none

Highlights

- Osteocalcin is a bone-derived hormone implicated in the acute stress response.
- We assessed the cross-sectional and prospective association between osteocalcin and psychopathology in youth
- Lower osteocalcin was associated with meeting criteria for a psychiatric disorder and with higher psychopathology symptoms over time
- Findings introduce osteocalcin as a biomarker of psychopathology in youth