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Calcium Supplementation and Bone Health - Baseline Network Analysis of Anthropometric, Biochemical, Dietary, and Bone Health Variables in Adolescent Girls: A Foundational Assessment Prior to Nutritional Intervention

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Abstract

BACKGROUND/AIMS: Adolescence is a sensitive phase of skeletal and metabolic growth. Elucidation of the interlinkages between physiological and nutritional factors can guide specific interventions for enhancing bone health and overall well-being. The main objective of this study was to analyze baseline relationships among major indicators of health in adolescent girls by investigating intra- and inter-cluster relationships among anthropometric indices, blood biomarkers, dietary intake, bone health markers, and urinary markers using baseline measurements, and to provide a baseline reference against which to measure post-intervention changes.

MATERIALS AND METHODS: A cross-sectional baseline dataset from adolescent volunteers participating in a nutritional intervention trial was compared. Variables were subdivided into five clusters: anthropometry (weight, body mass index, % body fat, grip strength), blood chemistry [C-reactive protein (CRP), hemoglobin, total cholesterol, alkaline phosphatase, osteocalcin, serum calcium, total protein), bone health (T-score, bone quality index), dietary intake (protein, fat, carbohydrate, total energy), and urinary markers (calcium, sodium). Pairwise correlations formed the basis for building a network graph, which was then supplemented by the calculation of centrality measures (betweenness, closeness, strength, expected influence) to identify key variables.

RESULTS: Network analysis revealed strong intra-cluster connections linking anthropometric and dietary variables. Serum calcium, osteocalcin, and CRP served as central nodes, indicating mechanistic links among inflammation, bone turnover, and mineral metabolism. Dietary macronutrients were associated with both anthropometric and biochemical variables. Urinary calcium was moderately correlated with serum calcium and with total protein consumption, reflecting the physiological coupling between renal excretion and dietary absorption. Bone health markers correlated closely with blood biomarkers, specifically serum osteocalcin and ALP.

CONCLUSION: The baseline network offers a holistic representation of the physiological interdependencies in adolescent girls prior to intervention. It emphasizes the role played by inflammatory, dietary, and biochemical interactions in determining bone and metabolic health. The model can serve as a reference point for assessing the effects of calcium supplementation and dietary interventions in future follow-up studies.

Keywords: Adolescents, network analysis, bone health, diet, calcium

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INTRODUCTION

Adolescence is an important period for the accumulation of bone mass, musculoskeletal development, and general physiological maturation. The adolescent growth spurt and hormonal changes significantly affect bone mineralization and peak bone mass, which are key determinants of long-term skeletal health and resistance to osteoporosis in old age.[1] Adolescent girls in low- and middle-income countries, including Pakistan, are at increased risk of nutritional deficiencies, particularly inadequate dietary calcium intake, micronutrient deficiencies, and physical inactivity, all of which weaken bone health and overall well-being.[2,3] All these factors are amenable, though challenging to address. Physical inactivity, particularly in girls, is usually attributed to a number of factors, among which social and cultural constraints are the most common.[4] Emerging evidence suggests that bone status is also related to systemic aspects of metabolic and inflammatory health, indicating that skeletal growth is inextricably linked to systemic physiological processes.[5]

Despite the established role of calcium in supporting maximal bone mineral density and bone quality, limited information is available on the impact of calcium supplementation on indices of bone health and on anthropometric, hematologic, inflammatory, and metabolic variables in real-world community practice. Conventional statistical methods may be unable to detect the complex, mutually conditional associations among these variables, thereby underestimating the multisystemic nature of adolescent health. Here, network analysis provides a new data-driven approach to depict and measure the interconnectedness of various health measures, to detect central variables, and to reveal important mechanisms underlying bone health outcomes.[6,7]

This report is part of a large study designed to examine the effects of calcium supplementation on the bone health of adolescent girls in a socio-economically disadvantaged population.[2,8] Added by the fact that calcium content of the local food is deficient in calcium contents.[9] Employing a network-analytical strategy, we examined the interplay among anthropometric, blood, bone-health, urinary, and dietary factors. We used baseline measurements in the current study to analyze patterns of intra- and interrelations among clusters of pertinent variables. These were anthropometric assessments [weight, body mass index (BMI), percentage of body fat, and grip strength]; blood chemistry indicators [C-reactive protein (CRP), hemoglobin (Hb), total cholesterol, alkaline phosphatase (ALP), osteocalcin, and serum calcium]; bone health parameters (T-score and index of bone quality); dietary intake factors (protein, fat, carbohydrate (CHO), and total dietary energy); and urinary indicators [urinary sodium (Urinary Na) and urinary calcium (Urinary Ca)]. This baseline network analysis seeks to establish an understanding of how these variables are connected before the intervention. It will be used as a point of comparison to identify structural alterations or new patterns of associations among variables following the intervention at 3- and 6-month follow-ups.

MATERIALS AND METHODS

Study Design and Participants

This study is part of a single-blind, randomized controlled trial designed to assess the effects of calcium supplementation on bone health and immune status among adolescent girls in a semi-rural community in Khyber Pakhtunkhwa, Pakistan. For the present study, a cohort of a total of 150 adolescent girls aged 11 to 17 years were recruited through community schools and local health centers using purposive sampling. Written informed consent was obtained from the participants' parents or legal guardians.

The Advanced Study and Research Board granted approval for this study (No. 1430/Agri/BKUC/2024, date: 21.05.2024). The study received ethical approval from the Bacha Khan University Charsadda Research Ethics Commite (approval no: 12/EIRB/ORIC/BKUC/2024, date: 07.05.2024). The research design strictly adhered to ethical standards for research involving human participants. To protect the safety, privacy, and rights of participants, ethical principles were upheld throughout the study. The parents or legal guardians of all participants provided written informed consent before enrollment. The trial is registered with the government of Japan Registry for Clinical Trials [UMIN-CTR (ID: UMIN000056977)], available online at https://center6.umin.ac.jp/cgi-open-bin/ctr_e/index.cgi.

Baseline Assessment

At baseline, all participants underwent an extensive health assessment, including anthropometric measurements, bone quality assessment, blood biochemical analysis, and urinalysis. The following cluster of variables was included: anthropometric measures-weight (kg), height (cm), (BMI, kg/m^2), body fat percentage (measured by bioelectrical impedance analysis), and grip strength (kg). Bone quality was measured by quantitative ultrasound of the calcaneus using the T-score and bone quality index (BQI). Blood specimens were drawn in the fasting state to measure serum calcium (mg/dL), phosphorus (mg/dL), (ALP, U/L), (Hb, g/dL), [parathyroid hormone (PTH), pg/mL], osteocalcin (ng/mL), and CRP (CRP, mg/L). Twenty-four-hour urinary excretion of calcium and sodium was measured by standard spectrophotometry.

Detailed anthropometric measurements, such as height, weight, BMI percentiles, and their Z-scores, have been analyzed using World Health Organization AnthroPlus software and reported in a companion manuscript. These are not reported in the current study to avoid duplication and have instead been included in a separate manuscript that is in publication. The current study reports only data on bone and nutrition outcomes that are suitable for network analysis.

Network Analysis Methods

To investigate the intricate relationships between biological, anthropometric, and clinical variables in adolescent girls receiving calcium supplementation, a symptom network analysis was performed. The method allows visualization of the way variables interact, cluster, and affect each other, especially in the context of a nutritional intervention.[10]

The network model was built using baseline measurements from all participants prior to the start of supplementation. In the network, variables were categorized into five main domains: anthropometrics (weight, BMI, body fat percentage, grip strength), biochemical (e.g., serum calcium, Hb, osteocalcin, PTH, ALP, CRP), urinary (e.g., Urinary Ca and sodium excretion), bone health indices (T-score, BQI), and dietary (energy, protein, and CHOs). The inflammatory marker CRP was also added to capture immune-related dynamics.

An undirected weighted network was approximated using regularized partial correlation models via the Graphical Least Absolute Shrinkage and Selection Operator, which shrinks small correlations to zero to improve interpretability and reduce false positives. The Extended Bayesian Information Criterion was employed for model estimation. The network was estimated and visualized with the R package, which provides an interactive platform for psychological and health-related network analysis.

Centrality measures-strength, betweenness, closeness, and expected influence-were calculated to evaluate the relative significance of every node (variable) in the network. 1) Strength is the sum of the absolute values of the weights of edges incident on a node; 2) Betweenness is the number of shortest paths passing through a node and signifies its bridging function; 3) Closeness is a measure of how close a node is to every other node in the network, with proximity determined based path lengths; and 4) Expected influence accounts for positive and negative edge weights and is particularly significant in psychological and biological networks. Case-dropping subset bootstrapping was applied to measure the stability of the centrality indices, while non-parametric bootstrapping with 1,000 resamples was used to assess the accuracy of edge weights. Detection of clusters in the network was also carried out using community-detection algorithms, such as walktrap to identify naturally occurring groupings of variables that may indicate underlying physiological mechanisms or functional domains.

This method yields a nuanced, evidence-based perspective on how calcium supplementation is likely to affect interconnected physiological systems and can inform the development of intervention hypotheses beyond conventional univariate or bivariate analytical paradigms.[9]

Statistical Analysis

Baseline characteristics were summarized using descriptive statistics. Network analysis was performed in R to graphically represent inter-variable relationships. Centrality measures of betweenness, closeness, strength, and expected influence were computed to establish the significance of variables within and across clusters (e.g., anthropometric, biochemical, urinary, and bone health domains).

RESULTS

Table 1 shows the baseline data of the participants. The study sample comprised adolescent girls from diverse sociodemographic backgrounds, with a balanced age distribution: 30% aged 9-10 years, 40% aged 10-11 years, and 30% aged 12-14 years. Educationally, 57% of participants were in primary school and 43% in middle school. Respondents were approximately balanced between urban (53%) and rural (47%) areas. The income pattern showed that 33% of households had incomes below PKR 30,000, 40% had incomes between PKR 30,000 and PKR 60,000, and 27% had incomes above PKR 60,000. In terms of nutrition, 23% of the girls had dietary limitations and 67% were 7-8% overweight, indicating an increasing trend in unhealthy weight gain. These variables collectively point to underlying socioeconomic, educational, and dietary differences that could affect the overall health and development of adolescent girls.

Results on Network Analysis

As shown in Figure 1, the network analysis revealed a highly interconnected structure in which dietary consumption, anthropometric indicators, blood biochemistry, and bone health variables exhibited strong conditional dependencies. Fat mass, CRP, PTH, and Urinary Na were identified as central nodes, suggesting that they mediate the effects of nutrition and metabolism on bone health. Of particular interest were the close relationships between dietary energy, protein, and fat and both body composition indicators and inflammatory markers. Bone health markers such as T-score and osteocalcin were incorporated into this network through serum calcium, PTH, and Urinary Ca, demonstrating the multifactorial regulation of bone metabolism in the study population. The anthropometric cluster, including weight, BMI, fat mass, and grip strength, showed dense internal connectivity, with weight, BMI, and fat mass forming a tightly coupled triad. Grip strength, although associated with these variables, had a relatively peripheral location, indicating its partial autonomy as a functional outcome. Inter-cluster analysis identified robust associations between anthropometrics and blood chemistry (specifically CRP) and between anthropometrics and energy intake, emphasizing the role of body composition in inflammatory status and metabolic markers. In addition, anthropometric measurements were strongly associated with food intake, particularly with energy and fat intake, suggesting a direct pathway from diet to body size. Associations with bone health indicators such as T-score and PTH support the dominant role of body composition in regulating both endocrine and excretory processes that affect skeletal integrity. The blood chemistry cluster displayed an intricate network of pathways indicative of inflammation, bone metabolism, and nutritional status. CRP was a central hub, showing extensive associations with fat mass, protein intake, Hb, and bone markers, indicating overlap between systemic inflammation and metabolic load. PTH was a crucial endocrine regulator, modulating the effects of dietary calcium and protein intake on bone turnover markers, such as osteocalcin and ALP, and mediating relationships with Urinary Ca and sodium. Serum calcium, at the nexus of dietary and skeletal systems, was highly

Table 1: Sociodemographic characteristics of the subjects

Variable	Categories	%	n
Age (years)	9-9.5	30	60
	9.6-10.0	40	80
	10.1-11.0	30	60
Grade level	Primary (grade 3-5)	57	114
	Middle (grade 6-8)	43	86
Residence	Urban	53	106
	Rural	47	94
	Higher education	13	26
Household monthly income	Low (<30,000 PKR)	33	66
	Middle (30,000-60,000 PKR)	40	80
	High (>60,000 PKR)	27	54
Father's occupation	Laborer/daily wage	27	54
	Private job	33	66
	Government job	27	54
	Businessman	13	26
Mother's occupation	Housewife	60	120
	Private job	20	40
	Government job	20	40
Nutritional status	Normal weight	67	134
	Overweight	23	46

PKR: Pakistani rupee.

correlated with both PTH and bone density scores, affirming its central role in mineral homeostasis. ALP and osteocalcin tightly clustered with PTH, creating a cohesive bone turnover sub-network. Hb and serum total protein although more peripheral, reflected the impact of protein status and inflammation on well-being. This cluster highlights the biochemical relationships among nutrition, inflammation, renal disposition, and bone metabolism. While this is a small cluster, these variables have significant roles as downstream markers of dietary intake, renal disposition, and systemic control-particularly of minerals such as sodium and calcium, and hormones such as PTH. The urinary cluster, which includes Urinary Na and Urinary Ca, is a physiological endpoint reflecting dietary intake and systemic control. These two factors were significantly associated, highlighting the well-known renal mechanism whereby increased sodium excretion promotes Urinary Ca excretion. Urinary Na exhibited significant associations with anthropometric markers, such as weight, BMI, and fat mass, suggesting effects of dietary intake or impaired renal function among those with higher adiposity. Both urinary markers were significantly associated with PTH, reflecting the hormone's pivotal role in regulating renal reabsorption of calcium and sodium. Urinary Ca was also associated with serum calcium, bone mineral density (T-score), and ALP, indicating its importance in calcium homeostasis and skeletal health. Associations with dietary protein and fat consumption support the inference that these urinary markers are indicative not only of endocrine regulation but also of nutrient-driven actions and serve as major mediators between diet, metabolism, and bone health.

The dietary cluster, including energy, protein, fat, and CHO intake, demonstrated strong internal consistency, reflecting the interrelated nature of macronutrient consumption. Energy consumption was strongly associated with intakes of all macronutrients, reinforcing the notion that eating habits cluster within individuals. Overall, dietary intake was strongly associated with anthropometric indices such as BMI, fat mass, and weight, indicating its central role in influencing body composition. Protein consumption was additionally associated with grip strength and serum total protein levels, reflecting its importance for muscular and nutritional health. In addition, dietary factors were correlated with markers of systemic inflammation (CRP) and of bone health (PTH and serum calcium), indicating a multisystem effect. Interestingly, protein intake correlated with Urinary Na and calcium excretion, consistent with the reported physiological effects of protein on renal mineral handling. Overall, the dietary cluster was an important driver across metabolic, skeletal, and renal domains, validating its central role in determining health outcomes in the population examined.

The bone health cluster, consisting of T-score and BQI, represents both the density and the microstructural integrity of bone tissue. These two markers covaried with bone mineral content and quality in healthy individuals. Inter-cluster analysis indicated that T-score and BQI were strongly associated with anthropometric markers of fat mass, weight, and grip strength, highlighting the biomechanical advantage conferred by body size and muscle function on bone strength. T-score was also associated with major metabolic and hormonal regulators, such as PTH, serum calcium, ALP, and CRP, suggesting multifactorial regulation

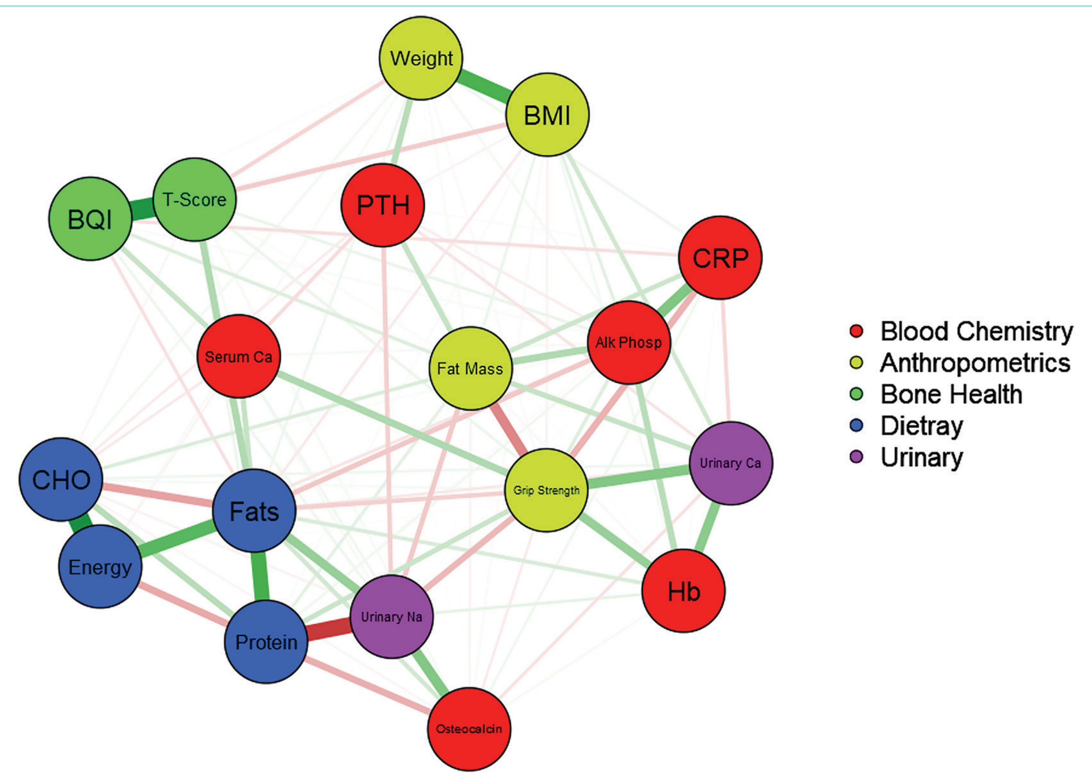

Figure 1. Social network analysis variables shows a simplified version of: Anthropometrics (BMI, weight, grip strength, fat mass) Blood chemistry (CRP, PTH, Hb, osteocalcin) Bone health (Green nodes: T-score I) Urinary (Yellow nodes: U Na, U Ca) Dietary intake (Pink nodes: Protein, fats, CHO, energy,).

Urinary Na: Urinary sodium, Urinary Ca: Urinary calcium, PTH: Parathyroid hormone, Hb: Hemoglobin, CRP: C-reactive protein, BQI: Bone quality index, BMI: Body mass index, CHO: Carbohydrate.

of bone density by endocrine and inflammatory mechanisms. Nutrient variables, especially protein and fat, correlated positively with T-score and BQI, emphasizing the role of nutritional sufficiency in maintaining bone strength. The negative correlation between Urinary Ca excretion and T-score also suggested that renal loss of calcium could jeopardize bone mineral reserves. Overall, the bone-health cluster serves as an integrated endpoint reflecting nutritional, metabolic, hormonal, and physical health factors.

Figure 2 shows the centrality measures of the network. Network centrality measures demonstrate each variable's role in the overall structure of the network using three measures: betweenness (the extent to which a variable spans gaps between others), closeness (how close a variable is to all other variables in the network), and strength (the aggregate size of its direct connections). Based on the data (Figure 2; Supplementary Table 1), grip strength and fats show significantly positive scores across all three indices and are therefore the most central and influential nodes in the network. Urinary Na has consistently high closeness values, implying that it is well connected and relatively important in bridging relationships. The protein has high closeness and strength but low betweenness, indicating that it is well integrated within the network but does not function as a bridging node. Conversely, variables such as CRP, osteocalcin, PTH, and serum calcium show negative values in the measurements, suggesting that they play peripheral roles in the network. BMI and weight also have particularly low closeness centrality, indicating that they are relatively distant from the network center. In general, the network is centered on a small set of highly interconnected variables (fats, grip strength, protein, Urinary Na), while numerous biochemical markers (CRP, osteocalcin, PTH, serum Ca) are located more peripherally and have less impact on network connectivity.

DISCUSSION

The network analysis identified distinct yet interrelated clusters that indicate the intricate relationships among adolescent girls' anthropometric status, biochemical markers, urinary excretion, and bone health indicators. The anthropometric cluster, comprising body weight, BMI, percentage body fat, and grip strength, showed strong interrelationships, with positive correlations among weight, BMI, and percentage body fat, reflecting known associations among these measures. Muscular strength, however, was negatively correlated with body fat and positively correlated with bone health indicators (T-score and BQI), indicating that higher muscular strength could contribute to or indicate healthier bone mineral density and quality.[11] This is consistent with findings from earlier studies showing that muscular loading from physical activity activates osteoblasts and increases bone density in adolescents.[12]

The bone health cluster, characterized by T-score and BQI, was positively associated with serum calcium, Hb, and osteocalcin, emphasizing their essential roles in bone remodeling and mineralization. Serum calcium is an indispensable substrate for the development of hydroxyapatite crystals within the bone, and osteocalcin is an indicator of active bone turnover and osteoblastic function.[13] The positive association with Hb indicates a relationship between overall nutritional and oxygenation status and bone health. Earlier studies have reported similar associations between Hb concentration and bone mineral content, which may be mediated by common nutritional deficiencies such as iron and vitamin D.[1-16] Conversely, the bone health cluster had negative correlations with CRP and Urinary Ca. CRP is an inflammatory marker, and long-term, low-grade inflammation has been shown to contribute to increased osteoclastic activity and reduced bone formation.[17] The inverse association with

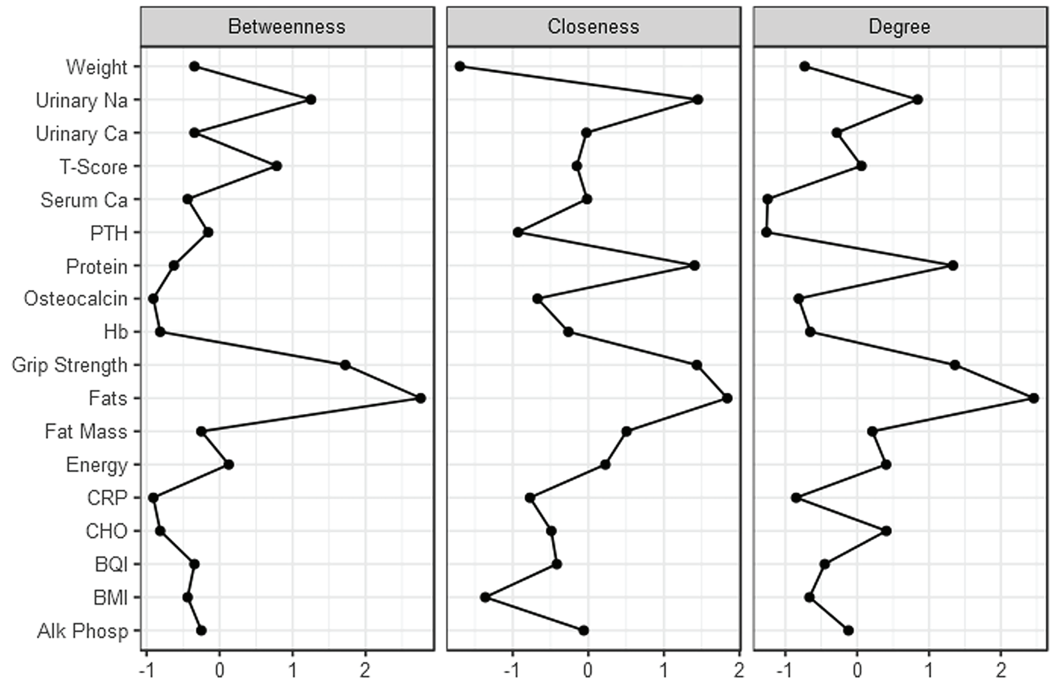

Figure 2. Centrality measures of the network.

Urinary Na: Urinary sodium, Urinary Ca: Urinary calcium, PTH: Parathyroid hormone, Hb: Hemoglobin, CRP: C-reactive protein, BQI: Bone quality index, BMI: Body mass index.

Urinary Ca indicates that inappropriately high renal calcium excretion can deplete calcium required for bone mineralization, a finding also noted in adolescents with adverse calcium balance.[18] Urinary Na was similar to both Urinary Ca and CRP, as would be expected given the established actions of excess sodium ingestion on calcium excretion via natriuretic mechanisms.[19] This may suggest a mechanistic process in which excessive dietary sodium may indirectly compromise bone health via enhanced calciuria and inflammatory stress. However, this causal relationship warrants further investigation.

The biochemical cluster (CRP, PTH, serum calcium, ALP, TP, osteocalcin, Hb) was characterized by both within-cluster coherence and connectivity with other systems. PTH, for example, is inversely related to serum calcium, reflecting the physiological feedback mechanism whereby declining serum calcium triggers PTH release, mobilizing skeletal calcium deposits.[20] ALP, a marker of bone formation and liver function, was moderately associated with osteocalcin and total protein, suggesting potential shared biosynthetic and metabolic pathways.[21] Significantly, total protein was weakly associated with bone and inflammatory markers, suggesting that it reflects a general aspect of nutritional status that influences multiple physiological systems.[22]

The centrality statistics from the network analysis (Figure 2) are indicative of how strongly every variable is linked to other variables in the system and how much influence it has within the network. Grip strength had the highest betweenness, closeness, strength, and expected influence, indicating that it is highly central and influential within the network; it may serve as a bridge between other variables, signifying strong relationships among multiple clusters. T-score also exhibited high strength and proximity, underpinning its central role in the bone health cluster, although its anticipated impact was negative, indicating it could be inversely correlated with some related variables. Urinary cawas another central node, showing high strength and a positive anticipated influence, supporting its significance in the physiological interaction between nutritional intake and bone metabolism. Conversely, measures such as PTH, CHO, and protein exhibited low or negative centrality across multiple centrality metrics, indicating less integration and influence in the system at baseline. CRP and energy intake also had relatively low centrality, reflecting reduced interconnectedness or influence within the system. Notably, serum total protein exhibited high closeness and betweenness centrality, suggesting that it serves as a nexus between otherwise disparate variables. These centrality profiles not only help identify the most central variables but also indicate which factors are potentially more peripheral, offering a baseline map of interactions against which post-intervention networks can be compared to detect changes in system dynamics. Supplementary File 1 (Supplementary Tables 1-4) provides detailed data for further reference.

Overall, the network indicates a highly integrated system in which anthropometric indices, biochemical status, and urinary losses regulate and report on bone health outcomes. This is consistent with a biopsychosocial model of adolescent bone development in which musculoskeletal load, nutritional sufficiency, endocrine status, and low-grade inflammation collectively regulate skeletal integrity.[23]

Mechanistically, increased body fat can compromise bone quality via adipocyte-derived inflammatory cytokines, whereas greater grip strength favors osteogenesis by mechanotransduction pathways. Similarly, calcium homeostasis emerges as an central mediator, affected by diet (sodium), renal processing (Urinary Ca), hormonal regulation (PTH), and systemic inflammation (CRP), all ultimately influencing bone health.

The cross-sectional analysis identified complex intra- and interrelationships among anthropometric, biochemical, dietary, bone-health, and urinary variables in adolescent girls, providing insight into their underlying health profile. Striking intra-cluster correlations-e.g., between BMI, weight, body fat, and grip strength-emphasize the coordinated development of physical growth and functional ability during adolescence, a period characterized by marked somatic changes.[24] Notably, blood biochemical markers, including CRP, serum calcium, osteocalcin, and ALP were intercorrelated, reflecting the interrelated nature of systemic inflammation, bone turnover, and mineral balance. Increased CRP might suggest low-grade inflammation, which has been linked to altered bone remodeling and nutrient uptake.[25] Correlations between dietary macronutrients and clusters of anthropometric and blood chemistry measures could reflect the contribution of energy and protein to growth and bone development, whereas imbalances might interfere with metabolic and inflammatory homeostasis.[26] The association between the urinary cluster and dietary and serum calcium levels indicates the importance of excretion and absorption processes in calcium balance and bone accretion. The association between the bone health cluster and the biochemical markers osteocalcin and serum calcium suggests a physiological link among bone density, mineral availability, and metabolic activity.[27] This unified map of variable relationships not only places the participants' baseline physiological status in context but also offers a critical framework for assessing how the intervention can alter these dynamics over time, perhaps pointing toward principal levers for enhancing adolescent bone health and, more broadly, well-being.

These findings provide a complete picture of the multifactorial predictors of bone strength and suggest that interventions need to target not only calcium intake but also physical activity, inflammation reduction, and Urinary Ca conservation to improve skeletal outcomes in adolescent girls.

Study Limitations

This study used a cross-sectional study design with relatively a small sample size which may limit the study findings to be generalized.

CONCLUSION

The network intra- and interconnections among variables present structural connectivity, representing coordinated physiological interactions in adolescent girls and serving as a reference point for detecting intervention-induced change in subsequent measurements at 3 and 6 months.

MAIN POINTS

- Very strong intra-cluster associations were observed, especially between anthropometric and dietary variables.
- Serum calcium, osteocalcin, and C-reactive protein appeared as key, highly connected nodes, revealing interrelations among bone metabolism, inflammation, and nutrition.

- Food macronutrients, particularly protein and fat, were associated with both anthropometric and biochemical indicators.
- Urinary calcium was correlated with serum calcium and total protein intake, demonstrating a physiological coupling among intake, absorption, and elimination.
- Bone health indicators (T-score, bone quality index) were closely associated with blood markers such as alkaline phosphatase and osteocalcin.

ETHICS

Ethics Committee Approval: The study received ethical approval from the Bacha Khan University Charsadda Research Ethics Commite (approval no: 12/EIRB/ORIC/BKUC/2024, date: 07.05.2024).

Informed Consent: The parents or legal guardians of all participants provided written informed consent before enrollment.

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Footnotes

Authorship Contributions

Concept: S.S., I.A., Design: S.S., I.A., Data Collection and/or Processing: S.S., I.A.B., S.F., H.S., I.A., Analysis and/or Interpretation: S.S., I.A.B., S.F., H.S., I.A., Literature Search: S.S., I.A.B., S.F., H.S., I.A., Writing: S.S., I.A.B., S.F., H.S., I.A.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Pinheiro J, Ribeiro L, Teixeira D, Ribeiro A, Coelho-E-Silva MJ. Skeletal maturity in adolescence: evaluating bone development and age metrics. Diagnostics (Basel). 2025; 15(8): 970.
2. Shah S, Alktebi A, Alam I. Low calcium intake of pre-adolescent girls from customary diets in a semi-rural setting in Khyber Pakhtunkhwa, Pakistan: low calcium intake of pre-adolescent girls from customary diets. DIET FACTOR (Journal of Nutritional and Food Sciences). 2025; 6(2): 15-9.
3. Zafar N, Mahmood S, Anjum N, Saldera KA, Zafar S, Hafiz SA. Association between vitamin D, P1NP, and BMD as major indicators of bone health in local population of Pakistan. International Journal Of Health Sciences. 2023; 7(S1): 271-83.
4. Alam S, Khan SB, Khattak QW, Abidin SZU, Farooqi S, Khan Z, et al. Level of physical activity in undergraduate students in Peshawar, Pakistan. Asian Journal of Health Sciences. 2021; 7(1): ID20.
5. Dong Y, Yuan H, Ma G, Cao H. Bone-muscle crosstalk under physiological and pathological conditions. Cell Mol Life Sci. 2024; 81(1): 310.
6. Meier M, Summers BJ, Buhlmann U. Which symptoms bridge symptoms of depression and symptoms of eating disorders?: A network analysis. J Nerv Ment Dis. 2024; 212(1): 61-7.
7. Ralph-Nearman C, Williams BM, Ortiz AML, Levinson CA. Investigating the theory of clinical perfectionism in a transdiagnostic eating disorder sample using network analysis. Behav Ther. 2024; 55(1): 14-25.
8. Shah S, Al Ktebi A, Alam I. Knowledge about calcium-rich foods in adolescent girls in Charsadda–a cross-sectional study: knowledge about calcium-rich foods in adolescent girls. DIET FACTOR (Journal of Nutritional and Food Sciences). 2024; 5(4): 25-30.
9. Shah S, Alam I. Determination of calcium content in a cocktail of calcium supplemented biscuits and dry milk: cocktail of calcium supplemented biscuits and dry milk. DIET FACTOR (Journal of Nutritional and FoodSciences). 2024; 5(04): 31-7.
10. Alam I. Exploring relationships between common healthy behaviors in adolescents using innovative social network analysis: adolescent health behaviors and network links. DIET FACTOR (Journal of Nutritional and Food Sciences). 2024; 5(3): 29-34.
11. Riviati N, Darma S, Reagan M, Iman MB, Syafira F, Indra B. Relationship between muscle mass and muscle strength with bone density in older adults: a systematic review. Ann Geriatr Med Res. 2025; 29(1): 1-14.
12. Faienza MF, Urbano F, Chiarito M, Lassandro G, Giordano P. Musculoskeletal health in children and adolescents. Front Pediatr. 2023; 11: 1226524.
13. Schini M, Vilaca T, Gossiel F, Salam S, Eastell R. Bone turnover markers: basic biology to clinical applications. Endocr Rev. 2023; 44(3): 417-73.
14. De Martinis M, Allegra A, Sirufo MM, Tonacci A, Pioggia G, Raggiunti M, et al. Vitamin D deficiency, osteoporosis and effect on autoimmune diseases and hematopoiesis: a review. Int J Mol Sci. 2021; 22(16): 8855.
15. Shoemaker ME, Salmon OF, Smith CM, Duarte-Gardea MO, Cramer JT. Influences of vitamin D and iron status on skeletal muscle health: a narrative review. Nutrients. 2022; 14(13): 2717.
16. Yang J, Li Q, Feng Y, Zeng Y. Iron deficiency and iron deficiency anemia: potential risk factors in bone loss. Int J Mol Sci. 2023; 24(8): 6891.
17. Epsley S, Tadros S, Farid A, Kargilis D, Mehta S, Rajapakse CS. The effect of inflammation on bone. Front Physiol. 2021; 11: 511799.
18. Zhukouskaya VV, Linglart A, Lambert AS. Disorders of Calcium homeostasis in childhood and adolescence. InPaediatric Endocrinology: Springer International Publishing. 2024; 283-324.
19. Liu M, Wu J, Gao M, Li Y, Xia W, Zhang Y, et al. Lifestyle factors, serum parameters, metabolic comorbidities, and the risk of kidney stones: a mendelian randomization study. Front Endocrinol (Lausanne). 2023; 14: 1240171.
20. Stanley S, Martin A. Thyroid, parathyroid hormones and calcium homeostasis. Anaesthesia & Intensive Care Medicine. 2023; 24(10): 639-43.
21. Miriouni E, Trifonidi I, Chronopoulos E, Makris K. Alkaline phosphatase-biochemistry, biological functions measurement and clinical relevance. JRPMS. 2025; 9(1): 33-45.
22. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr. 2013; 109 (Suppl 1): S1-34.
23. Collins KH, Herzog W, MacDonald GZ, Reimer RA, Rios JL, Smith IC, et al. Obesity, metabolic syndrome, and musculoskeletal disease: common inflammatory pathways suggest a central role for loss of muscle integrity. Front Physiol. 2018; 9: 112.
24. Pařízková J. Physical activity, fitness, nutrition and obesity during growth: secular changes of growth, body composition and functional capacity in children and adolescents in different environment. Bentham science publishers. 2015.

25. Torres HM, Arnold KM, Oviedo M, Westendorf JJ, Weaver SR. Inflammatory processes affecting bone health and repair. Curr Osteoporos Rep. 2023; 21(6): 842-53.

26. Kotas ME, Medzhitov R. Homeostasis, inflammation, and disease susceptibility. Cell. 2015; 160(5): 816-27.

27. Kini U, Nandeesh BN. Physiology of bone formation, remodeling, and metabolism. Radionuclide And Hybrid Bone Imaging. 2012; 29-57.

Supplementary File 1

Social Network Analysis (SNA) Guide

Guide to Describing Social Network Analysis (SNA)

1. What is SNA?

- Social Network Analysis (SNA) is a technique applied to the study of relationships among entities (referred to as nodes) and the ties (referred to as edges) among them.
- Rather than concentrating on individual attributes alone, SNA emphasizes where variables are located in a system and how they interact.
- It is highly applied in sociology, epidemiology, psychology, organizational studies, and health sciences.

2. Key Elements

- Nodes: The units being studied (e.g., individuals, behaviors, or variables).
- Edges (ties/links): The connections between nodes (e.g., correlations, interactions, or communications).

Network: The whole set of nodes and their connections.

3. Centrality Measures (Common in SNA Results)

These tell us how "important" or "influential" a node is within the network:

- Degree/strength: How many direct connections a node has (or the sum of connection weights).
- Closeness: The proximity of a node to all the others; greater closeness = quicker access to information or influence.
- Betweenness: The frequency of occurrence of a node being on the shortest path between two others; high betweenness = a bridging or brokerage role.
- Expected influence: For both positive and negative edges; indicates whether a variable supports or resists other variables.

4. Why use SNA?

- Determines the most central or influential factors in a system.
- Aids in discovering clusters or communities (sets of nodes highly linked).
- Unveils structural patterns that may not otherwise be apparent through conventional statistics.
- In health/nutrition, assists to observe how behaviors, biomarkers, and risk factors are interlinked.

5. Steps in Conducting SNA (Typical Workflow)

- Data gathering - collect variables or relational data (e.g., correlations, survey answers).
- Matrix building - express the data as an adjacency matrix (rows and columns = nodes, values = strength of links).
- Network estimation - apply statistical or graphical models (e.g., Gaussian graphical models, partial correlations).
- Visualization - plot the network (nodes + edges), usually with clustering and color schemes.
- Centrality analysis - compute indices (strength, closeness, betweenness, expected influence).
- Interpretation - connect network structure back to theory and research questions (e.g., which variables are central, which are peripheral).

6. How to Explain Results in Plain English

- "A highly strength variable is directly connected to numerous others."
- "A highly closeness variable can rapidly affect or be affected by the remaining network."
- "A highly betweenness variable behaves like a "bridge" between various parts of the system."
- "Negative centrality scores indicate weaker or reverse roles within the complete system."

7. SNA Limitations

- SNA exhibits associations rather than causation.
- Network pattern can vary based on variable choice and sample size.
- Centrality measures' interpretation must be theory-informed and careful.

8. SNA Example

Materials and Methods - Social Network Analysis

In this current study, SNA was used to investigate the interconnection among clinical, biochemical, dietary, and anthropometric variables of concern for adolescent bone and nutritional outcomes. In this method, every variable was considered a node, and the statistical relationships among variables were indicated as edges between nodes. The network was predicted based on a correlation matrix, where edge thickness and direction symbolize the strength and sign of relationships.

To measure the relative significance of every variable in the network, centrality measures were computed:

- Strength: The total direct connections of a node, representing the general degree of engagement of the node in the network.

- Closeness: The mean distance of a node to all others, indicating how effectively a variable is linked to the system.
- Betweenness: How much a node falls on the shortest path from others, indicating its bridging role.
- Expected influence (positive/negative): Capturing whether a node supports or hinders other nodes, enabling interpretation of inhibitory versus facilitative connections.

The resulting network was mapped using color-coded clusters, indicating variables with greater interconnections. Centrality plots were also created to compare relative positions of variables. Interpretation involved identifying those variables with high centrality, which could potentially be key drivers or hubs in adolescent bone health, and those with low or negative influence, which could potentially be peripheral or less influential factors.

Supplementary Table 1. Network analysis

Summary of network

	Network
Number of nodes	18
Number of non-zero edges	37
Sparsity	0.242

Supplementary Table 2. Weight matrix

BQI	CHO	CRP	Energy	Fat mass	Fats	Grip strength	Hb	Osteocalcin	PTH	Protein	Serum Ca	T-score	Urinary Ca	Urinary Na	Weight
-0.02	0.02	0.37	-0.07	0.23	-0.15	0.08	0.20	0.00	-0.05	0.04	0.03	0.06	0.01	0.00	0.03
0.02	0.00	0.00	0.00	0.05	-0.06	-0.04	0.04	-0.02	-0.03	-0.02	0.00	-0.14	0.12	0.00	0.55
0.00	0.00	-0.08	0.01	0.09	-0.08	0.00	0.00	0.00	0.00	0.00	0.15	0.75	0.00	0.05	0.02
0.00	0.00	0.00	0.80	0.09	-0.26	0.03	0.00	-0.03	-0.05	0.20	-0.06	-0.03	0.05	-0.04	0.04
-0.08	0.00	0.00	0.00	0.14	0.00	-0.21	-0.02	0.04	-0.02	-0.02	-0.01	-0.01	-0.10	0.01	0.05
0.01	0.80	0.00	0.00	0.01	0.49	0.00	0.00	0.00	0.00	-0.24	-0.04	0.00	0.02	0.00	0.03
0.09	0.09	0.14	0.01	0.00	0.00	-0.34	-0.03	-0.04	0.16	0.07	0.00	0.02	0.16	-0.15	0.02
-0.08	-0.26	0.00	0.49	0.00	0.00	-0.13	0.10	0.12	0.00	0.56	0.16	0.23	-0.06	0.30	0.05
0.00	0.03	-0.21	0.00	-0.34	-0.13	0.00	0.29	-0.06	0.03	0.15	0.23	0.01	0.36	-0.19	-0.02
0.00	0.00	-0.02	0.00	-0.03	0.10	0.29	0.00	0.03	0.02	0.00	0.00	0.05	0.33	0.07	0.01
0.00	-0.03	0.04	0.00	-0.04	0.12	-0.06	0.03	0.00	-0.01	-0.22	0.04	0.03	-0.08	0.36	-0.01
0.00	-0.05	-0.02	0.00	0.16	0.00	0.03	0.02	-0.01	0.00	0.00	-0.09	0.00	-0.07	-0.13	0.20
0.00	0.20	-0.02	-0.24	0.07	0.56	0.15	0.00	-0.22	0.00	0.00	0.00	0.05	0.00	-0.60	0.00
0.15	-0.06	-0.01	-0.04	0.00	0.16	0.23	0.00	0.04	-0.09	0.00	0.00	0.04	0.00	0.03	0.00
0.75	-0.03	-0.01	0.00	0.02	0.23	0.01	0.05	0.03	0.00	0.05	0.04	0.00	0.00	0.00	-0.11
0.00	0.05	-0.10	0.02	0.16	-0.06	0.36	0.33	-0.08	-0.07	0.00	0.00	0.00	0.00	0.00	0.00
0.05	-0.04	0.01	0.00	-0.15	0.30	-0.19	0.07	0.36	-0.13	-0.60	0.03	0.00	0.00	0.00	0.00
0.02	0.04	0.05	0.03	0.02	0.05	-0.02	0.01	-0.01	0.20	0.00	0.00	-0.11	0.00	0.00	0.00

Urinary Na: Urinary sodium, Urinary Ca: Urinary calcium, PTH: Parathyroid hormone, Hb: Hemoglobin, CRP: C-reactive protein, BQI: Bone quality index, BMI: Body mass index, CHO: Carbohydrate.

Supplementary Table 3. Layout

x	y
Alk phosp=0.6957	Alk phosp=0.1526
BMI=0.4522	BMI=0.8319
BQI=-0.9119	BQI=0.5216
CHO=-1	CHO=-0.2554
CRP=0.9678	CRP=0.4056
Energy= 0.8826	Energy=-0.5149
Fat mass=0.2232	Fat mass=0.0753

CRP: C-reactive protein, BQI: Bone quality index, BMI: Body mass index, CHO: Carbohydrate.

Supplementary Table 4. Centrality measures per variable

	Network		
Variable	Betweenness	Closeness	Strength
Alk phosp	-0.25	-0.06	-0.121
BMI	-0.438	-1.363	-0.664
BQI	-0.344	-0.417	-0.451
CHO	-0.814	-0.49	0.406
CRP	-0.908	-0.772	-0.85
Energy	0.125	0.225	0.403
Fat mass	-0.25	0.506	0.211
Fats	2.754	1.838	2.459
Grip strength	1.722	1.436	1.361
Hb	-0.814	-0.263	-0.653
Osteocalcin	-0.908	-0.672	-0.814
PTH	-0.157	-0.93	-1.263
Protein	-0.626	1.406	1.334
Serum Ca	-0.438	-0.017	-1.247
T-score	0.783	-0.152	0.06
Urinary Ca	-0.344	-0.026	-0.283
Urinary Na	1.252	1.45	0.842
Weight	-0.344	-1.699	-0.73

Urinary Na: Urinary sodium, Urinary Ca: Urinary calcium, PTH: Parathyroid hormone, Hb: Hemoglobin, CRP: C-reactive protein, BQI: Bone quality index, BMI: Body mass index, CHO: Carbohydrate.