Geographic and Sexual Dimorphism in Calcium-Regulating Hormones: A Comparative Study of Urban and Rural Students at Tikrit University

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Abstract

Objective:

This study analyzed how residential setting (urban or rural) and sex influence key biochemical regulators of calcium homeostasis serum calcium, vitamin D3, PTH, and calcitonin—in students at Tikrit University.

Materials and Methods:

A total of 90 healthy students aged 20 to 35 years were enrolled and randomly assigned to urban and rural groups, with equal gender representation. The study took place from December 2022 to February 2023. Blood samples were collected to measure serum levels of vitamin D3, PTH, and calcitonin using commercial ELISA kits, and calcium was measured by colorimetric analysis. Data were analyzed with SPSS using one-way ANOVA and Duncan's test, with significance set at $p \leq 0.05$ and $p \leq 0.01$.

Results:

Residential location significantly affected certain parameters: rural students had higher calcium ($p \leq 0.01$) and PTH ($p \leq 0.05$) levels than urban students. Sex-based differences were also notable, with males exhibiting higher calcium levels ($p \leq 0.01$) and females exhibiting higher PTH levels ($p \leq 0.01$). No significant differences related to residence or sex were observed for calcitonin or vitamin D3. Interaction analysis indicated that rural males had the most favourable profile, with higher calcium and vitamin D3 levels, whereas rural females had higher PTH levels.

Conclusion:

The study indicates that geographic location and sex influence calcium regulation, reflecting possible physiological or lifestyle adaptations. Rural men exhibited superior biochemical profiles, while women showed elevated PTH levels, likely due to environmental or dietary factors. Calcitonin remained stable, suggesting a minor role.

Keywords: Calcitonin, Calcium, Parathyroid Hormone (PTH), Rural vs. Urban Residence, Vitamin D3.

1. Introduction:

The regulation of calcium balance is essential for maintaining skeletal integrity and supporting a wide range of physiological functions throughout adulthood. Although bone disorders are commonly associated with advanced age, recent evidence indicates that mineral balance and hormonal regulation during early adulthood play a decisive role in determining long-term bone health [1, 3]. This stage of life is characterized by continued bone remodeling and metabolic stability, making it a suitable period for investigating factors that influence calcium homeostasis. calcium is a fundamental mineral in the human body, with the majority stored in bone tissue and a smaller fraction involved in critical biological processes such as muscle contraction, nerve conduction, and intracellular signaling [4]. The maintenance of stable serum calcium levels depends on coordinated hormonal activity, mainly involving vitamin D3 and parathyroid hormone (PTH), while calcitonin contributes to calcium regulation under specific physiological conditions [5, 7]. Vitamin D3 facilitates intestinal calcium absorption and supports bone mineralization, whereas PTH acts to maintain circulating calcium levels by regulating bone turnover and renal calcium handling [8, 9]. Calcitonin, in contrast, exerts a more limited role by inhibiting excessive bone resorption [10]. Environmental conditions and lifestyle patterns are increasingly recognized as influential factors in calcium metabolism. Differences between urban and rural living environments may affect dietary quality, physical activity, and exposure to sunlight, all of which are relevant to calcium and vitamin D3 status [11 , 12] . Rural populations often engage in greater levels of outdoor activity and physical labor, potentially enhancing bone loading and calcium utilization, while urban lifestyles may be associated with reduced sunlight exposure and more sedentary behavior [13] . Boilogical sex also plays an important role in calcium regulation due to differences in hormonal profiles, body composition, and metabolic requirements. Variations in sex hormones may influence both calcium levels and PTH secretion, contributing to observable differences between males and females [14, 15]. However, previous studies have reported inconsistent findings regarding sex-related differences in vitamin D3 and calcitonin concentrations, suggesting that environmental and lifestyle factors may interact with biological sex in shaping these outcomes [16, 17]. Despite the relevance of these factors, data examining the combined effets of residence and sex on calcium – regulating hormones among young adults remain limited, particularly within the Iraqi population. Most available research has focused on children or older adults, leaving a gap in understanding hormonal regulation during early adulthood [18, 19]. Addressing this gap is important for clarifying how environmental and biological influences contribute to calcium homeostasis in healthy individuals. In light of these considerations, the present study examined the influence of residential setting (urban versus rural) and sex on serum calcium, vitamin D3, parathyroid hormone, and calcitonin levels among students at Tikrit University, with the aim of providing clearer insight into factors affecting calcium regulation during early adulthood.

2. Materials and Methods

Study Design and Sample

The current study was conducted among students at Tikrit University to assess the levels of calcium, vitamin D3, parathyroid hormone (PTH), and calcitonin among students residing in rural and urban areas from December 27, 2022, to February 2, 2023. A sample of 90 patients was selected and randomly assigned to two groups. The first cohort comprised 45 students residing in the urban area (21 males and 22 females). The second cohort comprised 45 students living on the rural outskirts (24 rural males and 23 rural females).

Participants were randomly selected using specific criteria to ensure the accuracy of the findings. Participants who were consuming vitamin D3 tablets, calcium tablets, or dietary supplements, and pregnant women, were excluded. Participants were aged 20–35 years, and selection was limited to healthy individuals without chronic disease. Approximately 2–3 mL of venous blood was collected using clean, anticoagulant-free tubes. The samples were incubated at room temperature (25 °C) until clotting was complete, then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was stored at -20 °C until analysis to preserve the stability of biochemical parameters.

Precautions were strictly implemented to reduce the risk of hemolysis and its direct impact on biochemical analysis results.

Laboratory Measurements

Commercial ELISA kits (Sunlong Biotech Co., Ltd, China) were used in measuring the concentration of the following parameters:

Human 25-hydroxy vitamin D (25-OH-D) ELISA Kit

Catalog No.: SL/1898Hu

Human Parathyroid Hormone (PTH) ELISA Kit

Catalog No.: SL/1342Hu

Human Calcitonin (CT) ELISA Kit

Catalog No.: SL/0391Hu

A 10 µL sample volume was used for each assay, and absorbance was measured at 450 nm using a microplate reader. Standard calibration curves were generated for each assay. The serum calcium concentration was measured using a colorimetric kit (BioLabo, France), which employs a thymol blue dye reaction with calcium in an alkaline medium. The color intensity, proportional to calcium concentration, was measured spectrophotometrically at 570 nm.

Ethical Considerations:

The study protocol received approval from the Institutional Review Board (IRB) of the College of Science at Tikrit University (Approval Number: sciTuH 0005, dated 14-12-2025). This approval ensured compliance with the ethical standards set forth in the Declaration of Helsinki. Given the study's retrospective design, which uses pre-existing, anonymized data, the IRB waived the requirement for individual informed consent.

Statistical Analysis

Data were analyzed using SPSS (SPSS, 2001). One-way analysis of variance (One-way ANOVA) was used to compare group differences. Means were compared and differences specified in detail using Duncan's multiple-range test. The study applied a statistical significance level of ($p \leq 0.05$) to identify a significant difference, and ($p \leq 0.01$) to specify a highly significant difference.

3. Results:

This study presents serum biochemical parameters among students at the University of Tikrit residing in rural and urban areas.

Table 1: Effect of Residence on Biochemical Parameters

Test	Urban (Mean ± SD)	Rural (Mean ± SD)	Significance	P Value
Calcium (Ca, mg/dl)	8.47 ± 1.45	9.06 ± 0.62	**	$p \leq 0.01$
Parathyroid Hormone (PTH, mg/dl)	61.0 ± 20.8	72.1 ± 23.5	*	$p \leq 0.05$
Calcitonin (CT)	25.3 ± 21.8	30.2 ± 23.8	NS	Not significant

Vitamin D3 (D3)	*43*.3 ± 17.7	44.8 ± 16.8	NS	Not significant
Note : * Significant difference at (p ≤ o.o5) , ** Highly significant difference at (p ≤ o.o5)				

Table 2: Effect of Gender on Biochemical Parameters

Test	Males (Mean ± SD)	Females (Mean ± SD)	Significance	P Value
Calcium (Ca, mg/dl)	9.08 ± 0.72	8.48 ± 1.37	**	p ≤ 0.01
Parathyroid Hormone (PTH, mg/dl)	59.8 ± 16.4	73.8 ± 26.2	**	p ≤ 0.01
Calcitonin (CT)	28.4 ± 24.5	27.3 ± 21.5	NS	Not significant
Vitamin D3 (D3)	46.0 ± 18.5	42.1 ± 15.7	NS	Not significant
Note : * Significant difference at (p ≤ o.o5) , ** Highly significant difference at (p ≤ o.o5)				

Table 3 Interaction of Gender and Residence on Biochemical Parameters

Test	Urban Males	Urban Females	Rural Males	Rural Females
	21	22	24	23
Calcium (Ca, mg/dl)	8.74 ± 0.63 Ab	8.21 ± 1.91 B	9.37 ± 0.68 A	8.74 ± 0.35 Ab
Parathyroid Hormone (PTH, mg/dl)	57.54 ± 13.47 B	64.30 ± 25.85 B	61.81 ± 18.66 B	82.80 ± 23.60 A
Calcitonin (CT)	28.01 ± 6.40 A	22.71 ± 7.66 A	28.66 ± 8.45 A	31.71 ± 8.69 A
Vitamin D3 (D3)	39.93 ± 18.89 B	46.47 ± 16.28 Ab	51.33 ± 16.79 A	37.95 ± 14.24 B
Note: Different superscript letters (a,b.ab) indicate significant differences at (p ≤ o.o5)				

4. Discussion:

Effect of Residence on Some Blood Indices:

As can be seen from Table 1, rural students had higher levels of calcium (9.06 ± 0.62 mg/dl) compared to urban students (8.47 ± 1.45 mg/dl) with a highly significant difference ($p < 0.01$). This finding may be attributed to several interrelated factors, including differences in dietary habits, food quality, and increased exposure to sunlight among rural inhabitants. Sunlight is required for the skin to synthesize vitamin D, which is central to calcium absorption.[1] Yet, in the study by [12] , no differences in calcium levels were detected between urban and rural residents, highlighting the precision of hormonal regulation in maintaining serum calcium within a narrow physiological range. This underscores the importance of regulatory hormones, particularly parathyroid hormone (PTH), in calcium homeostasis [13] . Work [14] reported on the relationship between vitamin D3, calcium, and the enzyme ALP, indicating that the absence of vitamin D3 is generally associated with low calcium and elevated alkaline phosphatase.

Rural pupils in Table 1 also had higher PTH levels (72.1 ± 23.5) than their urban peers (61.0 ± 20.8), with a significant difference ($p < 0.05$). Given their higher calcium status and normal vitamin D3 levels, and in the absence of prevalent diseases or pathological conditions such as renal failure, vitamin D3 deficiency, or parathyroid tumors, these findings are best interpreted through functional or physiological mechanisms rather than disease-related causes [15] . One of the most relevant explanations is an altered calcium set point, whereby individual variations in the calcium-sensing threshold result in sustained PTH secretion even at calcium levels considered normal in other individuals. This is not a disease per se, but rather a physiological variation that is genetic or secondary to prolonged adaptation to lifestyle [16] . Another contributing factor may be increased physical stress or prolonged physical activity (such as farm labor, herding, and daily walking), which is more prevalent in rural populations. This mechanical loading stimulates bone remodeling, necessitating elevated PTH secretion to regulate calcium and phosphate mobilization from bone [17] .

Another probable reason is peripheral resistance to PTH (PTH resistance), a relatively uncommon condition where tissues are less responsive to PTH. In compensation, the parathyroid gland produces additional PTH despite apparently normal biochemical profiles. This is not necessarily a disease and may be transient or partial [18] . Subclinical magnesium deficiency is another plausible contributor, as magnesium is required to prevent excessive PTH secretion. Even with normal vitamin D3 and calcium levels, magnesium deficiency can lead to elevated PTH levels [19] . This condition may be more prevalent in rural settings due to limited dietary diversity or reliance on low-mineral water sources [20] .

Another choice is normocalcemic primary hyperparathyroidism, in which the parathyroid gland secretes excess PTH for no evident reason. The disorder is often clinically silent and may arise from microscopic adenomas or intrinsic dysregulation of the gland [21] .

There were no significant differences between urban and rural students in calcitonin concentration, indicating that environmental factors in this population do not significantly influence calcitonin secretion. Calcitonin primarily inhibits osteoclast activity and bone resorption, thereby contributing to stabilization of bone density; bones do not usually undergo marked changes except under pathological conditions [22] . It regulates intracellular processes via cAMP and PKA and suppresses the development of new osteoclasts [23] . Calcitonin is clinically used to reduce fracture risk [24] . A study by [25] demonstrated that calcitonin is regulated by multiple factors, including age, sex, weight, lifestyle, diet quality, physical activity, and smoking. These findings suggest that lifestyle differences may exist, but are insufficient to produce statistically significant differences in calcitonin levels between rural and urban residents.

There was no significant variation in Table 1 in the level of vitamin D3 between urban students (43.3 ± 17.7) and rural students (44.8 ± 16.8). This may reflect comparable sun-exposure habits or widespread supplement use in both groups [26] . This result contrasts with [14, 27], which reported that urban participants had lower vitamin D3 levels than rural participants, perhaps due to reduced sun exposure in urban regions resulting from enclosed lifestyles and skyscrapers. Conversely, rural dwellers are more susceptible to sun exposure due to farm work, outdoor exposure, and a lifestyle more attuned to the environment [28, 29] . In a study by [30] Children in rural areas were reported to have lower vitamin D levels than urban children, with inadequate sun exposure and insufficient dietary sources of vitamin D identified as the main reasons.

The Effect of Sex on Certain Biochemical Parameters:

Table 2 shows that male students (9.076 ± 0.723) had significantly higher calcium levels than female students (8.480 ± 1.370) ($P \leq 0.01$), consistent with [31]. This may partly be attributed to higher total bound calcium in men than in women, particularly pregnant women, due to higher albumin levels. Women, especially during pregnancy, experience a physiological decrease in total and plasma protein levels due to blood volume expansion. This change reduces the protein-bound calcium capacity, underscoring the compensatory role of hormones in maintaining stable ionized calcium. Other contributing factors include increased muscle and bone mass in males, which leads to higher metabolic demands and a more dynamic exchange of calcium between bones and blood. However, the metabolic needs of women, particularly pregnant women, necessitate a more dynamic exchange of calcium between maternal bone and blood to support fetal skeletal growth [32] . Testosterone also enhances intestinal calcium absorption and bone formation, thereby raising serum calcium levels [33, 34] . Changes in lifestyle patterns and physical activity can also play a role, since men are likely to have higher calcium intake and greater physical activity, both of which are favorable for bone formation [35] . On the other hand, women lose calcium from menstruation, pregnancy, and lactation, and such losses are not necessarily compensated. [36] . In addition, estrogen may suppress intestinal calcium absorption, resulting in lower calcium levels in the premenopausal period. After menopause, calcium redistribution in bone becomes deranged, further reducing calcium [37].

Table 2 also shows significantly higher PTH levels in female students than in male students ($P \leq 0.01$), consistent with [38] This is because of hormonal change, as estrogen induces the parathyroid gland to secrete more PTH directly, particularly at puberty and menopause [39] . Estrogen indirectly affects PTH levels by inducing fibroblast growth factor 23 (FGF23), a protein secreted by maximally differentiated osteocytes that reduces the production of $1,25(OH)_2D_3$ in the kidney by suppressing the 1α-hydroxylase enzyme, thereby modulating calcium-phosphorus metabolism and controlling PTH secretion [40] . In addition, greater muscle mass in men would help stabilize calcium and phosphorus levels, thereby reducing PTH secretion [41] . Genetic and transcriptional control may also be involved, as [42] using single-cell transcriptomics showed higher expression of PTH-related genes, such as FABP5 and VIM, in females. Lifestyle factors such as alcohol consumption, smoking, and physical exercise have also been shown to influence PTH [16] . These findings, however, contrast with [43] , who found no sex-dependent differences in PTH.

As indicated in Table 2, there is no difference between male and female students in calcitonin hormone levels. The finding agrees with [44] , and may be attributed to the fact that calcitonin is made by C cells of the thyroid gland and works to decrease blood calcium by inhibiting the activity of osteoclasts. This control system is directly sensitive to calcium and is not regulated by sex hormones such as estrogen or testosterone [45] . Calcitonin is not considered a primary regulator of calcium homeostasis because the body relies more on parathyroid hormone (PTH) and vitamin D3. As a result, calcitonin is less sensitive to gender hormonal variations [46] . The results are, nevertheless, inconsistent with observations by [25] , which identified elevated calcitonin levels in males compared with females. They add that this may be because of the larger size of the thyroid gland in men, which can heighten the secretion of calcitonin, as well as lifestyle factors such as smoking. [47] determined that sex does not influence fasting calcitonin levels but does affect the physiological response to food intake. Specifically, they reported a pronounced postprandial increase in calcitonin secretion in men, but not in women. Similarly, [48] reported clear structural discrepancies in the distribution of C cells, with a greater number in the thyroid glands of men than in those of women. This suggests a potential sex hormone effect on the histological features underlying calcitonin secretion.

Furthermore,[49] wrote that sex hormones, such as testosterone and estrogen, can exert indirect effects on C cells by regulating calcitonin secretion or by affecting C cell growth. This adds evidence to the hypothesis that sex may have both structural and functional effects on calcitonin kinetics. Secondly, with respect to vitamin D3 status, Table 2 shows no significant sex differences, consistent with [50] This is largely explained by lifestyle matching, sun-exposure patterns, and matching environmental, dietary, and hormonal determinants. Therefore, sex does not appear to be an important or independent predictor of vitamin D3 status in young adults [51] . These results conflict with those of [52] Smoking and an unhealthy lifestyle in men have a dual effect. While smoking causes a severe deficiency in vitamin D levels due to impaired metabolism and its retention in fatty tissues, it also abnormally increases blood viscosity and red and white blood cell counts. This imbalance increases oxidative stress and vascular inflammation in young men, making vitamin D deficiency a contributing factor to the worsening of cardiovascular risks associated with abnormal blood markers.

Furthermore,[53] reported higher vitamin D3 levels in men than in women. They attributed this on the grounds of cultural and behavioral determinants, such as decreased exposure to sunlight by women due to covering clothes, decreased activity levels, and dietary differences. Women also have higher body fat, which further increases vitamin D3 storage and limits its release into the circulation [54, 55] also demonstrated sex differences in gene expression in vitamin D metabolism in adipose tissue. Women were found to have higher levels of CYP27B1 and VDR, indicating more efficient conversion of vitamin D3 to its active form.

The Effect of Sex by Residence on Some Biochemical Parameters

Table 3 evidently indicates a statistically significant difference ($P \leq 0.05$) in calcium levels in favor of rural male students (9.367 ± 0.681) when compared with urban female students (8.209 ± 1.917). This can be attributed to conventional physical activity (e.g., farm work), which leads to greater lean mass and lower fat mass among rural residents. This, in turn, increases the mechanical loading of bones, leading to greater calcium absorption and increased bone strength, particularly in the femoral neck region [56] . Additionally, calcium-rich diets directly affect blood calcium levels and bone mineral density [35] .

Table 3 also indicates a statistically significant increase ($P \leq 0.05$) in PTH levels among female students in rural areas compared with their urban counterparts, as well as among male students in both rural and urban areas. It may be due to vitamin D3 deficiency, which leads to a compensatory increase in PTH secretion [57] . The second probable reason is wearing full-body coverings (hijab) or long clothing, which block ultraviolet rays, thereby significantly reducing vitamin D3 production in the skin and decreasing calcium absorption, thereby increasing PTH [58] .

With respect to calcitonin levels, Table 3 shows no statistically significant differences. This may be because calcitonin is a second-line regulator of calcium homeostasis relative to PTH and vitamin D3, and its concentration in healthy subjects is relatively stable. Calcitonin is secreted primarily in response to stimuli, such as an increase in calcium levels. Calcitonin's concentration is not immediately regulated by sex hormones [59] . Moreover, calcitonin is indirectly affected by nutrition and environmental pollution, which are standard, statistically controlled variables in data analysis [16] .

Table (3) also shows a statistically significant increase ($P \leq 0.05$) in rural male students' level of vitamin D3 compared to urban male students and rural females. This outcome is consistent with [60] and is attributable to significantly higher levels of sun exposure among rural populations [28] . In addition, reduced air pollution in rural environments limits exposure to ultraviolet light, which is necessary for the synthesis of vitamin D [61] . Rural dwellers are more likely to engage in agriculture, livestock production, or walking long distances, activities that necessarily expose them to more sunlight than urban dwellers, who spend most of their time indoors or in vehicles [62] .

Conclusion

The study demonstrated that rural students tend to have higher levels of calcium and parathyroid hormone (PTH), while urban students showed similar concentrations of calcitonin and vitamin D3, indicating differences influenced by residence. Male students exhibited significantly higher calcium levels, whereas females displayed elevated PTH levels; no significant sex-related differences were observed in calcitonin or vitamin D3. The most favorable biochemical profile was identified among rural males, characterized by elevated calcium and vitamin D3, whereas rural females showed increased PTH levels. Calcitonin levels remained stable across groups, reflecting its secondary role in calcium homeostasis. These findings highlight the combined impact of residence and sex on calcium regulation and underscore the importance of developing tailored health interventions, especially for urban populations and rural females.

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