Clinical studies about the influence of calcium intake on the biochemical parameters of bone metabolism

N W Rasheed* and R I Ahmad¹

¹AL-Rafidain University College, Medical Laboratory Techniques, Baghdad- Iraq

*Corresponding: noora.waal@ruc.edu.iq

Abstract. Calcium is a major component of bone, an adequate dietary supply of calcium during growth being considered critically important for the development of strong and healthy bone. The aim of the study was to evaluate the short term effects of dietary calcium intake on the biochemical diagnostic of the hyperparathyroidism in healthy people Subjects. The study was conducted on 36 subjects (age between 21 and 71 years, 4 men and 32 women) recruited from patients refereed to the Endocrinology Department of Elias Hospital who were not diagnosed with diseases proved to influence calcium or bone metabolism and agreed with the study for inclusion in the study, all subjects were checked for thyroid dysfunctions, hypercortisolism, hypogonadism, and active liver diseases which might interfere with total alkaline phosphatase values. The data obtained through this study sustain the significant effect of short term variations in calcium dietary intake on the biochemical diagnosis of hyperparathyroidism.

Keywords: osteoporosis, calcium, hyperparathyroidism

1. Introduction

Calcium is a major component of bone, an adequate dietary supply of calcium during growth being considered critically important for the development of strong and healthy bone. Moreover, after the period of growth and development, an adequate calcium intake is critical for preserving the integrity of the skeleton in humans and animals. Evidence from many studies support the important role of calcium optimal intake; consequently there are clinical guidelines for the calcium supplementation in order to assure and maintain healthy bones [1].

Low calcium intake certainly increases the risk of osteoporosis both in animals and humans, presumably as the result of exaggerated bone resorption and decreased bone formation. The cause-effect relationship between low calcium intake and bone resorption is so documented that low calcium diet has been accepted as a method of increasing bone resorption and inducing osteoporosis in experimental models. [2]

Calcium absorption is a function of active transport that is controlled by 1,25(OH)₂ vitamin D, which is particularly important in the case of low calcium intakes, and passive diffusion, which dominates at high calcium intakes. Calcitriol or 1,25(OH)₂ vitamin D, the active form of the vitamin D is very constant in the blood, so evaluation of the vitamin D status relies on serum 25 (OH) vitamin D which reflects more accurately its deposits. Besides the vitamin D reserve, calcium absorption from the gut is dependent also on the calcium content but also on the overall component of the diet, a relatively high protein content increasing the calcium absorption from the intestine [3]. The relationship between calcium...
intake, circulating calcium level, hormones, and bone status is subject of tight control of homeostatic mechanisms, parathyroid hormone (PTH) and vitamin D being the most two important players. [4] In clinical practice, calcium balance can be easily evaluated through measurement of serum calcium and phosphorus, 24-hour urinary calcium excretion, total alkaline phosphatase, serum intact PTH, simple but important biochemical and hormonal parameters recommended as the first line evaluation for the differential diagnosis of osteoporosis.[5] Thus, due to increasing incidence of osteoporosis and high prevalence of parathyroid diseases among these patients, in clinical practice we frequently face the difficulty in discriminating between primary and secondary hyperparathyroidism. Primary hyperparathyroidism (PHP), is defined by autonomously secretion of PTH by one or multiple parathyroid glands with increased serum levels of calcium. [6] Recently, a more subtle form was described, the normocalcemic PHP, with increased serum PTH but normal levels of calcium in the blood [7,8]. On the other hand, secondary hyperparathyroidism (SHP), defined as a physiologic response of the parathyroid secretion to low long term calcium intake (mostly due to low vitamin D availability) is diagnosed on the basis of low or normal calcium blood levels with increased serum PTH. [9,10]

2. Study presentation

The aim of the study was to evaluate the short term effects of dietary calcium intake on the biochemical diagnostic of the hyperparathyroidism in healthy people

Subjects. The study was conducted on 36 subjects (age between 21 and 71 years, 4 men and 32 women) recruited from patients refereed to the Endocrinology Department of Elias Hospital who were not diagnosed with diseases proved to influence calcium or bone metabolism and agreed with the study.

Methods: For inclusion in the study, all subjects were checked for thyroid dysfunctions, hypercortisolism, hypogonadism, and active liver diseases which might interfere with total alkaline phosphatase values. Baseline evaluation was done on a normal diet followed by reevaluation after one day of low calcium content of the diet and another day of high calcium content diet, respective. Low calcium diet without any dairy product or any calcium supplement, administered for one day under a specialized personnel supervision; high calcium diet was obtained through supplementation with dairy products with an average content of 2000 mg calcium a day. Serum osteocalcin , betacrosslaps , 25 (OH) vitamin D were measured using ELISA kits only at baseline to exclude abnormal bone or calcium turnover. Other parameters measured at baseline and day I and II respectively, were: sodium, phosphorus, total proteins, blood urea nitrogen (creatinine), total alkaline phosphatase (ALKP) and parathyroid hormone (PTH) from serum. Other routine measurements were performed from the 24 hour urine collection (sodium, phosphorus, calcium and creatinine

3. Results and Discussion

The mean age of the subjects was 48.7 years, with 4 men and 6 postmenopausal women included. All the subjects had normal thyroid function, normal cortisol secretion rhythm. Serum crosslaps and osteocalcin levels were within the normal range for gender in all subjects and for menopausal status respectively in women (table 1.1).

| Table 1.1 Baseline characteristics of the study group. |
|--------------------------------------------------------|
| **Baseline**                                           |
| Age (years)                                            | 48.7±13.6 |
| BMI (kg/m²)                                            | 28.3±5   |
| 25 OH vitamin D (ng/mL)                                | 34±13.6  |
| Osteocalcin (ng/mL)                                    | 11.2± 7.1|
| β crosslaps (ng/mL)                                    | 0.119±0.3|
| Serum total proteins (g/dL)                            | 7.1±0.1  |
| Parameter                                    | Value        |
|----------------------------------------------|-------------|
| Blood urea nitrogen/creatinine (mg/dL)       | 0.7±0.1     |
| Serum total calcium (mg/dL)                  | 9.65 ±0.37  |
| Serum phosphorus (mg/dL)                     | 3.8 ±0.3    |
| Serum total ALKP (U/L)                       | 55±35       |
| iPTH (pg/mL)                                 | 41.5±25.4   |
| Urine creatinine (g/24hr)                    | 0.86±0.39   |
| Calciuria(mg/24h)                            | 145.7±102   |
| Urine phosphorus (g/24hr)                    | 0.51±0.39   |
| Urinary Ca/creatinine                        | 169.46±22.1 |

Based on the baseline 25 (OH) vitamin D serum levels, 16 out of 36 subjects (see figure 1.1) fulfilled the criteria for vitamin D mild insufficiency. The cut off value used for defining mild insufficiency was below 30 ng/mL.

![Figure 1.1. Subjects distribution according to the baseline serum 25 (OH) vitamin D evaluation.](image)

Variations of the biochemical parameters measured at baseline and after low and high calcium diet respectively are illustrated in table 1.2.

**Serum calcium.** There was no significant change in the mean level of serum total calcium between normal and low/high calcium diet days respectively. This aspect is not surprising since serum calcium levels are tightly controlled through PTH secretion in order to protect the body against hypocalcaemia in the absence of dietary intake.

**PTH serum levels.** It can be noticed an increase of the mean serum intact PTH after one day low calcium intake by 11.67% (46.38 vs. 41.54 pg/mL, p=0.07), and a decrease after one day of high calcium diet intake by 6.3% compared to baseline, but significantly lower comparing to day I (38.9 vs. 46.38, p< 0.05, see figure 5.2).

| Parameter                                    | Baseline       | 24 hr low calcium diet | 24 hr high calcium diet |
|----------------------------------------------|----------------|------------------------|-------------------------|
| Calcemia(mg/dL)                              | 9.65 ±0.3      | 9.7±0.3                | 9.7±0.3                 |
| Serum phosphorus (mg/dL)                     | 3.8 ±0.3       | 3.7 ±0.3               | 3.9±0.3                 |
| ALKP                                         | 55±35          | 57±43                  | 54±44                   |
The International Conference of Chemistry 2020

Journal of Physics: Conference Series 1853 (2021) 012001 doi:10.1088/1742-6596/1853/1/012001

### Table 1.2

| Parameter                  | Baseline       | Low Calcium Diet | High Calcium Diet |
|----------------------------|----------------|------------------|-------------------|
| iPTH (pg/mL)               | 41.5±25.4      | 46.38±24.4       | 38.9±22.3 *       |
| Urine creatinine (g/24hr)  | 0.86±0.39      | 1.49±2.26        | 0.9±0.9           |
| Calciuria (mg/24h)         | 145.7±102      | 149.2±88         | 190.1±80          |
| Urine phosphorus (g/24hr)  | 0.51±0.39      | 0.61±0.27        | 0.58              |
| Urinary Ca/creatinine      | 169.46±22.1    | 100.16±17.9 *    | 211.22±23.8 *#    |

*p<0.05 compared to baseline
#p<0.05 compared to low calcium diet day (day I)

**Figure 1.2.** PTH variability related to the calcium content of the diet (mean values with the lowest and highest value).

A significant difference was found between day I and day II (low and high calcium diet).
The short term increase in serum PTH levels is explained by the physiologic response to the low calcium intake meant to sustain normal calcemia. There were important variations of the individual changes in PTH level ranging from -26% to +99%, but 27 out of 36 (75%) subjects experienced an increase in serum intact PTH level after calcium intake restriction. The explanation of this aspect lies in individual background, especially the vitamin D reserve. Subjects with normal vitamin D levels are more efficient in calcium absorption from the gut compared to those vitamin D deficient[11]. Thus, in our study group, 16 out of 36 subjects had mild vitamin D deficiency with a higher baseline serum intact PTH (55.6 pg/mL) compared to those with normal vitamin D (33.2 pg/mL), suggesting a subclinical PTH stimulation. Among the vitamin D insufficient subjects, the mean PTH increase after low calcium diet was lower compared to normal vitamin D subjects (2.4% vs. 16.4%), probably due to previous chronic stimulation of the PTH.

Finally, an important issue for the routine measurements of serum PTH levels, after one day of low calcium diet, 7 out of 36 subjects have levels PTH levels out of the normal range, with moderation in 6 cases in second day after a high calcium intake[12]. This aspect was significantly more frequent among patients with low vitamin D serum levels (figure 1.3) suggesting that false positive results for normocalcemic PHP diagnostic could be obtained when serum PTH is measured on a low calcium intake in patients with previous vitamin D insufficiency. As described also in other non calcemic parameters [13], vitamin D treatment could alleviate this effect, with implications on the prevalence of abnormal calcium balance biochemical parameters. On the other hand, serial measurements of serum calcium and PTH on one day `normal` versus one day `low` calcium diet could be performed as a rapid reliable way to discriminate between normocalcemic PHP and SHP compared to more slowly installing effect of vitamin D repletion. Treatment with vitamin D supplements would be recommended anyway in cases with increased serum PTH due to its salutary effects not only to the skeleton but also as adjuvant in the anti-infective therapy [14,19]; nevertheless, the discrimination between these two diseases is important when choosing the type of vitamin D product (active or not) and for dose and dietary adjustments [15,20].

![Figure 1.3. The Pearson Correlation’s coefficient between PTH (pg/mL) and age (years)](image-url)
Low vitamin D subjects were more affected by the low dietary calcium in terms of abnormal values of PTH (prevalence of normal PTH on low calcium diet distributed according to baseline vitamin D).

**Calciuria.** Low calcium diet caused a significant decrease in the urinary calcium loss when the value was reported to urine creatinine excretion, by 41% (p<0.05) after one day of low calcium intake (see table II); high calcium diet caused a significant increase in urinary calcium loss by 24% compared to baseline (p<0.05). The high variability of this parameter was highlighted by other studies mostly in chronic renal failure patients [14,19], but our data suggest its important variations also in healthy subjects. It is an important parameter, and basic evaluation for calcium overall balance relies on its measurements. Our data suggests that increased sensibility for calciuria based evaluation could be achieved if measured in standard dietary conditions or associated to a dietary questionnaire.

4. Conclusions
Serum parathyroid hormone (PTH) level is increased by the low calcium intake in vitamin D insufficient subjects, falsely increasing the prevalence of normocalcemic primary hyperparathyroidism. Serial measurements of serum calcium and PTH on one day ‘normal’ versus one day ‘low’ calcium diet could be performed as a rapid reliable way to discriminate between normocalcemic primary hyperparathyroidism (PHP) and secondary hyperparathyroidism (SHP) compared to more slowly installing effect of vitamin D repletion.

Changes in calcium excretion have been shown to be consistent with the content of calcium in the diet and in inverse proportion to changes in PTH, suggesting its value in discriminating abnormal calcium balance when measured in standard calcium intake or in conjunction with a dietary questionnaire.

Serial measurements of serum blood calcium, urinary calcium excretion and serum intact parathyroid hormone (PTH) were done in 36 healthy subjects (age 21 -71 years) during a three days follow up period with a controlled dietary intake of calcium: baseline evaluation on a normal diet followed by reevaluation after one day of low dietary calcium intake and another day of high dietary calcium intake, respective.

Serum calcium was not significantly modified during these three days, but the calcium urine excretion when reported to creatinine was significantly modified by the dietary calcium content (decreased on low calcium diet and increased on high calcium diet).[11,16, 18]
The low calcium diet induced an increase in serum PTH levels in 7 out of 36 subjects, hyperparathyroidis diagnosis, aspect more frequent found in the vitamin D insufficient subjects.

Our data sustain the significant effect of short term variations in calcium dietary intake on the biochemical diagnosis of hyperparathyroidism.
5. References

[1] Tai, V.; Leung, W.; Grey, A.; Reid, I.R.; Bolland, M.J. Calcium intake and bone mineral density: Systematic review and meta-analysis. BMJ 2015, 351, h4183.

[2] Burt, L.A.; Billington, E.O.; Rose, M.S.; Raymond, D.A.; Hanley, D.A.; Boyd, S.K. Effect of High-Dose Vitamin D Supplementation on Volumetric Bone Density and Bone Strength: A Randomized Clinical Trial. Effect of High-Dose Vitamin D Supplementation on Volumetric Bone Density and Bone Strength Effect. JAMA 2019, 322, 736–745.

[3] Ambroszkiewicz J, Sands D, Gajewska J, Chelchowska M, et al. Bone turnover markers, osteoprotegerin and RANKL cytokines in children with cystic fibrosis. Journal: Advances in Medical Sciences. 2013;58:338-343.

[4] Reid, I.R.; Horne, A.M.; Mihov, B.; Stewart, A.; Garratt, E.; Wong, S.; Wiessing, K.R.; Bolland, M.J.; Bastin, S.; Gamble, G.D. Fracture prevention with zoledronate in older women with osteopenia. N. Engl. J. Med. 2018, 379, 2407–2416. [CrossRef] [PubMed]

[5] Harvey, N.C.; Biver, E.; Kaufman, J.M.; Bauer, J.; Branco, J.; Brandi, M.L.; Bruyere, O.; Coxam, V.; Cruz-Jentoft, A.; Czerwinski, E.; et al. The role of calcium supplementation in healthy musculoskeletal ageing: An expert consensus meeting of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Foundation for Osteoporosis (IOF). Osteoporos Int. 2017, 28, 447–462.

[6] Scragg, R.; Stewart, A.W.; Waayer, D.; Lawes, C.M.M.; Toop, L.; Sluyter, J.; Murphy, J.; Khaw, K.-T.; Camargo, C.A., Jr. Effect of monthly high-dose vitamin D supplementation on cardiovascular disease: The vitamin D assessment (ViDA) study (a randomized controlled trial). JAMA Cardiol. 2017, 2, 608–616.

[7] Manson, J.E.; Cook, N.R.; Lee, I.M.; Christen, W.; Bassuk, S.S.; Mora, S.; Gibson, H.; Gordon, D.; Copeland, T.; D’Agostino, D.; et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. N. Engl. J. Med. 2018, 380, 33–44. [CrossRef] [PubMed]

[8] Atkins, G. J., Welldon, K. J., Halbout, P., et. al. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. Osteoporos Int. 2009; 20: 653–664.

[9] Shoback D, Rosen CJ, Black DM, Cheung AM, Murad MH, Eastell R. Pharmacological Management of Osteoporosis in Postmenopausal Women: An Endocrine Society Guideline Update. J Clin Endocrinol Metab. 2020;105: dgaa048. doi:10.1210/clinem/dgaa048

[10] Bacchetta, J. --- Ranchin, B. --- Dubourg, L. --- Cochat, P. Vitamin D revisited: A cornerstone of health?. Arch Pediatr. 2010; 17:1687-1695.

[11] Kanis JA, Harvey NC, McCloskey E, Bruyère O, Veronesen N, Lorentzon M, et al. Algorithm for the management of patients at low, high and very high risk of osteoporotic fractures. Osteoporosis Int 2020; 31:1-12.

[12] Meeta M, Harinarayan C V, Marwah R, Sahay R, Kalra S, Babhulkar S. Clinical practice guidelines on postmenopausal osteoporosis: *An executive summary and recommendations – Update 2019–2020. J Mid-life Health 2020;11: 96-112.

[13] Baudhuin M, Duplomb L, Teletchea S, et al. Osteoprotegerin: Multiple partners for multiple functions. Journal: Cytokine and Growth Factor Reviews. 2013;24:401-409.

[14] R. Koda, J. J. Kazama, K. Matsuo et al., “Intact parathyroid hormone and whole parathyroid hormone assay results disagree in hemodialysis patients under cinacalcet hydrochloride therapy,” Clinical and Experimental Nephrology, vol. 19, no. 4, pp. 710–717, 2015.

[15] B. Hocher and S. Zeng, “Clear the fog around parathyroid hormone assays: what do iPTH assays really measure?” Clinical Journal of the American Society of Nephrology, vol. 13, no. 4, pp. 524–526, 2018.

[16] J. G. Zhao, X. T. Zeng, J. Wang, and L. Liu, “Association between calcium or Vitamin D supplementation and fracture incidence in community-dwelling older adults a systematic review and meta-analysis,” JAMA, vol. 318, no. 24, pp. 2466–2482, 2017.
[17] J. E. Compston, M. R. McClung, and W. D. Leslie, “Osteoporosis,” The Lancet, vol. 393, no. 10169, pp. 364–376, 2019.
[18] J. A. Kanis, N. Harvey, N. C. Harvey et al., “A systematic review of intervention thresholds based on FRAX,” Archives of Osteoporosis, vol. 11, no. 1, 2016.
[19] W. F. Elbossaty, “Mineralization of bones in osteoporosis and osteomalacia,” Annals of Clinical and Laboratory Research, vol. 5, no. 4, pp. 3–6, 2017.
[20] S. F. Boettger, B. Angersbach, C. N. Klimek et al., “Prevalence and predictors of vitamin D-deficiency in frail older hospitalized patients,” BMC Geriatrics, vol. 18, no. 1, pp. 1–6, 2018.