Orginial Communication

A Vitamin D–Calcium-Fortified Yogurt Drink Decreased Serum PTH but did not Affect Osteocalcin in Subjects with Type 2 Diabetes

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Received: January 29, 2015; Accepted: March 27, 2015

Abstract: *Introduction:* There is sparse evidence of the effect of vitamin D on bone biomarkers in diabetic patients, and therefore, in a randomized clinical trial, we evaluated the effects of the daily intake of vitamin D, either with or without extra calcium, on selected bone biomarkers. *Materials and Methods:* Ninety women and men aged 30–50 years old with type 2 diabetes were randomly divided into three groups in a double-blind manner. Group 1 (PD), the control group, received a plain yogurt drink. Groups 2 (DD) and 3 (CDD) received 1000 IU vitamin D3, and 1000 IU vitamin D3 plus 500 mg calcium, respectively, via drinking two 250 mL bottles a day of a fortified yogurt drink for twelve weeks. Anthropometric and biochemical assessments were made, including 25(OH), intact parathyroid hormone (iPTH), osteocalcin, and calcium. *Results:* Although the time and time×group interaction effects on the bone biomarkers were not statistically significant, there was a modest decrease in iPTH concentrations in both DD and CDD groups over twelve weeks. The subgroups with initial vitamin D deficiency/insufficiency in the CDD group had greater and significant decrease in serum iPTH concentrations after twelve weeks of treatment compared to the PD group (–9.0 ± 21.2 v.s 8.6 ± 21.8 pg/mL, p = 0.042). *Conclusion:* The improvement in vitamin D status following the daily intake of fortified *doogh* for twelve weeks was accompanied by a decrement in iPTH, mostly in those subjects with poor initial vitamin D status.

Key words: vitamin D, fortified yogurt drink, bone, diabetes

Introduction

Vitamin D deficiency is a worldwide epidemic [1, 2]. Many studies have suggested that this deficiency is associated with an increased risk of diabetes, cancer, cardiovascular and autoimmune diseases [3–5].

It is known that vitamin D is a key regulator of calcium homeostasis and bone metabolism. Well-known consequences of vitamin D deficiency include a decrease in calcium absorption, secondary hyperparathyroidism, accelerated bone loss, and an increase in falls, osteoporosis, and fractures [6–8].

Diabetes is among the secondary causes of osteoporosis [9] and the number of patients with diabetes mellitus and osteoporosis is rapidly increasing [10]. A relatively large number of studies have evaluated bone mineral density in patients with type 2 diabetes (T2D). The results are very controversial: some studies report increased bone mineral density (BMD) but others have failed to confirm this finding [11–13]. Traditionally, an increased risk of fractures was not a concern in patients with T2D due to high bone mineral density, however, recent studies have consistently reported that patients with T2D are at higher risk of fractures in the presence of high bone mass [14, 15].

Some possible contributing factors to bone loss in diabetes include insulin resistance, the accumulation of advanced glycation end products (AGEs) in bone, inflammatory status, medication affecting bone metabolism, hypercalciuria due to uncontrolled diabetes [16] and vitamin D deficiency [17], which has been reported as prevalent in patients with diabetes [18].

Due to the high prevalence and health care costs of bone problems, efficient, economic and safe preventive strategies are needed [19].

Recently, we demonstrated the ameliorating effects of a daily intake of 1000 IU of both vitamin D and 1000 IU vitamin D + 500 mg calcium intake via a fortified Persian yogurt drink (*doogh*) on glycemic, inflammatory and oxidative stress status in subjects with T2D [20–22]. There is sparse evidence of the effect of vitamin D on bone biomarkers in diabetic patients, and therefore, we evaluated the effects of daily intake of vitamin D either with or without extra calcium on certain selected bone biomarkers using the same study population and protocols.

Materials and Methods

The study design and inclusion criteria have been described elsewhere [20]. Briefly, 90 women and men with T2D, aged 30–50 years , were recruited and enrolled in the study if they met the inclusion criteria: 1) fasting blood glucose concentration above 126 mg/dL on the first visit; 2) not taking dietary supplements including calcium, vitamin D, or omega-3 for the past 3 months prior to the intervention; 3) not receiving medications that could potentially influence vitamin D metabolism or insulin; and 4) not having any other clinical disease that could influence vitamin D metabolism (e.g. renal, hepatic, other endocrinology disorders and malignancies).

In a 2-week run-in period, the participants were instructed to have 2–3 exchanges of low-fat dairy products (milk and yogurt), 2–3 exchanges of vegetables and 2–3 exchanges of fresh fruits a day. They were then randomly divided into three groups in a double-blind manner to receive two 250 mL bottles a day of plain Persian yogurt drink or *doogh* (PD), containing 150 mg calcium and no detectable vitamin D3/250 mL; vitamin D-fortified *doogh* (DD), containing 150 mg calcium and 500 IU vitamin D3/250 mL; or calcium + vitamin D3-fortified *doogh* (CDD), containing 250 mg calcium and 500 IU vitamin D3/250 mL. Two bottles of *doogh* (500 mL) replaced one exchange of dairy product a day (Figure 1). The study was conducted over twelve weeks in February–March, when there is minimal sunlight exposure, and serum 25-hydroxycholecalciferol (25 (OH)D) concentrations were expected to be at their lowest. The dietary intake assessment was made using 24 hour recall questionnaire for two days (including a weekend), as described elsewhere [23].

This study was part of the Calcium + vitamin D-fortified yogurt drink (*doogh*) and Diabetes Project, which has been approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute (NNFTRI), and patients provided written informed consent to participate in the study. This trial was registered at clinicaltrails.gov: NCT01229891.

Laboratory investigations

Fasting venous blood samples were obtained at baseline and after twelve weeks intervention. The procedure for blood sampling and handling has been described previously [20].

Serum 25(OH)D was measured by high-performance liquid chromatography [24]. 25(OH)D concentrations (nmol/L) were defined using the following categories: deficiency (<27.5), inadequacy (27.5 to <50), and adequacy (>50) [25].

Serum intact parathyroid hormone (iPTH; Biomerica, USA), and osteocalcin (OC; BioSource, Belgium)

were assessed using the enzyme-linked immunoassay method.

Serum and urinary concentrations of calcium, phosphorous and magnesium were measured via colorimetric methods using commercial kits (Pars-Azmoon, Tehran, Iran) and an auto-analyzer (Selecta E; Vitalab, Holliston, Netherlands). Percent of body fat mass (FM) was evaluated using a bioelectrical impedance analysis (BIA) system (Quadscan 4000; BodyStat, Douglas, United Kingdom). Initial and final urine and serum samples were kept at –80 °C until the day of analysis and then all laboratory tests were performed at the end of the intervention period at the Laboratory of Nutrition Research, NNFTRI.

Statistical analyses

The data was expressed as mean ± SD. The distribution of all variables was checked for normality by using the Kolmogorov-Smirnov test.

Two-factor repeated-measures analysis of variance (ANOVA) was used to test time×group interactions. ANOVA and Kruskal–Wallis tests were used to compare between-group changes of independent, normally and non-normally distributed variables, respectively. The within-group comparison of values was performed by paired-samples t test (normal distributed) and Wilcoxon test (non-normally distributed). Correlations between normally distributed data were evaluated using Pearson (r) correlation coefficients. Spearman (r_s) coefficients were used to correlate non-normally continuous variables. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc., Chicago, IL).

Results

The baseline characteristics of the subjects have been completely described elsewhere [20], however, some anthropometric and biochemical values of the three groups are provided in Table I. Baseline data did not differ significantly among the groups. As reported earlier [23], dietary intake (except for calcium and vitamin D) did not show any significant within- or between-group difference (data not shown).

After twelve weeks, both DD and CDD groups showed significant improvement in both vitamin D status [20], and serum calcium concentrations. The time effect on serum calcium was significant ($p < 0.001$). Although the time and time × group interaction effects on the rest of the bone biomarkers were not statistically significant, there was a modest decrease in iPTH concentrations in both DD and CDD groups over twelve weeks (Table II).

The subgroups with initial vitamin D deficiency/insufficiency in the CDD group had a greater and significant decrease in serum iPTH concentrations after twelve weeks of treatment compared to the PD group (–9.0 ± 21.2 v.s 8.6 ± 21.8 pg/mL, $p = 0.042$).

The other bone biomarkers did not differ significantly in DD and CDD groups after twelve weeks, compared with the baseline values (Table II).

In our study, a significant inverse association was found between 25(OH)D and serum iPTH at baseline ($r = -0.348$, $p = 0.001$) and changes in 25(OH)D and changes in iPTH during the study ($r = -0.323$, $p = 0.002$).

Simple correlation analyses were also performed between serum OC versus anthropometric and glycemic parameters at baseline. Serum osteocalcin significantly and negatively correlated with BMI ($r = -0.267$, $p = 0.011$), FM ($r = -0.274$, $p = 0.009$), serum glucose

Table I: Comparison of the values of some variables in three intervention groups at baseline. [1]

	PD	DD	CDD	P value[a]
Age (yr)	50.8 ± 6.7	51.5 ± 5.4	49.9 ± 6.2	0.613
Weight (kg)	77.4 ± 16.7	75.0 ± 14.1	75.7 ± 11.9	0.844
BMI (kg/m^2)	29.9 ± 4.7	29.2 ± 4.4	29.1 ± 5.5	0.779
WC (cm)	97.9 ± 11.0	95.6 ± 11.4	98.0 ± 10.7	0.621
FM (%)	35.7 ± 7.8	33.0 ± 9.5	35.1 ± 9.8	0.479
FSG (mg/dL)	187.0 ± 57.1	184.1 ± 63.8	184.0 ± 57.3	0.976
HbA1c (%)	7.5 ± 1.5	7.4 ± 1.8	7.8 ± 1.9	0.754

Data are expressed as means ± SD. BMI, Body mass index; FM, fat mass; FSG, fasting serum glucose; HbA1c, glycated hemoglobin; WC, waist circumference, PD, plain yogurt; DD, vitamin D-fortified yogurt drink; CDD, calcium + vitamin D-fortified yogurt drink.

[a] Significance among three groups (one-factor ANOVA)

Table II: Comparison of changes within and between groups after intervention.

Variable	PD			DD			CDD			P^b	P^c	P^d	P^e
	Before	After	P^a	Before	After	P^a	Before	After	P^a				
25(OH)D3 (nmol/L)	41.6±44.5	37.2±44	0.136	44.4±28.7	77.7±28.6	<0.001	44.5±43.7	74.6±39.5	<0.001	<0.001	<0.001	0.578	<0.001
iPTH (pg/mL)	69.8±32.0	76.0±28.0	0.181	52.7±26.4	50.2±21.2	0.667	61.1±25.8	54.9±22.7	0.169	0.427	0.178	0.851	0.372
OC (ng/mL)	8.4±5.6	8.5±5.5	0.878	7.1±3.2	7.5±3.2	0.551	8.3±3.9	8.4±4.1	0.781	0.991	0.997	0.940	0.909
Serum Ca (mg/dL)	9.5±0.4	9.6±0.6	0.376	9.4±0.4	9.8±0.3	<0.001	9.4±0.4	9.7±0.3	0.001	0.285	0.309	0.999	0.229
Serum P (mg/dL)	3.7±0.3	3.8±0.4	0.180	3.7±0.5	3.8±0.5	0.053	3.7±0.4	3.7±0.3	1.00	0.812	0.613	0.270	0.298
Serum Mg (mg/dL)	1.9±0.2	2.0±0.2	0.105	2.0±0.2	2.0±0.2	0.562	1.9±0.2	2.0±0.1	0.211	0.571	0.879	0.858	0.600
Urinary Ca (mg/g creat)	157.7±100.5	143.1±95.3	0.411	152.7±106.2	175.7±112.5	0.354	165.0±85.1	175.8±86.3	0.594	0.413	0.666	0.910	0.282
Urinary P(mg/g creat)	493.8±286.3	492.9±347.1	0.990	415.0±214.1	352.5±164.9	0.232	410.6±221.8	352.5±164.9	0.406	0.749	0.888	0.462	0.799
Urinary Mg (mg/g creat)	63.3±50.6	51.8±28.4	0.219	54.4±23.3	47.9±22.0	0.297	49.2±19.1	50.8±18.5	0.655	0.855	0.351	0.670	0.552

Data are expressed as means ±SD. 25(OH)D3, 25-hydroxyvitamin D3; FM, fat mass; iPTH, intact parathyroid hormone; OC, osteocalcin, PD, plain yogurt; DD, vitamin D–fortified yogurt drink; CDD, calcium + vitamin D–fortified yogurt drink.
a Significance of within-group changes (paired-samples *t* test).
b Significance between the plain (PD) and vitamin D-fortified (DD) groups (one-factor ANOVA).
c Significance between the calcium+vitamin D-fortified (CDD) and PD groups (one-factor ANOVA).
d Significance between the CDD and DD groups (one-factor ANOVA).
e Time*group interaction (two-factor ANOVA).

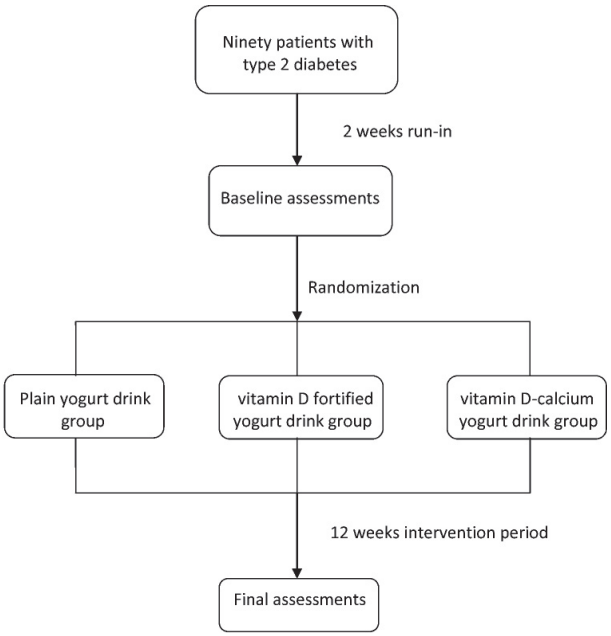

Figure 1: Summary of the study design.

(r = –0.235, p = 0.026), and low-density lipoprotein cholesterol (LDL-C) (r = –0.210, p = 0.047).

Discussion

The present investigation showed that a daily intake of 1000 IU vitamin D, with or without extra calcium, could prevent a seasonal increase in serum iPTH [26] but had no significant effects on the studied bone biomarkers.

It is evident that vitamin D deficiency or insufficiency is prevalent worldwide and that the problem may be exaggerated in patients with either type 1 or 2 diabetes [27, 28], however, the benefits of vitamin D supplementation on bone metabolism in patients with T2D are not well documented.

Measurement of serum bone biomarkers is helpful to determine the effect of an intervention on bone quality prior to any significant change in bone density [29]. Many researchers have studied the correlation between these markers of turnover and BMD [30, 31].

Previous studies have shown that although patients with T2D show a BMD reduction, fracture risks increase up to approximately one and a half times [32, 33], although it is still unclear why subjects with T2D have an increased risk of fracture despite normal BMD.

Indeed, recent studies have shown that diabetes may be the major predictor of osteoporotic hip fracture in non-elderly patients [34,35]. The higher risk of bone problems in diabetes may be caused by chronic hyperglycemia, osteoblast dysfunction, inflammation, diabetes medications and hypovitaminosis D [36].

In the present study, no significant improvement in the selected bone markers was observed, however, when we reanalyzed the between-group changes of the variables in the subjects with initial undesirable vitamin D status, we found that the serum iPTH decrease in the CDD group was slightly but significantly greater compared to the PD group.

It is noteworthy that the final mean 25(OH)D in both DD and CDD groups was < 78 nmol/L and 71.1 % of our patients had 25(OH)D < 75 nmol/L. It is suggested that bone resorption tends to increase with age and thus higher doses of vitamin D may be required to slow down the bone resorption. Some clinical trials suggest that serum 25 (OH) D concentrations of 80 – 125 nmol/L are required to show any benefit in bone turnover [19, 37, 38].

It is estimated that with a late summer and 25(OH) D concentrations of 70 nmol/L, 1000 IU vitamin D/d is needed during winter months to maintain the starting level, while with baseline levels between 20 and 40 nmol/L, a daily dose of 2200 IU vitamin D may be required to reach and maintain 80 nmol/L [39, 40]. These results indicate that individuals with a lower baseline level may need a higher dose of vitamin D to achieve desirable levels [41].

We found an inverse relationship between 25(OH) D and iPTH at baseline, in keeping with several studies [42 – 44]. PTH helps maintaining normal serum calcium concentrations and is regulated itself by the levels of $1,25(OH)_2D$ and serum calcium, but an increase in PTH results in increased bone turnover and bone loss [45]. An inverse association between serum 25(OH)D and serum PTH is well recognized [46].

It is suggested that PTH suppression may only depend on a higher calcium intake if serum 25-hydroxyvitamin D levels are very low [46]. As habitual calcium intake was relatively low in our subjects (710 ± 338 mg/d) [23], it is likely that the significant decrease in serum iPTH in CDD compared to the PD group was due to higher calcium intake (200 mg extra calcium via fortified *doogh*).

There were no significant changes in serum OC after intervention. Interestingly, a significant negative correlation was found between osteocalcin and BMI, FM, serum glucose, and LDL-C at baseline. These findings further confirm those of previous studies [47 – 49].

Serum concentrations of OC, an osteoblast-specific protein, generally reflect mature osteoblastic activity and bone formation. Recent studies have shown that the serum OC level is related to not only bone metabolism, but also plasma glucose levels, fat mass and atherosclerosis parameters in individuals with or without T2D [50–52]. Several studies indicated that hyperglycemia induced a low bone turnover with osteoblast dysfunction and caused suppression of serum OC levels [53, 54]. Lower serum OC concentrations have been reported in diabetic women [53].

Some limitations in this study are acknowledged. Although calcium, magnesium and phosphorous are all necessary nutrients for bone [55, 56], and vitamin D and PTH are major determinants of bone health [57, 58], osteocalcin and urinary calcium were the major biomarkers of bone metabolism in this study [59]. The possible effects of vitamin D and calcium intake on other circulating bone biomarkers remain to be elucidated by further studies. As *doogh* is a dairy product, it was impossible to fortify it with higher amounts of calcium without organoleptic changes.

In conclusion, the intervention itself may have led to a significant improvement in vitamin D status, but not enough to significantly affect the selected bone biomarkers. Present data suggests that the greatest impact of vitamin D intervention might be seen in individuals with initial hypovitaminosis D, and extra calcium intake via consumption of fortified *doogh* slightly adds to the PTH-suppressing effect of vitamin D.

There are few studies on vitamin D/calcium supplementation and bone in patients with T2D, and future studies are needed to examine the strength of the association between vitamin D status and measures of bone metabolism in these subjects.

Abbreviations

25OHD: 25-Hydroxy Vitamin D
CDD: calcium + vitamin D3-fortified *doogh*
DD: vitamin D3-fortified *doogh*
iPTH: intact parathyroid hormone
OC: osteocalcin
PD: plain yogurt drink or *doogh*
T2D: type 2 diabetes

Conflicts of interest

Nothing to declare.

Funding

This work was funded by National Nutrition and Food Technology Research Institute (NNFTRI).

Author contributions

TRN designed and supervised the study; TRN and BN were involved in all stages of the research, including all laboratory bench work; BN was involved in statistical analyses; AK, NS and MZ were involved in the anthropometric measures and laboratory assays.

Acknowledgements

We acknowledge the Iranian Diabetes Society for their cooperation, with special thanks to its head, Asadollah Rajab. We also thank all of the subjects who participated in this study. All the laboratory bench work was conducted at the Laboratory of Nutrition Research.

References

1. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, et al. (2009) Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20: 1807–1820.

2. Ben-Shoshan M (2012) Vitamin D deficiency/insufficiency and challenges in developing global vitamin D fortification and supplementation policy in adults. International Journal for Vitamin and Nutrition Research 82: 237–259.

3. Holick MF (2007) Vitamin D deficiency. New England Journal of Medicine 357: 266–281.

4. Garland CF, Gorham ED, Mohr SB, Garland FC (2009) Vitamin D for cancer prevention: global perspective. Annals of Epidemiology 19: 468–483.

5. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, et al. (2011) Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. The Journal of Clinical Endocrinology & Metabolism 96: 1931–1942.

6. Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine Reviews 22: 477–501.

7. Bischoff-Ferrari H (2012) Vitamin D-from essentiality to functionality. International Journal for Vitamin and Nutrition Research 82: 321–326.

8. Genc S, Ömer B, Aycan-Üstyol E, Kumral A, Gürdöl F (2012) Bone Turnover Markers and Vitamin D Status in Postmenopausal Turkish Women. International Journal for Vitamin and Nutrition Research 82: 27–33.

9. Rosen CJ, Compston JE, Lian JB (2009) Primer on the metabolic bone diseases and disorders of mineral metabolism: John Wiley & Sons.

10. Inzerillo AM, Epstein S (2004) Osteoporosis and diabetes mellitus. Reviews in Endocrine and Metabolic Disorders 5: 261–268.

11. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, et al. (2006) Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. The Journal of Clinical Endocrinology & Metabolism 91: 3404–3410.

12. Leidig-Bruckner G, Ziegler R (2001) Diabetes mellitus a risk for osteoporosis? Experimental and Clinical Endocrinology & Diabetes 109: S493-S514.

13. Yaturu S, Humphrey S, Jain SK, Landry C (2008) Decreased bone mineral density in men with metabolic syndrome alone and with type 2 diabetes. Medical Science Monitor Basic Research 15: CR5-CR9.

14. Antonopoulou M, Bahtiyar G, Banerji MA, Sacerdote AS (2013) Diabetes and bone health. Maturitas 76: 253–259.

15. Janghorbani M, Van Dam RM, Willett WC, Hu FB (2007) Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. American Journal of Epidemiology 166: 495–505.

16. RASKIN P, STEVENSON MR, BARILLA DE, PAK CY (1978) The hypercalciuria of diabetes mellitus: its amelioration with insulin. Clinical Endocrinology 9: 329–335.

17. Yaturu S (2009) Diabetes and skeletal health. Journal of Diabetes 1: 246–254.

18. Yoho RM, Frerichs J, Dodson NB, Greenhagen R, Geletta S (2009) A comparison of vitamin D levels in nondiabetic and diabetic patient populations. Journal of the American Podiatric Medical Association 99: 35–41.

19. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, et al. (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. Jama 293: 2257–2264.

20. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, et al. (2011) Daily consumption of vitamin D–or vitamin D+ calcium–fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. The American journal of clinical nutrition 93: 764–771.

21. Neyestani TR, Nikooyeh B, Alavi-Majd H, Shariatzadeh N, Kalayi A, et al. (2012) Improvement of vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism 97: 2005–2011.

22. Nikooyeh B, Neyestani T, Tayebinejad N, Alavi-Majd H, Shariatzadeh N, et al. (2014) Daily intake of vitamin D-or calcium vitamin D-fortified Persian yogurt drink (doogh) attenuates diabetes-induced oxidative stress: evidence for antioxidative properties of vitamin D. Journal of Human Nutrition and Dietetics 27: 276–283.

23. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, et al. (2011) Daily consumption of vitamin D- or vitamin D + calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. Am J Clin Nutr 93: 764–771.

24. Neyestani TR, Gharavi A, Kalayi A (2007) Determination of serum 25-hydroxy cholecalciferol using high-performance liquid chromatography: a reliable tool for assessment of vitamin D status. International Journal for Vitamin and Nutrition Research 77: 341–346.

25. Saintonge S, Bang H, Gerber LM (2009) Implications of a new definition of vitamin D deficiency in a multiracial us adolescent population: the National Health and Nutrition Examination Survey III. Pediatrics 123: 797–803.

26. Viljakainen HT, Väisänen M, Kemi V, Rikkonen T, Kröger H, et al. (2009) Wintertime vitamin D supplementation inhibits seasonal variation of calcitropic hormones and maintains bone turnover in healthy men. Journal of Bone and Mineral Research 24: 346–352.

27. Isaia G, Giorgino R, Adami S (2001) High prevalence of hypovitaminosis D in female type 2 diabetic population. Diabetes Care 24: 1496–1496.

28. Svoren BM, Volkening LK, Wood JR, Laffel L (2009) Significant vitamin D deficiency in youth with type 1 diabetes mellitus. The Journal of Pediatrics 154: 132–134.

29. Delmas PD, Eastell R, Garnero P, Seibel M, Stepan J (2000) The use of biochemical markers of bone turnover in osteoporosis. Osteoporosis International 11: S2-S17.

30. Bjarnason N, Christiansen C (2000) Early response in biochemical markers predicts long-term response in bone mass during hormone replacement therapy in early postmenopausal women. Bone 26: 561–569.

31. Pi Y-Z, Wu X-P, Liu S-P, Luo X-H, Cao X-Z, et al. (2006) Age-related changes in bone biochemical markers and their relationship with bone mineral density in normal Chinese women. Journal of Bone and Mineral Metabolism 24: 380–385.

32. Vestergaard P (2007) Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporosis International 18: 427–444.

33. Lipscombe LL, Jamal SA, Booth GL, Hawker GA (2007) The Risk of Hip Fractures in Older Individuals With Diabetes A population-based study. Diabetes Care 30: 835–841.

34. Pasoto SG, Yoshihara LA, Maeda LC, Bernik MM, Lotufo PA, et al. (2012) Osteoporotic hip fractures in non-elderly patients: relevance of associated co-morbidities. Rheumatology International 32: 3149–3153.

35. Holmberg AH, Johnell O, Nilsson PM, Nilsson J-A, Berglund G, et al. (2005) Risk factors for hip fractures in a middle-aged population: a study of 33,000 men and women. Osteoporosis International 16: 2185–2194.

36. Thrailkill KM, Lumpkin Jr CK, Bunn RC, Kemp SF, Fowlkes JL (2005) Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. American Journal of Physiology-Endocrinology and Metabolism 289: E735-E745.

37. Grant A, Avenell A, Campbell M, McDonald A, MacLennan G, et al. (2004) Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet 365: 1621–1628.

38. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, et al. (2006) Calcium plus vitamin D supplementation and the risk of fractures. New England Journal of Medicine 354: 669–683.

39. Heaney RP (2005) The vitamin D requirement in health and disease. The Journal of Steroid Biochemistry and Molecular Biology 97: 13–19.

40. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ (2003) Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. The American Journal of Clinical Nutrition 77: 204–210.

41. Bischoff-Ferrari H (2009) Vitamin D: what is an adequate vitamin D level and how much supplementation is necessary? Best Practice & Research Clinical Rheumatology 23: 789–795.

42. Vanderschueren D, Pye SR, O'Neill TW, Lee DM, Jans I, et al. (2013) Active vitamin d (1, 25-dihydroxyvitamin d) and bone health in middle-aged and elderly men: The European Male Aging Study (EMAS). The Journal of Clinical Endocrinology & Metabolism 98: 995–1005.

43. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, et al. (2009) Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. The Journal of Clinical Endocrinology & Metabolism 94: 1244–1250.

44. Bates C, Carter G, Mishra G, O'shea D, Jones J, et al. (2003) In a population study, can parathyroid hormone aid the definition of adequate vitamin D status? A study of people aged 65 years and over from the British National Diet and Nutrition Survey. Osteoporosis International 14: 152–159.

45. Sigurdsson G, Franzson L, Steingrimsdottir L, Sigvaldason H (2000) The association between parathyroid hormone, vitamin D and bone mineral density in 70-year-old Icelandic women. Osteoporosis International 11: 1031–1035.

46. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G (2005) Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. Jama 294: 2336–2341.

47. Bao Y, Ma X, Yang R, Wang F, Hao Y, et al. (2012) Inverse relationship between serum osteocalcin levels and visceral fat area in Chinese men. The Journal of Clinical Endocrinology & Metabolism 98: 345–351.

48. Movahed A, Larijani B, Nabipour I, Kalantarhormozi M, Asadipooya K, et al. (2012) Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: the crosstalk between bone and energy metabolism. Journal of Bone and Mineral Metabolism 30: 683–691.

49. Al-Daghri NM, Alkharfy KM, Al-Othman A, Yakout SM, Al-Saleh Y, et al. (2013) Effect of non-pharmacologic vitamin D status correction on circulating bone markers in healthy overweight and obese Saudis. Molecules 18: 10671–10680.

50. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, et al. (2009) Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism 94: 45–49.

51. Kindblom JM, Ohlsson C, Ljunggren Ö, Karlsson MK, Tivesten A, et al. (2009) Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. Journal of Bone and Mineral Research 24: 785–791.

52. Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B (2009) Association between serum osteocalcin and markers of metabolic phenotype. The Journal of Clinical Endocrinology & Metabolism 94: 827–832.

53. Gerdhem P, Isaksson A, Akesson K, Obrant KJ (2005) Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus. Osteoporosis International 16: 1506–1512.

54. Rosato M, Schneider S, Shapses S (1998) Bone turnover and insulin-like growth factor I levels increase after improved glycemic control in noninsulin-dependent diabetes mellitus. Calcified Tissue International 63: 107–111.

55. Abrams SA (2011) What are the risks and benefits to increasing dietary bone minerals and vitamin D intake in infants and small children? Annual Review of Nutrition 31: 285–297.

56. Nishimuta M (2000) The concept of intracellular-, extracellular- and bone-minerals. Biofactors 12: 35–38.

57. Song Q, Sergeev IN (2015) High vitamin D and calcium intakes increase bone mineral (Ca and P) content in high-fat diet-induced obese mice. Nutrition Research 35: 146–154.

58. Thaler HW, Oudshoorn C, Hartholt KA, van der Cammen TJ (2015) Parameters of bone health and fracture risk in older female fall victims: what do they tell us? Z Gerontol Geriatr 48: 539–542.

59. Bonjour JP, Kohrt W, Levasseur R, Warren M, Whiting S, et al. (2014) Biochemical markers for assessment of calcium economy and bone metabolism: application in clinical trials from pharmaceutical agents to nutritional products. Nutrition Research Reviews 27: 252–267.

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