Osteocalcin and Calcium Homeostasis in Type I Diabetes: Effect of Vitamin D Bolus

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Summary This work was designed to clarify the influence of a vitamin D₃ bolus on bone metabolic turnover as manifested by serum osteocalcin, calcium, and other parameters. Bone, like other tissues, in diabetics displays microangiopathy, particularly in the case of type I diabetes. Insulin-dependent diabetics (IDD) without retinopathy (Group I, *n*=18), with retinopathy (Group II, *n*=16), and healthy persons (Group III, *n*=16) were studied. Fasting and 2-h postprandial serum glucose, Ca²⁺ (total and ionized), albumin, alkaline phosphatase (ALP), osteocalcin (OC), parathormone (PTH), 25(OH)D₃, 1,25(OH)₂D₃, and 24-h urinary calcium were evaluated. Diabetics were injected I.M. with 600,000 IU of vitamin D₃, and were maintained on their dietary and antidiabetic regimen. There was a significant decrease in serum Ca²⁺ (total and ionized), urinary calcium, OC, 1,25(OH)₂D₃ and serum albumin and significant increases in glucose and ALP in both diabetic groups compared with the control. Vitamin D₃ injection in diabetics led to increase in plasma 25(OH)D₃ and 1,25(OH)₂D₃ and decrease in PTH levels together with normalization of serum Ca²⁺ (total and ionized) and 24-h urinary calcium, ALP, and OC. This could be due to the specific action of vitamin D₃. The insignificant difference in calcium levels and its controlling hormones between complicated and uncomplicated diabetics suggests that development of angiopathy neither exaggerates osteopathy nor impairs the osteogenic effect of vitamin D₃. Hepatic and renal metabolism of the latter were intact. Vitamin D₃ injection could ameliorate diabetic hyperglycaemia and improve hypoalbuminaemia.

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Osteocalcin (OC) or bone GLA protein is a small 49-amino acid, non-collagenous, vitamin K-dependent protein synthesized by osteoblasts [1]. It binds to hydroxyapatite and accumulates in the bone matrix. Nanomolar concentrations of OC are released into the blood, and its measurement in serum or plasma is a convenient method for the study of bone turnover [2]. $1,25(\text{OH})_2\text{D}_3$ stimulates synthesis of OC [3], and insulin [4] and growth factors [5] also promote bone formation and OC synthesis. On the other hand, modulators of bone resorption also affect OC synthesis; e.g., PTH decreases it.

A growing body of evidence indicates some reduction of bone mass (osteopenia) in many people with diabetes mellitus, both type I and type II. Multiple factors may be involved in the development of disturbed calcium homeostasis in diabetes; these are hyperglycaemia [6], insulin deficiency [7], increased plasma prostaglandins [8], reduced somatomedins [9], phosphate and magnesium depletion due to increased urinary loss [10], adrenal hyperfunction [11], and disturbed production of vitamin D metabolites [12]. Microangiopathy may be responsible for defective tissue perfusion with subsequent deleterious metabolic effects. This study was planned to evaluate serum calcium and related factors in type I diabetes as well as to examine the effect of microangiopathy on such parameters. Influence of vitamin D bolus was also studied to assess its value as additive therapy.

SUBJECTS AND METHODS

Subjects. This study was performed on 34 patients and 16 healthy controls. The patients were selected from the medical wards of Cairo University Hospital.

The subjects were divided into 3 groups: Group I ($n=18$): Insulin-dependent diabetes mellitus (IDDM), with controlled hyperglycaemia and without evidence of vascular or other diabetic complications. Their age was 21 ± 4.68 years ($\pm$ SD). Group II ($n=16$): IDDM with uncontrolled hyperglycaemia and diabetic retinopathy. Other diabetic complications were excluded. Their age was 34.06 ± 6.68 years. Group III ($n=16$): Healthy subjects as a control group. Their age was 27.25 ± 7.21 years.

Sampling and analytical methods. Venous blood samples were taken after an overnight fast, and 2 h after an oral glucose load (1.0 g/kg body weight). Intramuscular injection of 600,000 IU of vitamin D_3 was done (Devarol, Memphis Co., Cairo, Egypt). Another injection was repeated after 1 week. Fasting and 2-h postprandial samples were taken 1 week later. Serum glucose, total calcium, albumin, and alkaline phosphatase were measured in fresh samples by ordinary colorimetric methods using kits produced by Biomerieux, Cedex, France. Ionized calcium was measured with an ICAI Radiometer (Copenhagen, Denmark) [13].

Sera for determination of OC, PTH, and $25(\text{OH})\text{D}_3$ were kept frozen at -20°C till the time of assay. Both osteocalcin [14] and $25(\text{OH})\text{D}_3$ [15] were measured by the double antibody technique of radioimmunoassay using the corresponding ^{125}I -labelled antigens. PTH (intact) was measured by use of ^{125}I -labelled goat antibody specific for PTH 1-34 [16]. Determination of plasma $1,25(\text{OH})_2\text{D}_3$ was preceded by chromatographic cleaning-up by microcolumns packed with octadecyl silane (C_{18})OH (Millipore, Milford, MA). The technique followed was the radioreceptor assay, and the receptors were prepared from calf thymus by the manufacturer. The tracer used was $1,25(\text{OH})_2\{^3\text{H}-26,27\}\text{D}_3$ [17]. The kits for the assay of OC, PTH, $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ were produced by Incaster Corp., Stillwater, MN.

The data obtained were statistically analyzed according to Student's *t*-test for unpaired and paired data. Correlation coefficient (*r*) was calculated among the investigated parameters.

RESULTS

In both diabetic Groups (I and II) serum calcium (total and ionized) and the 24-h urinary calcium levels were significantly reduced compared with those of the control group. They were significantly elevated to almost the level of the controls after vitamin D injection (Table 1). The same pattern was observed for osteocalcin (OC) and albumin i.e., their serum levels were decreased in Groups I and II before vitamin D injection and significantly elevated after the vitamin D bolus (Tables 1, 2).

ALP activity was significantly higher in diabetics than in controls before the vitamin D injection, and was corrected after vitamin D administration. ALP and total calcium levels were significantly positively correlated ($r=0.624$, $p<0.01$) in controlled diabetics. $1,25(\text{OH})_2\text{D}_3$ was significantly lower in complicated diabetics than in the controls, but vitamin D administration led to a significant increase in both $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ levels ($p<0.001$) and to a decrease in the PTH level in both diabetic groups compared with the controls. Meanwhile, a significant positive correlation was observed between $1,25(\text{OH})_2\text{D}_3$ and OC in the complicated diabetic patients (Group II) ($r=0.523$, $p<0.001$) after vitamin D injection.

Serum PTH was not altered significantly in Groups I and II vs. Group III before vitamin bolus administration. Its level was significantly reduced thereafter.

Serum glucose levels were significantly ($p<0.05$) decreased after vitamin D administration in both diabetic groups (Table 3).

DISCUSSION

Insulin-dependent diabetes mellitus (IDDM) is frequently associated with osteoporosis. Impaired bone formation due to osteoblast deficit has been proposed as a cause of diabetic osteopenia. Chronic insulin deficiency may well prove to be a key factor in the development of diabetic osteopenia [7]. Hence, we chose this

Table 1. Serum albumin, total calcium, ionized calcium and 24-h urinary calcium before and after vitamin D bolus.

Group	Parameter	Albumin (g/dl)		Total calcium (mg/dl)		Ionized calcium (mg/dl)		Urinary calcium (mg/24 h)	
		Before	After	Before	After	Before	After	Before	After
Group I (n=18)	Mean±SE	3.5±0.1	4.1±0.1	8.4±0.2	9.8±0.2	3.6±0.1	4.7±0.1	140±19	206±28
	P1	<0.005	NS	0.001	NS	0.01	NS	0.001	NS
	P3	<0.001		<0.001		<0.001		<0.005	
Group II (n=16)	Mean±SE	3.7±0.2	4.2±0.2	8.4±0.2	9.5±0.2	3.8±0.1	4.6±0.1	118±23	234±47
	P1	<0.001	NS	<0.001	NS	<0.01	NS	0.001	NS
	P2	NS	NS	NS	NS	NS	NS	NS	NS
Group III (n=16)	Mean±SE	4.5±0.1		10±0.1		4.2±0.2		267±22	

Values are means±SE. P1, results vs. the control group; P2, results vs. group I; P3, comparison of pre- vs. post-vitamin D bolus values.

Table 2. Serum 25(OH)D₃, 1,25(OH)₂D₃, osteocalcin, parathormone (PTH), and alkaline phosphatase (ALP) before and after vitamin D bolus.

Group	Parameter	25(OH)D ₃ (ng/dl)		1,25(OH) ₂ D ₃ (pg/ml)		Osteocalcin (ng/ml)		PTH (pg/ml)		ALP (units/liter)	
		Before	After	Before	After	Before	After	Before	After	Before	After
Group I (n=18)	Mean±SE	30±2	54.1±5.3	23.7±0.6	30.4±2.1	1.8±0.2	3.8±1.0	17.1±0.7	13.3±0.7	63.2±7.4	40.9±2.5
	P1	NS	<0.001	NS	<0.01	<0.001	NS	NS	<0.05	<0.005	NS
	P3	<0.001		<0.001		<0.001		<0.001		<0.001	
Group II (n=16)	Mean±SE	25.5±3.2	59.4±7.3	22±0.5	26.8±0.6	2.6±0.5	4.2±0.8	21±2.5	12.9±0.8	65±9	38.9±3.8
	P1	NS	<0.001	<0.05	<0.05	<0.001	NS	NS	<0.05	<0.005	NS
	P2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Group III (n=16)	Mean±SE	25±1.8		24.6±1.0		3.7±0.1		16.4±1.2		40.1±1.2	

Values are means±SE. P1, results vs. the control group; P2, results vs. group I; P3, comparison of pre- vs. post-vitamin D bolus values.

Table 3. Serum glucose (mg/dl), fasting and postprandial, before and after vitamin D bolus in diabetics.

Group	Parameter	Before		After	
		Fasting	Postprandial	Fasting	Postprandial
Group I (n=18)	Mean $\pm$ SE	111 $\pm$ 5	159 $\pm$ 5	111 $\pm$ 4	137 $\pm$ 6
	P1	<0.001	<0.001	<0.001	<0.001
	P3			NS	<0.05
Group II (n=16)	Mean $\pm$ SE	228 $\pm$ 36	291 $\pm$ 28	179 $\pm$ 23	223 $\pm$ 30
	P1	<0.001	<0.001	<0.001	<0.001
	P2	<0.001	<0.001	<0.001	<0.001
	P3			<0.05	<0.025
Group III (n=16)	Mean $\pm$ SE	86 $\pm$ 4	115 $\pm$ 23	81 $\pm$ 3.1	104 $\pm$ 3.5

P1, results vs. the control group; P2, results vs. group I; P3, comparison of pre- vs. post-vitamin D bolus values.

type of diabetes to study.

Our results demonstrated an insignificant increase in 25(OH)D₃ in diabetic groups compared with the control and a significantly lower level of 1,25(OH)₂D₃ in complicated diabetics and an insignificant alteration in controlled diabetics compared with healthy controls. This may indicate that insulinopenia is the mechanism by which circulating 1,25(OH)₂D₃ is reduced and suggests a relationship between 1,25(OH)₂D₃ and diabetic metabolic control, as indicated earlier [18]. Moreover, in a previous study a decreased activity of 1 α -hydroxylase was suggested [19, 20]. Also, reduced circulating vitamin D-binding protein reported in diabetes may be responsible for inhibition of the enzyme [21].

Elevated serum ALP activity has been found in diabetics. Our findings of low serum OC, total and ionized calcium levels before vitamin D administration and the normalization of ALP together with its positive correlation with Ca²⁺ after vitamin D administration suggest a bone source of the originally elevated ALP. Before vitamin D administration PTH levels showed insignificant changes in the two diabetic groups compared with the control. This agrees with some previous reports [22] and is contradictory to others [7]. The observed significant reduction in serum OC in diabetics compared with the control supports previous reports [23]. In diabetic patients a severe reduction in the number and/or function of osteoblasts along with decreased osteoid surface, mineral apposition rate, and plasma OC was reported [24].

Serum calcium, total and ionized, and 24-h urinary calcium were decreased in both diabetic groups compared with their levels in the control group. No significant difference was observed between the diabetic groups. The lower serum ionized calcium levels may be attributed to increased calcium binding to plasma proteins [25]. The osteoblast deficit, demonstrated in the present study by low levels of OC, may be a contributing factor. On the other hand, intestinal absorption of calcium has been shown to be increased [26] or normal [22] in diabetic subjects. The

finding of an insulin-reversible increase in intestinal absorption of calcium in insulinopenic diabetes was interpreted as a compensatory mechanism for the marked renal calcium loss observed in these patients.

After vitamin D supplementation, normalization of serum total and ionized calcium, 24-h urinary calcium, serum OC, and ALP together with decreased serum PTH was observed. This may indicate improvement of bone turnover in diabetics. The associated increase in $1,25(\text{OH})_2\text{D}_3$ and the positive correlation between OC and $1,25(\text{OH})_2\text{D}_3$ encountered in Group II of complicated diabetics after vitamin D supplementation support this explanation. The reduced levels of serum albumin in diabetics could be due to defective hepatic synthesis or associated diabetic albuminuria. Decreased serum albumin possibly explains parallel changes in serum calcium. The significant increase in serum albumin after vitamin D bolus suggests an enhancing effect of the latter on albumin synthesis, probably by a mechanism similar to the effect of this vitamin on calbindin (calcium binding protein) and vitamin D-binding protein genes [27].

Lowering of blood glucose level observed in diabetics (Groups I and II) after vitamin D administration could be attributed to increased plasma insulin. Studies of Tanaka *et al.* showed a direct action of vitamin D metabolites on pancreatic B-cell function [28]. Rise of serum C-peptide and improvement of peripheral glucose utilization after vitamin D administration was also reported in our laboratory [29]. Meanwhile, the significant elevation of the levels of $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ in Groups I and II after vitamin D_3 administration may be due to improvement of glucose tolerance and increased insulin secretion, which lead to improved renal 1α -hydroxylase function.

In conclusion, a beneficial action of vitamin D on bone turnover may be suggested from this study, which action leads to normalization of serum calcium, PTH, ALP, and OC. Development of microangiopathy is not necessarily exaggerating diabetic changes in bones nor delaying the effect of vitamin D on bone turnover. It is noteworthy to stress that diabetics may have a reduced plasma $1,25(\text{OH})_2\text{D}_3$ concentration, especially if the disease is poorly controlled. Plasma $1,25(\text{OH})_2\text{D}_3$ levels should be periodically checked; and if they are found to be low, vitamin D supplementation could be of benefit.

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