J Med Biochem 2016; 35 (1) DOI: 10.1515/jomb-2015-0011

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 35: 34–42, 2016

Original paper
Originalni naučni rad

CTX CORRELATION TO DISEASE DURATION AND ADIPONECTIN IN EGYPTIAN CHILDREN WITH T1DM

KORELACIJA IZMEĐU CTX-a I TRAJANJA BOLESTI I ADIPONEKTINA KOD EGIPATSKE DECE SA T1DM

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Summary

Background: In this study, we investigated the relationship of adiponectin with bone marker changes in Egyptian children and adolescents with T1DM and the effect of disease duration on these markers, as well as the possible correlations between adiponectin and bone markers in these patients.
Methods: Sixty Egyptian children and adolescent patients with T1DM were studied. Serum adiponectin and collagen breakdown products (cross-linked C-terminal telopeptide of collagen type I »CTX«) were measured and compared to the results of 20 age-matched healthy controls.
Results: After adjustment for age, BMI, Tanner stage and gender; (total) adiponectin was significantly higher in all T1DM patients. Serum level of CTX and 25(OH)D showed a marked decrease in diabetics with disease duration > 5 years. Serum level of (total) calcium and inorganic phosphorus (P_i) did not show significant difference from control. CTX was inversely correlated to FBG and T1DM duration. P_i was inversely, while 25(OH)D was directly correlated to FBG. Total calcium showed an inverse correlation with HbA_{1c}. FBG, TC, TAG, LDL-C were independent predictors of CTX in T1DM.
Conclusions: Adiponectin showed no correlation with either CTX or bone homeostatic indices. FBG, TC, TAG, LDL-C were independent predictors of CTX in T1DM. We recommend further investigation of adiponectin isoforms in a population-based study, to establish a good age- and sex-related reference.

Keywords: clinical, T1DM, adiponectin, bone biochemical markers, CTX, T1DM

Kratak sadržaj

Uvod: U ovoj studiji istraživali smo odnos između adiponektina i markera koštanih promena kod egipatske dece i adolescenata sa T1DM, uticaj trajanja bolesti na ove markere, kao i potencijalne korelacije između adiponektina i koštanih markera kod ovih pacijenata.
Metode: Proučavano je šezdesetoro dece i adolescenata egipatske nacionalnosti sa T1DM. Mereni su serumski adiponektin i produkti razgradnje kolagena (*cross-linked* C-terminalni telopeptid kolagena tip I, CTX) i upoređeni sa rezultatima 20 zdravih kontrolnih subjekata odgovarajućeg uzrasta.
Rezultati: Posle prilagođavanja za uzrast, indeks telesne mase, Tanerovu skalu i pol; (ukupni) adiponektin bio je značajno viši kod svih pacijenata sa T1DM. CTX je pokazao izrazit pad kod dijabetičara sa trajanjem bolesti > 5 godina. Serumski nivo 25(OH)D, (ukupni) kalcijum i neorganski fosfor (P_i) nisu se značajno razlikovali u odnosu na kontrolu. CTX je bio u inverznoj korelaciji sa FBG i trajanjem T1DM. P_i je bio u inverznoj, a 25(OH)D u direktnoj korelaciji sa FBG. Ukupni kalcijum bio je u inverznoj korelaciji sa HbA_{1c}. FBG, TC, TAG, LDL-C bili su nezavisni prediktori CTX-a u T1DM.
Zaključak: Adiponektin nije pokazao korelaciju ni sa CTX-om ni sa indeksima koštane homeostaze. FBG, TC, TAG, LDL-C bili su nezavisni prediktori CTX-a u T1DM. Preporučujemo dalje istraživanje izoformi adiponektina u populacionoj studiji kako bi se ustanovio validan referentni opseg u odnosu na uzrast i pol.

Ključne reči: klinički, T1DM, adiponektin, koštani biohemijski markeri, CTX, T1DM

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Introduction

Diabetes mellitus is the most common endocrine metabolic disorder of childhood. An increased incidence of Type 1 diabetes mellitus »T1DM«, is expected worldwide constitutes about 5–10% of diabetes mellitus cases (1). It spreads widely all over Egypt, with prevalence of 1.09 per 1000 among school aged children (2).

Osteoporosis is a common long-term complication of T1DM, and recent observations suggest that children and adolescents with T1DM are at risk for decreased bone mineral acquisition (3). Bone metabolism is a coupled process of bone formation and resorption. Uncontrolled bone resorption is a major cause of fast decrease in bone mass. Organic bone matrix consists of 90% type I collagen, the most abundant protein in the human body, primarily synthesized in bone (4). During bone regeneration, small peptide fragments of type I collagen are degraded by bone resorption (the amino- or carboxyterminal telopeptides) (5). Cross-linked C-terminal telopeptide of collagen type I »CTX« – a small 12 kDa, trivalent cyclic pyridinium structure – has been extensively used as a surrogate measure of bone resorption. It is released from bone collagen into circulation following its degradation by osteoclasts (6).

The most obvious characteristic of hard tissue is the organized deposition of protein, mainly collagen type I, and proper mineralization (7). Many studies have reported that elevated body weight or body mass index, »BMI«, are positively correlated to increased bone mineral density with reduced risk of fragility fractures (8).

There is a complex network of interaction among adipose tissue, liver, and bone, which reciprocally modulate the function of each other. The main mediators of such crosstalk include hormonal/cytokine signals from bone (osteopontin, osteocalcin, and osteoprotegerin), liver (fetuin-A), and adipose tissue (leptin, TNF-α and adiponectin). Thus, bone and glucose metabolisms are probably connected through this complex pathway (8).

Several lines of evidence suggest that obesity and bone metabolism are interrelated. First, both osteoblasts and adipocytes are derived from a common bone marrow mesenchymal stem cell (7), and agents inhibiting adipogenesis stimulate osteoblast differentiation and vice versa. Second, decreased bone marrow osteoblastogenesis with aging is usually accompanied by increased marrow adipogenesis (9).

Adiponectin – one of the most abundant adipocyte derived proteins – circulates at high concentrations in humans, typically 2–30 μg/mL (10) and plays a crucial role in maintaining the balance of energy metabolism, bone metabolism and inflammatory responses (11). Its plasma level is inversely correlated with: metabolic syndrome markers and markers of adiposity (including BMI and total fat mass) (12), regulating energy homeostasis and insulin sensitivity, and decreasing postprandial blood glucose (11). It correlates with basal and insulin-suppressed endogenous glucose production. It is elevated in T1DM regardless of disease duration (13).

Adiponectin was reported to act directly on bones, inducing human osteoblast proliferation, differentiation and mineralization (14). Adiponectin also increases osteoclast formation indirectly by stimulating the receptor activator of nuclear factor $\kappa\beta$ ligand (RANKL) and inhibiting osteoprotegerin production in osteoblasts. However, other studies suggest that adiponectin seems to exert a negative net effect on bone mass and to be an independent predictor of lower bone mass (15).

The aim of the present study was to investigate the relationship of adiponectin with CTX and other serum bone turnover markers in Egyptian children and adolescents with T1DM, and to explore the effect of disease duration on these markers. Also, the possible correlations between adiponectin and other bone turnover markers were evaluated.

Materials and Methods

Subjects

The study comprised 80 Egyptian subjects (43 males and 37 females), recruited from the pediatric outpatient clinic of the National Institute of Diabetes and Endocrinology (NIDE). All subjects underwent careful physical examination, detailed history, and laboratory investigations before inclusion in this study to exclude any condition that may interfere with the studied parameters. Patients were clinically diagnosed T1DM according to the American Diabetic Association (16). None of the patients was receiving any medications other than insulin, nor complaining from other chronic/acute illness, or nutritional derangements that might cause changes in bone metabolism. All subjects had no new fractures during this study.

Subjects were divided into two main groups: 20 healthy subjects as the control group (C), and 60 patients with T1DM who were further subdivided into 3 subgroups according to the duration of the disease: »D_1« with duration of diabetes < 1 year, »D_2« with duration of diabetes ≥ 1 year and < 5 years and »D_3« with duration of diabetes ≥ 5 years. All groups were age- and sex-matched. The study was approved by the ethics committee of the National Institute of Diabetes and Endocrinology, Cairo, Egypt.

Samples preparation and biochemical analyses

Blood samples were collected from all subjects in vacutainer tubes in the early morning after overnight fasting.

Glycated hemoglobin (HbA_{1c}) was determined in the whole blood, using the ion exchange HPLC technique (17).

Serum lipid profile including total cholesterol (TC), triacylglycerol (TAG), high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) was determined using a fully automated Dimension® RxL MAX Integrated chemistry system (Dade Behring instruments inc. USA). Fasting blood glucose level (FBG) was measured using hexokinase and glucose-6-phosphate dehydrogenase UV methods (18). Serum total calcium »Ca« was determined by the o-cresolphtalein complexone method, CPC (19). Serum inorganic phosphorous (Pi) was measured using the Molybdenum Blue method (20). Determination of total adiponectin was done by AssayMax Human Adiponectin ELISA kits (Assaypro® Research Laboratories, USA) (21). The assay of the degradation products of C-terminal telopeptides of type I collagen (CTX) was done using CrossLaps® ELISA assay (REF; AC-02F1, provided by Immunodiagnostic Systems Ltd »IDS Ltd«, UK) (22). Serum 25(OH)D concentration was determined using commercially available ELISA kits (Immunodiagnostic AG, Stubenwald-Allee 8a, D64625 Bensheim) (23).

Fresh morning urine samples were collected from each subject and used for determination of albuminuria using the turbidimetric assay (24) and urinary creatinine (25). ADVIA® 1650 clinical chemistry system was used for automatic estimation of the albumin to creatinine ratio (ACR) in randomly collected samples according to Justesen et al. (26). BMI was calculated as weight/height^2 (kg/m^2) (27).

Statistical analysis

Statistics were done using GraphPad Instat tm (© 1992–2000 Graph software Inc., V 3.05, Ralf Stahlman, Purdue Univ. 931897 S) to test the significance of differences between groups. Data were expressed as M±SE (average mean ± standard error). Appropriate graphs were plotted using GraphPad Prism 6 (Graphpad software Inc., V 6.00, USA). Comparisons between the studied groups were performed by one-way analysis of variance (ANOVA). Correlation co-efficient was done using the least square method. P value less than 0.05 was considered statistically significant. Spearman's correlation analysis was used to analyze the interrelationship between serum adiponectin and CTX levels and other clinical parameters.

Results

Clinical data, demographic variables and glycemic indices of the studied population are shown in *Table I*. The results of our study showed that there was no significant difference between the diabetic subgroups and the control group concerning gender, Tanner stage, BMI and age ($P>0.05$). Both FBG and HbA_{1c} were significantly elevated in all T1DM patient subgroups compared to control group ($p<0.001$), without any discrimination among the subgroups ($p>0.05$).

Metabolic variables of the studied subjects are shown in *Table II*. In our study, the serum levels of TAG, HDL-C and LDL-C showed no significant difference between the diabetic subgroups and control group, while serum levels of TC were significantly higher in all diabetic subgroups compared to control group ($p<0.05$ and <0.01 respectively). Liver enzymes (ALT, AST) and kidney function tests (urea, creatinine, albuminuria) showed no significant difference in all diabetic subgroups compared to control group ($p>0.05$). Although ACR was elevated in all diabetic subgroups with respect to control, only D1 subgroup showed significant increase in the level of ACR compared to control ($p<0.001$).

Analyses of adiponectin and indices of skeletal homeostasis revealed that serum levels of (total) adiponectin were significantly elevated in all T1DM subgroups compared to control group ($p<0.001$) with no significant difference between the three diabetic subgroups. Serum level of CTX was significantly increased in the D1 subgroup compared to control group ($p<0.01$), but then the levels decreased significantly in D2 and D3 subgroups compared to D1 subgroup ($P<0.05$). Serum level of 25(OH)D was decreased in all subgroups of T1DM, but it reached statistical significance only in D3 subgroup ($P<0.05$). Also, serum levels of both total Ca and Pi did not show significant difference between the three subgroups and control group *(Table III)*.

Simple linear regression analysis revealed that the serum level of adiponectin showed a statistically significant inverse correlation only with TAG ($P<0.001$). Serum level of CTX showed a significant inverse correlation with T1DM duration ($p<0.001$) and FBG ($P<0.01$). Serum level of 25(OH)D was directly correlated to FBG ($P<0.01$), while the serum level of Pi was inversely correlated with FBG ($P<0.01$). Total Ca showed an inverse correlation with both HbA_{1c} and ACR ($p<0.05$ and $P<0.01$ respectively), as shown in *Table IV*.

Table I Clinical, demographic variables and glycemic indices of the studied population.

Parameter	C (№ 20)	D1 (№ 27)	D2 (№ 17)	D3 (№ 16)
Age (years)	11±0.8013 (5–17)	10.176±0.5971 (5–17)	10.294±0.7352 (4–14)	12.767±0.5624 (7–15.5)
Gender (Male/Female)	11 / 9	16 / 11	8 / 9	8 / 8
Lactation Breast	17	23	14	13
Synthetic	3	4	3	2
Cow	–	–	–	1
Height (m^2)	1.384±0.044	1.40±0.033	1.362±0.038	1.414±0.04
Weight (kg)	39.95±3.858	39.44±3.022	38.71±3.305	42.19±3.049
BMI (kg/m^2)	19.86±0.99	18.004±0.5756	19.92±0.6935	20.81±0.7318
T1DM duration (years)	–	0.294±0.069	2.82±0.201	7.06±0.528
FBG (mmol/L)	4.03±0.09	12.46±1.09***	14.69±1.44***	16.52±1.40***
HbA1c (%)	5.34±0.065	10.512±0.551***	8.96±0.447***	9.69±0.7072***
Insulin Dose (U/day)	–	35.95±4.242	45.07±4.925	54±7.056

D_1: Duration of diabetes < 1 year, D_2: duration of diabetes 1–5 years, D_3: duration of diabetes ≥ 5 years, №: total number inside each group, BMI: body mass index, FBG: fasting blood glucose, HbA1c: glycated hemoglobin, T1DM: type 1 diabetes mellitus. ***: $p<0.001$ compared to group C; using parametric one-way ordinary ANOVA followed by Tukey-Kramer multiple comparison tests.
Data are expressed as M ± SE (average mean ± standard error)

Table II Metabolic variables of the studied population.

Parameter	C (№ 20)	D1 (№ 27)	D2 (№ 17)	D3 (№ 16)
TAG, mmol/L	0.84±0.09	0.99±0.052	0.95±0.063	0.99±0.055
TC, mmol/L	4.13±0.12	4.83±0.19*	4.91±0.24*	5.20±0.24**
HDL-C, mmol/L	1.08±0.032	1.21±0.051	1.17±0.062	1.27±0.038
LDL-C, mmol/L	2.69±0.10	3.13±0.17	3.24±0.20	3.42±0.22*
AST, U/L	25.75±1.357	31.115±2.326	27.47±2.080	27.75±2.507
ALT, U/L	18.65±0.998	26.11±2.744	21.76±1.994	25.69±3.004
Urea, mmol/L	8.82±0.41	10.34±0.46	10.33±0.32	10.31±0.61
Serum Creatinine, μmol/L	59.23±2.23	60.24±2.06	57.20±3.14	64.64±3.50
Urine Creatinine, g/day	140.62±15.47	91.70±10.33*	89.18±8.95*	75.17±9.18**
Albuminuria, mg/L	2.68±0.2548	3.413±0.4020	2.53±0.3356	2.29±0.2888
ACR, mg/g	2.26±0.21	5.45±0.63 ***	3.07±0.33##	2.94±0.43 ##

D_1: Duration of diabetes < 1 year, D_2: duration of diabetes 1–5 years, D_3: duration of diabetes ≥ 5 years, №: number inside each group, ACR: urinary albumin to creatinine ratio, HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TC: total cholesterol, TAG: triacylglycerol.
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ compared to group C, ##: $p<0.01$ compared to »D_1« group using parametric one-way ordinary ANOVA followed by Tukey-Kramer multiple comparison tests.
Data are expressed as M±SE (average mean ± standard error).

Table III Circulating adiponectin and indices of skeletal homeostasis of the studied population.

Parameter	C (№ 20)	D_1 (№ 27)	D_2 (№ 17)	D_3 (№ 16)
Total Adiponectin, μg/mL	10.26±1.229	26.4±1.44 ***	24.45±1.041 ***	22.48±1.195***
CTX, ng/mL	0.952±0.068	1.932±0.292**	0.976±0.053#	1.065±0.164 #
Total Calcium, mmol/L	2.33±0.04	2.40±0.03	2.5±0.07	2.40±0.07
Inorganic Phosphorus (Pi), mmol/L	1.5±0.091	1.6±0.10	1.5±0.052	1.5±0.11
25(OH)D, nmol/L	194.6±10.94	175.46±8.87	157.4±9.51	155.13±11.05*

D_1: Duration of diabetes < 1 year, D_2: duration of diabetes 1–5 years, D_3: duration of diabetes ≥ 5 years, №: number inside each group, CTX: cross-linked C-terminal telopeptide of collagen type I, 25(OH)D: 25-hydroxy cholecalciferol.

*: P<0.05, ***: p<0.001 compared to C group, #: p<0.05 compared to »D_1« group using parametric one-way ordinary ANOVA followed by Tukey-Kramer multiple comparison tests.

Data are expressed as M±SE (average mean ± standard error).

Table IV Correlations between the investigated parameters.

Parameter (log transformed)	r	p value
CTX vs. Adiponectin	0.098	0.3869
CTX vs. FBG	-0.3380	0.0083
CTX vs. T1DM duration	-0.4378	0.0005
Adiponectin vs. TAG	-0.4211	0.0008
25(OH)D vs. FBG	0.3311	0.0145
Total Calcium vs. HbA1c	-0.2715	0.0358
Total Calcium vs. ACR	-0.3395	0.0080
Pi vs. FBG	-0.3276	0.0156

CTX: Degradation products of C-terminal telopeptides of type I collagen, 25(OH)D: vitamin D, FBG: fasting blood glucose level, T1DM: type 1 diabetes mellitus, TAG: triacylglycerol, ACR: urinary albumin to creatinine ratio, HbA1c: glycated hemoglobin.
Correlation coefficient (Pearson rank) assuming Gaussian distributions, for all diabetics (№=60).
All possible correlations were tested; those unmentioned were statistically non-significant (p>0.05).

Discussion

Diabetic osteopathy is a significant comorbidity of T1DM, characterized by osteoporosis, increased risk for bone fracture, and micro-architectural changes that increase brittleness of bone. Chronic hyperglycemia, hypoinsulinemia, disruption of growth hormone, IGF-1: IGFBP system and altered vitamin/mineral homeostasis are all thought to contribute to this skeletal pathology (28).

T1DM is associated with reduced bone mineral content (BMC) and appears to affect bone cross-sectional size and cortical rigidity (29). Adiponectin plays an important role in hyperglycemia, as well as dyslipidemia (30). In an attempt to discover more about the role of adiponectin and its relationship with bone homeostatic (total Ca, 25(OH)D, Pi) and resorption indices (CTX), demographic characteristics and other metabolic parameters, we conducted this study on Egyptian T1DM children and adolescents.

In the present study, lipid profile was measured to study its relation to homeostatic markers and CTX. Kidney and liver function tests were done to eliminate the effect of altered metabolism of the investigated parameters; especially CTX and 25(OH)D.

In our study, the serum levels of TAG and HDL-C showed no significant difference between the diabetic subgroups and control group; the serum level of LDL-C was slightly increased in the diabetic subgroups compared to control group although still non-significant, while only the serum level of TC was significantly increased in all the diabetic patient subgroups and its level increased with the increase in the diabetic duration of disease. Mitra et al. (32) stated that hypertriglyceridemia and hypercholesterolemia are the most common lipid abnormalities in diabetes. These results were supported by the studies of Gentilini et al. (24).

In this study, ACR showed an elevation in new onset diabetics, then declined to almost normal with longer durations, in contrast to the findings of Faulkner et al. (32) who demonstrated that albuminuria was significantly elevated in T1DM with longer duration (>5 years). This could be attributed to and aggravated by chronic uncontrolled hyperglycemia (33) (supported by our HbA1c values).

In the present study, serum (total) adiponectin was significantly elevated in all T1DM patient subgroups irrespective of disease duration and this came in accordance with Abd El-Mohsin et al. (13) who stated that in patients with T1DM, plasma adiponectin is reported to be usually increased. Our finding also agrees with Galler et al. (34), who demonstrated elevated levels of adiponectin in children and adolescents with T1DM compared to healthy subjects but contradicts Morales et al. (35) who stated that adiponectin levels in children and adolescents with T1DM did not differ from those in healthy subjects.

T1DM children and adolescents show several impairments of bone metabolism and structure, resulting in a higher risk of decreased bone mass, mineral acquisition (36), and its related complications later in life, compared with a non-diabetic reference population (37).

In our study, CTX showed a marked decrease in diabetics with longer disease duration (>5 years) compared to the newly diagnosed ones. These results came in line with Maggio et al. (38) who stated that T1DM children had lower levels of CTX compared to healthy controls. Bone turnover is altered in T1DM children, whereas BMD remains normal during growth stage. Also, our results are in agreement with Bonfanti et al. (39) who found that serum CTX in prepubertal children was within normal range at onset of T1DM and decreased during the follow-up to reach a significant difference compared to controls after months of insulin treatment. Confirming results were also found in previous studies (40) but our results came in contrast with Abd El Dayem et al. (41) who reported that T1DM diabetics had low BMD after adjustment (Z score), low bone formation and high bone resorption markers. Pubertal subjects (diabetics and controls) have higher BMD and BMC than the prepubertal.

Children and adolescents with poorly controlled T1DM are at risk for decreased bone mass, which could be due to abnormal bone turnover or disturbances in the Ca/parathyroid hormone/vitamin D axis or both (42). Alterations of the nuclear factor-kB ligand (RANKL)/osteoprotegerin (OPG) system have been implicated in several metabolic bone diseases characterized by increased osteoclast differentiation and activation, and enhanced bone resorption (37).

In our study, (total) Ca and Pi did not show significant difference from control. Serum level of 25(OH)D decreased in all diabetic subgroups but did not reach statistical significance unless in the D3 subgroup. These results came in contrast to Karagüzel et al. (43) who reported that T1DM children with lower serum levels of Ca and higher serum levels of 25(OH)D had reduced bone formation and increased bone resorption, while Greer et al. (44) reported that newly diagnosed T1DM children had lower 25(OH)D than controls or children with established diabetes. Thnc et al. also found that there was vitamin D deficiency in 28% and vitamin D insufficiency in 43% of T1DM patients, whereas 29% had normal serum 25OHD levels (45). Hamed et al. (46) stated that Egyptian children and adolescents with T1DM have abnormal bone status (osteopenia-osteoporosis) mostly in the axial skeleton, and diabetic patients showed significant increase in Pi and PTH levels and significant decrease in Ca, IGF-1, and 25(OH)D serum levels.

Correlation study after age, BMI, Tanner stage and gender adjustment showed that adiponectin was in an inverse correlation with TAG only, with no other correlation with neither T1DM duration, nor HDL-C, LDL-C and TC. Our results are in line with Von Eynatten et al. (47) and also with Goropashnaya et al. (48) who reported the presence of an inverse correlation between adiponectin and TAG levels. But our results are contrary to Lindström et al. (49) who reported a non-significant correlation of adiponectin with TAG but a direct association with TC. In our results, ACR was not correlated to adiponectin, which is supported by the results of Ljubic et al. (50).

Here, CTX was inversely correlated to FBG and T1DM duration, in line with Bechtold et al. (36), who reported a negative impact of disease duration on bone mass, and with Bonfanti et al. (39) but contradicting Chobot et al. (51) who observed no correlation between bone status of T1DM adolescents and diabetes duration. Also, contrary to Maggio et al. (38), no inverse correlations between HbA_{1c} and CTX were found.

Our results revealed that P_i was inversely, while 25(OH)D was directly correlated to FBG. Total Ca showed an inverse correlation with both HbA_{1c} and ACR. This is in agreement with De Schepper et al. (52) who reported that poor blood glucose control has been associated with lower bone mass in adults and adolescents with T1DM but not in children. Also, Chobot et al. (51) reported that osteoporosis as defined by the WHO criteria was diagnosed in 51.2% of T1DM children (at total body by DXA); reduction was significantly more marked in those patients whose HbA_{1c} >7.0% when compared with those whose HbA_{1c} was lower.

Conclusion

To our knowledge, this is on a few the first clinical research that investigates the relationship between adiponectin and bone biochemical and resorption markers in Egyptian children and adolescents with T1DM. Although adiponectin was reported to preserve bone both *in vivo* and *in vitro*, we were unable to find any correlation with CTX and the homeostatic indices, which could be attributed to the relatively short duration of T1DM in patients included in our study. CTX was inversely correlated with FBG and T1DM duration.

We recommend further investigation focusing on: (1) CTX, (2) adiponectin isoforms distribution between healthy and diabetics with longer duration of disease, in a large population-based study, to establish good age- and sex-related reference data.

Acknowledgment. We gratefully thank the staff of the National Institute of Diabetes and Endocrinology (NIDE) for technical support.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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Received: April 5, 2015
Accepted: June 16, 2015