

## Effect of green tea extract on bone turnover markers in type 2 diabetic patients; A double- blind, placebo-controlled clinical trial study

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### ABSTRACT

*Background and the purpose of the study:* This study was performed to investigate the effect of green tea extract (GTE) on bone turnover in type 2 diabetes mellitus (T2DM) patients.

*Methods:* Totally 72 T2DM patients with stratified randomize method were divided into two interventional and control groups in a double blind placebo- control clinical trial study. GTE, 500mg, and placebo were prescribed three times a day for 8 weeks. Laboratory and anthropometric measurements included fasting blood glucose (FBG), oral glucose tolerance test (G2h), glycosylated hemoglobin A1c (HbA1C) and lipid Profile, fasting serum osteocalcin, crosslaps and insulin, body mass index (BMI) and waist to hip ratio (WHR) before and after intervention.

*Results and major conclusion:* Assess of GTE effect on the bone markers revealed that change in osteocalcin was almost significant but log osteocalcin had significant alteration in GTE group. We found no significant changes in WHR, BMI, FBG, G2h, Hb1Ac, fasting insulin concentration and crosslaps levels in this group after intervention. Representational evidences demonstrated that decrease in crosslaps level was 10 times in green tea groups compared to the placebo group. Results also showed that improved bone turnover patients had significantly lower FBG and HbA1c levels ratio than non-improved patients as well as had higher fasting insulin concentration in GTE group. Our results suggest that GTE may reduces bone resorption marker more than placebo and probably modifies the bone turnover in T2DM patients.

**Keywords:** Green tea extraction, Bone turnover, Type 2 diabetes mellitus, Crosslaps, Osteocalcin

### INTRODUCTION

The main complications of diabetes include nephropathy, neuropathy, retinopathy, macrovascular and microvascular disease, and alterations of bone and mineral metabolism (1). Diabetic osteopenia causes an increase in bone fractures (2), delays the healing of fractures (3) and affects the quality of life. Bone remodeling is an ongoing cyclical process characterized by two coupling process; bone resorption by activated osteoclasts with subsequent deposition of new matrix by osteoblasts. These processes are revealed to some extent by biochemical markers of bone turnover. The mechanisms responsible for diabetic bone disease in diabetes are complex, although progression of the disease is consistently associated with alteration in bone turnover (4). Conflicting results with regard to bone remodeling markers have also been reported in type 2 diabetic patients (5). Evidences exposed from previous studies shown some factors derived from osteoblasts (6, 7) and osteoclasts (8). These factors may

be helpful as indexes of bone metabolism in type 2 diabetes patients. Circulating osteocalcin is a biological marker for osteoblastic function (9), It has been proposed that osteoblastic action decrease by progress of type 2 diabetes disease. Previous investigations on diabetic animal model demonstrated reduction of circulating osteocalcin. (10, 11). On the other hand, the subjects regarding osteoclastic function in the diabetic state have been still controversial (11, 12). Crosslaps (C-terminal cross- linked telopeptide of type I collagen) is a biological marker for osteoclastic function (12). Recently it has been reported that the concentrations of serum markers for osteoclastic function elevated significantly in male type 2 diabetic patients (12). One of the main applications for measurement of bone markers are in interventional studies. An early prediction of bone mass density (BMD) response by bone markers are apparent from previous intervention studies (13) as well as markers are useful

in monitoring treatment efficacy in patients. There is evidence from intervention studies that a surveillance of therapy by bone markers will probably provide a powerful tool in early diagnose of intervention effect (14). However, the interpretation of bone marker data is somewhat cumbersome. Findings of studies indicate that Green tea (GT) and its components have many pharmacological activities such as anti-obesity and anti-diabetic properties and somewhat the corresponding mechanisms of action have been clarified in some extent in previous studies. There are a number of investigations that examined the effect of some gradients of GTE on bone metabolism. Findings of previous studies demonstrated the effect of its components on suppressing bone resorption (15) inducing osteoclast apoptosis (16, 17) and suppressing its differentiation (18). Results of recent studies also demonstrated stimulation of osteoblastogenesis via increase in the expressions of osteogenic genes and mineralization (19) and stimulated osteoclastic cell death (20,21) and inhibition of the formation of osteoclastic cells (22). Previous cross-sectional epidemiological studies also have been conducted on the beneficial effects of green tea consumption on bone health (23). More recent study on women showed that GT components in prevention of bone loss are supportive factors (24). An experimental study showed the inducing effect of GT component on the mRNA expressions of osteocalcin in murine bone marrow mesenchymal stem cell line (25). Information relative to a possible role of GTE in bone turnover is presently scanty. We found no clinical trial study that investigated the effect of GTE on osteocalcin and crosslaps levels in Type 2 diabetes. The aim of the present study was to evaluate two parameters of bone turnover and bone structure and their possible modulations by GTE in characterized type 2 diabetic (T2D) patients.

## MATERIALS AND METHODS

### *Study population and anthropometric measurements*

As a double blind placebo- control clinical trial study, 72 patients with clinically proven type 2 diabetes mellitus diagnosed based on the World Health Organization criteria (26) (January to June 2008) in diabetes clinic were recruited in Endocrinology and Metabolism Research Center (EMRC). Inclusion criteria were age  $\geq 40$  years, BMI (Body mass index)  $\geq 25$  and at least 2 years of diagnosis of type 2 diabetes. Exclusion criteria were history of diabetes type 1, any chronic disease other than T2DM and its complications and insulin therapy. In addition, users of drugs inducing bone loss like corticosteroids were excluded. Informed written consent was obtained from all subjects before their participation in the study. The study protocol was approved by ethics committee of EMRC (Endocrinology and Metabolism Research Center). Demographic data, life style, medical history and medications that they took through last 3 month

were obtained by a questionnaire. For every subject anthropometric questionnaires was accomplished before and after intervention period. Evaluated parameters including weight (to the nearest 0.1 kg) that measured by a balance scale while the subjects were dressed lightly and bare foot. Height was measured using a stadiometer. Body mass index was calculated as weight divided by the square of height expressed in  $\text{kg/m}^2$ . Waist and hip circumferences (to the nearest 0.1 cm) were measured while the subjects were fasting and in light covered clothings. Subject's waists were measured with a soft tape midway between the lowest rib and the iliac crest; hip circumference was measured at the widest part of the gluteal region. BMI and WHR (waist to hip ratio) were calculated for all patients.

### *Laboratory tests*

The peripheral blood was taken after 10-12 hrs fasting. Serum after centrifugation was aliquoted and stored at  $-80^\circ\text{C}$ . All samples were run in the same assay. All measurements were performed in the EMRC laboratory of Shariatei hospital. HbA1C was measured using HPLC (High pressure liquid chromatography) exchange ion method (DS5 England), FBG was measured by GOD/ PAP method. OGTT was performed according to the World Health Organization standard procedure (27). After overnight fasting, the subjects were given a standard solution of 75 gr glucose in 250 ml of water. Blood samples were taken after 120 min to measure plasma glucose concentrations by utilizing the GOD/PAP and Randox method laboratory kits. Serum visfatin concentration was determined by ELISA method (Human visfatin ELISA kit, AdipoGen Pharmaceuticals, Belmont, Seoul, Korea). Minimum detectable concentration was 30 pg/ml, Intra CV was 4.3 % and Inter CV was 7.5 %. Serum insulin concentrations were measured by ELISA method (Human insulin ELISA kit, DRG Pharmaceuticals, GmbH, Germany). Minimum detectable concentration was 1.76  $\mu\text{IU/ml}$ , Intra CV was 2.19% and Inter CV was 4.4%. Serum adiponectin concentration was determined by ELISA method (Human adiponectin ELISA kit, AdipoGen Pharmaceuticals, Belmont, Seoul, Korea), minimum detectable concentration was 100 pg/ml, Intra CV was 5.15 % and Inter CV was 3.82 %. Markers of bone formation included osteocalcin, which was measured by immunoassay (ELISA) using a Bioscience kit (Nortec Bioscience Diagnostic A/S, Denmark). The intra- and inter-assay CV were 2.6% and 4.7%, respectively. Another marker of bone resorption is the serum C-terminal telopeptides of type I collagen: serum crosslaps. Crosslaps were measured by immunoassay (ELISA) using a Bioscience kit (Nortec Bioscience Diagnostic A/S, Denmark), with intra- and inter-assay CV of 5.1% and 6.6%, respectively.

### *Preparation of capsules and prescription*

Leaves of *Camellia sinensis* were collected from Lahijan, in May 2007. *C. sinensis* was authenticated,

and then a voucher specimen (No.F.P. 28) was deposited in the herbarium of faculty of pharmacy of Shahid Beheshti University of Medical Science, Tehran, Iran. The placebo given to the control group comprised pure microcrystalline cellulose. The subjects were asked to take one capsule containing 500 mg of GTE (50 mg caffeine, 80 mg polyphenoles) or cellulose three times a day for 8 weeks. The capsule was taken after main meal.

#### Statistical analysis

Numerical variables were reported as the mean  $\pm$  SD and categorical variables were presented as percentage. All statistical analyses were performed using the SPSS version 15 software. Comparisons between intervention and control groups were carried out using Pair t-test. Student t-test was used to compare quantitative variables. Chi-square test was used to compare the qualitative variables and ANOVA was used to compare the quantitative variable. P values less than 0.05 were considered to be statistically significant.

### RESULTS

Totally 72 subjects, 16 men (19.5%) and 66 women (80.5%), with T2DM participated in this study. The mean  $\pm$  SD of age, BMI, WHR of participants were  $54.56 \pm 11.23$  years,  $29.90 \pm 4.19$  kg/m<sup>2</sup> and  $0.9 \pm 0.06$  respectively. Age, sex and BMI and diabetic criterion distributions were similar between two groups. Numbers of patients in interventional and control groups were 36(43.9%) and 46(56.1) respectively. All participants' baseline demographic and biochemical characteristics are shown in tables 1. As shown in table 2, evaluation of GTE effect on bone markers revealed that change in osteocalcin was almost significant (pvalue=0.07) but log osteocalcin had significant alteration in GTE group. No significant changes was found in WHR, BMI, FBG, G2h, Hb1Ac, fasting insulin concentration and crosslaps levels in this group after intervention. Representational evidences demonstrated that decrease in crosslaps level was 10 times in green tea groups opposed to the placebo group (-0.11 in GTE group vs. -0.01 in placebo one). Analyses of the results also displayed that 22.2% patients in GTE group vs. 4.3% in placebo one had decrease in crosslaps levels (pvalue=0.019). According to the bone turnover definition, we classified the patients with increase in osteocalcin and decrease in crosslaps or decrease in both markers as improved turnover groups and the other patients as non-improved turnover groups. In accordance with classification groups, our results showed that improved turnover patients had significantly lower mean $\pm$ SD levels of FBG and HbA1c ratio to non-improved patients [  $142.5 \pm 57.19$  vs.  $189.57 \pm 63.76$  (pvalue=0.02) and  $5.93 \pm 0.58$  vs.  $7.79 \pm 1.59$  (pvalue=0.01) respectively] as well as had higher fasting insulin concentration [ $18.38 \pm 8.62$  vs.  $12.42 \pm 2.97$  (pvalue=0.01)] in GTE group. No

significant difference was found in parameter between improved and non improved turnover patients in placebo groups. Lastly, the analysis of the bone turnover status between good and poor control of blood glucose was analyses according to Hb1Ac levels (Hb1Ac  $\leq$  7% as good control and Hb1Ac  $>$  7% as poor- control). Our results showed that in 75% of subjects with GTE intervention the bone turnover status became better in good control groups although only 33.3% had the same condition in placebo one (pvalue=0.015). We found no significant differences between two groups in poor- control patients.

### DISCUSSION

The prevalence of diabetes mellitus has been rapidly increasing, stimulated by an epidemic increase in obesity and other metabolic risk factors (28). The association between diabetes and bone disorders is complex and this issue remains controversial. The mechanisms responsible for diabetic bone disease in diabetes are complex, although disease is associated with alteration in bone turnover (29). Previous study clearly showed that, green tea component has significant effects on obesity in type-2 diabetes but demonstrated partial Information relative to a possible role of GTE in bone turnover. The available information makes obvious controversial results about the effect of GT components on glucose control in T2DM patients. However some of them have demonstrated significant reduction in glucose levels, but others found no change or increase in blood glucose after intervention (20, 31). Evaluation of GTE effect on diabetic features such as FBG, G2h, HbA1c and fasting insulin levels in present study demonstrated no significant changes after intervention. Results of Fukino et al (32) of randomized-controlled study on borderline or established diabetes demonstrated no significant differences in blood glucose, Hb, A1C and insulin levels in the intervention group within 2 months. Also evidences of Ryu et al (33) in randomized cross-over study in type-2 diabetes patients showed no significant differences in blood glucose and insulin resistance at 4 weeks intervention with green tea. A recent study reported GTE had significant effect on the decrease of Hb, A1c, but their results demonstrated no significant differences in fasting insulin and glucose between intervention and control groups (30). The main reason for results of the previous study by researchers were that there was not any control on drinking green tea in the control group. It might have masked potential effect of intervention between groups.

Evidences of this study exhibited no significant alteration in osteocalcin levels after intervention. We have no information about the mechanisms by which GTE acts on osteocalcin production. Evidences of previous research demonstrated that expression of osteocalcin mRNA was lower in T2DM patients

**Table 1.** The baseline demographic and biochemical characteristics of participants in study

Demographic and biochemical characteristic of participants in study	
Age (years)	54.56±11.23
Diagnose of T2D disease (month)	68.71±53.54
BMI (kg/m <sup>2</sup> )	29.90±4.19
FBG (mg/dl)	170.42±62.96
G2h (mg/dl)	217.92±83.54
Hb A1C (%)	7.68±1.83
Fasting insulin (μIU/ml)	14.70±6.01
Adiponectin (μg/ml)	6.91±3.94
Visfatin (ng/ml)	17.27±11.83
Osteocalcin (ng/ml)	17.42± 10.51
Crosslaps (ng/ml)	1.03± 0.41

BMI; body mass index, FBG; fasting blood glucose, G2h; Oral glucose tolerance test, HbA1c; glycosylated hemoglobin A1c.

**Table 3.** The clinical characteristics and laboratory results of subjects with T2DM before and after in placebo group

Characteristics	Placebo (n=46)		P* value
	Before	After	
BMI (kg/m <sup>2</sup> )	29.78±4.03	29.87±4.06	0.454
WHR	0.89±0.05	0.90±0.07	0.057
FPG (mg/dl)	175.90±62.39	185.51±72.75	0.155
G2h (mg/dl)	214.64±84.01	206.15±63.59	0.285
Hb A1C (%)	7.61±2.04	8.17±2.09	0.054
Fasting Insulin (μIU/ml)	14.13±4.39	15.57±7.03	0.062
Adiponectin (μg/ml)	7.14±4.43	7.49±5.45	0.17
Visfatin (ng/ml)	18.66±5.65	16.42±4.98	0.292
Crosslaps (ng/ml)	0.97±0.38	0.96±0.49	0.42
Osteocalcin (ng/ml)	15.00±10.41	15.10±10.22	0.60
Log crosslaps	-0.13±0.64	-0.13±0.31	0.931
Log osteocalcin	1.13±0.36	1.16±0.45	0.104

P value between before and after intervention in placebo group \*

BMI; body mass index, WHR; waist to hip ratio, FBG; fasting blood glucose, G2h; Oral glucose tolerance test, HbA1c; glycosylated hemoglobin A1c.

(33). Results of Shen et al (34) in vivo research in middle-aged rats for 16-week demonstrated that GT component increased the mRNA expression of osteocalcin. Previous in vitro studies have shown that there were correlation between glucose concentration and osteocalcin levels and chronic hyperglycemia decreased osteocalcin expression (35). The same results also were obtained from the present clinical study that there was not significant alteration in both parameters after intervention. Previous clinical trial

**Table 2.** The clinical characteristics and laboratory results of subjects with T2DM before and after intervention in green tea group

Characteristics	Green tea group (n=26)		P* value
	Before	After	
BMI (kg/m <sup>2</sup> )	30.05±4.51	29.87±4.60	0.354
WHR	0.91±0.06	07.0.90±	0.391
FPG (mg/dl)	162.71±65.72	169.07±70.60	0.316
G2h (mg/dl)	217.96±85.36	220.82±77.20	0.862
Hb A1C (%)	7.21±1.63	7.25±1.87	0.724
Fasting Insulin (μIU/ml)	15.92±7.42	16.70±8.99	0.348
Adiponectin (μg/ml)	6.80±3.31	8.38±2.69	0.001
Visfatin (ng/ml)	17.48±8.52	17.83±3.44	0.68
Cross laps (ng/ml)	1.12±0.44	1.01±0.50	0.285
Osteocalcin (ng/ml)	12.67±10.04	15.40±9.37	0.078
Log crosslaps	0.018±0.17	-0.169±0.71	0.130
Log osteocalcin	0.86±0.53	1.11±0.26	0.004

P value between before and after intervention in Green tea group \*

BMI; body mass index, WHR; waist to hip ratio, FBG; fasting blood glucose, G2h; Oral glucose tolerance test, HbA1c; glycosylated hemoglobin A1c.

on 101 post-menopausal women that was randomized to a dairy intervention group, a calcium- supplemented group and a control group also demonstrated no significant differences among groups regarding the changes in serum osteocalcin and type I collagen cross- linked C- telopeptide levels. However other parameters had significant differences between groups (36). It appears that the episode of the present clinical intervention similar to other interventional studies were not sufficient to elucidate the significant alteration that has been shown in previous in vivo study.

Findings of a recent study by Kanazawa et al. (37) showed negative correlation between serum osteocalcin levels and BMI and HbA1c. We found also negative correlation between change of osteocalcin levels and BMI changes. Our results was in favor of kanazawa et al results regarding existent of correlation between BMI change and osteocalcin .

Although osteocalcin changes was not significant after intervention but our results exhibited decrease in crosslaps level approximately 10 times in green tea groups opposed to the placebo group. Decrease of crosslaps levels as an indicator of osteoclast function is novel finding of GT effect on reduction bone turnover in T2DM. Our finding also corroborates those reported effect of GT component on osteoclastic cell via well-known pathway such as motivating cell death via Fenton reaction, caspase activation and inhibition of the formation of osteoclastic cells (16, 22, 38).

However, the study of the effect of glycemic control on bone turnover led to controversial conclusions. The effect of the control of the glucose concentration on improved bone turnover with GT, showed significant differences between the number of patients that



improved bone turnover according to good control and poor control groups which indicates correlation between osteocalcin with HbA1c levels. These findings were similar to results of the previous reports (37, 39). Okazaki et al. (40) suggested that metabolic improvement of poorly controlled type 2 diabetes is associated with a decrease in urinary deoxypyridinoline and urinary calcium, but not with an increase in serum osteocalcin. Rosato et al. (41) found that both formation and resorption a bone markers were sensitive to a change in glycaemic control and serum osteocalcin increased after improved glycaemic control in diabetic patients, which is consistent with results of this study. We also observed a statistical correlation between crosslaps and osteocalcin changes with green tea and HbA1c alteration levels in patients, suggesting increased improvement in bone turnover in good-controlled diabetic patients.

## CONCLUSION

Comprehensively, whit regards to biochemical indices of bone remodelling no significant differences were observed among groups for serum osteocalcin and crosslaps levels. Nonetheless, a tendency for a greater decrease was observed for crosslaps in the GT group compared to placebo one. This particular change in crosslaps is consistent with recent evidence reporting similar short-term decreases in several bone resorption indices. This evidence suggests that effect of intervention in bone resorption marker is faster than the formation marker (42).

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