Alendronate improves fasting plasma glucose and insulin sensitivity and decreases insulin resistance in prediabetic osteopenic postmenopausal women: a randomized triple-blind clinical trial

KARIMI FARD, Maryam, AMINORROAYA, Ashraf <http://orcid.org/0000-0002-7550-1198>, KACHUEI, Ali, SALAMAT, Mohammad Reza, HADI ALIJANVAND, Moluk, AMINORROAYA YAMINI, Sima <http://orcid.org/0000-0002-2312-8272>, KARIMIFAR, Mansoor, FEIZI, Awat and AMINI, Massoud

Available from Sheffield Hallam University Research Archive (SHURA) at:
http://shura.shu.ac.uk/22842/

This document is the author deposited version. You are advised to consult the publisher's version if you wish to cite from it.

Published version


Copyright and re-use policy

See http://shura.shu.ac.uk/information.html
Alendronate improves fasting plasma glucose and insulin sensitivity and decreases insulin resistance in prediabetic osteopenic postmenopausal women: a randomized triple-blind clinical trial

Maryam Karimi Fard, MD,1,2 Ashraf Aminorroaya, MD,1 Ali Kachuei, MD,1 Mohammad Reza Salamat, PhD,3 Moluk Hadi Alijanvand, PhD,4 Sima Aminorroaya Yamini, PhD,5 Mansoor Karimifar, MD,6 Awat Feizi, PhD,1,4 Massoud Amini, MD,1

1 Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
2 Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
3 Department of Medical Physics and Medical Engineering, Isfahan University of Medical Sciences, Isfahan, Iran
4 Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.
5 Department of Engineering and Mathematics, Sheffield Hallam University, Sheffield, S1 1WB, UK.
6 Isfahan Rheumatology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jdi.12944
This article is protected by copyright. All rights reserved.
Corresponding author: Ashraf Aminorroaya. Postal address: Isfahan Endocrine and Metabolism Research Center, Sedigheh Tahereh Research Complex, Isfahan, Iran. Postal code: 8187698191.

Email: aminorroaya@med.mui.ac.ir
Tel: +98 31 33359933
Fax: +98 31 33373733

Abstract

Aims: Postmenopausal women receive bisphosphonates for osteoporosis treatment. The effect of these medications on developing diabetes mellitus (DM) in prediabetic patients is yet to be investigated. We aimed to determine the effect of alendronate on plasma glucose, insulin indices of postmenopausal women with prediabetes and osteopenia.

Methods: This triple-blind randomized controlled clinical trial included 60 postmenopausal women, aged 45–60 years. All patients were vitamin D sufficient. They were randomly enrolled in intervention (70 mg/week alendronate for 12 week) and control (placebo tablet per week for 12 weeks) groups. The morning 8 hour fasting blood samples were collected at the baseline and follow-up visits to measure the fasting plasma glucose (FPG) (mg/dl), insulin and hemoglobin A1c (HbA1c).

Plasma glucose and insulin concentration were measured 30, 60, and 120 minutes after glucose tolerance test. Matsuda index, homeostasis model assessment of insulin resistance (HOMA–IR), homeostasis model assessment of beta–cell function (HOMA–B) and the area under the curves (AUC) of glucose and insulin were calculated.

Results: Mean (SD) FPG (102.43 (1.46) mg/dl vs. 94.23 (1.17) mg/dl, P=0.001), 120-minutes insulin concentration (101.86 (15.70) μU/l vs. 72.60 (11.36), P=0.026), HbA1c (5.60 (0.06) % vs. 5.40 (0.05)%, P=0.001), HOMA-IR (3.57 (0.45) vs. 2.62 (0.24), P=0.021) and Matsuda index (7.7 (0.41) vs. 9.2 (0.4), P=0.001) significantly improved in the alendronate-treated group. There was statistically significant more reductions in FPG (-8.2 (8.63) mg/dl vs. -2.5 (14.26) mg/dl, P=0.002) and HbA1c (-
0.2 (0.23) % vs. -0.09 (0.26) %, P=0.015) were observed in alendronate-treated group than placebo group during the study course, respectively.

**Conclusions:** Administration of 70 mg/week alendronate improves fasting plasma glucose, HbA1c and insulin indices in postmenopausal women.

**Keywords:** Alendronate; Menopause; Prediabetic

This trial was registered with IRCT. (no. IRCT2016101530309N1)

**Introduction**

Diabetes is a serious global health issue and is a major cause of morbidity, mortality, and worldwide economic burden with an annual increase in the prevalence rate\(^1\). Although the prevalence of diabetes mellitus (DM) among the Iranian population is close to the global prevalence (8–9%), it has increased to roughly 20% in women, aged 55–65 years\(^2\). Therefore, any method that can reduce the chance of developing DM in this high-risk group of women by paying more attention to prediabetic patients is highly desirable.

DM is associated with increased prevalence of complications such as nephropathy, cardiomyopathy, retinopathy, and vasculopathy\(^3\), mainly caused by generalized capillary dysfunction\(^4\). Hence, several interventions have been suggested to reduce the incidence rate of DM, such as lifestyle or pharmacological interventions to reduce the risk factors\(^5,6\). The prediabetes is mainly diagnosed by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)\(^7\), defined by hemoglobinA1c (HbA1c) levels of 5.7%–6.4%\(^3\). Generally, 25–
50% of patients in the prediabetic state develop DM\textsuperscript{8}, thus appropriate risk reduction methods and interventions in prediabetic state can prevent its progression to DM and assist in reducing diabetes–related complications\textsuperscript{9,10}.

Another significant and prevalent cause of morbidity and mortality in postmenopausal women is osteoporosis, characterized by substantial bone loss, resulting in increased risk of fractures\textsuperscript{11}, that imposes a great cost and impairs the quality of life of the affected patients\textsuperscript{12}. Bisphosphonates, specifically alendronate and risedronate\textsuperscript{13}, are in the first line of treatment for osteoporosis with few adverse effects\textsuperscript{14} that efficiently suppress bone resorption and prevent fractures. Bisphosphonates have also shown antitumor effects\textsuperscript{15}, and lipid profile modification\textsuperscript{16}. Recent population–based studies have suggested 50% reduced risk of type 2 diabetes mellitus (T2DM) with the use of bisphosphonate, but it has not yet been proven clinically\textsuperscript{17,18}. Studies performed on mouse models have suggested that reduced bone turnover can regulate insulin sensitivity\textsuperscript{19,20}. A population based cohort study indicated that the use of alendronate decreased the incidence of diabetes\textsuperscript{21}. However, no clinical trial study has yet investigated the effect of anti-resorptive therapies on fasting glucose, weight and diabetes incidence. The aim of the present study was to determine the effect of bisphosphonates on metabolic indices (FPG, Insulin, HOMA–IR, HOMA–B, Matsuda Index) of postmenopausal women with prediabetes and osteopenia. We opted for patients with osteopenia because it is unethical to deprive osteoporotic patients of proven treatment.
Materials and methods

Study design

The present triple-blind randomized controlled trial (RCT) was carried out in the Isfahan Endocrine and Metabolism Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran, between 20 March 2016 and 21 December 2016. The study was approved by Ethics Committee of the Isfahan University of Medical Sciences (code: Ir.mui.rec.1395.3.292) and registered in the Iranian Registry of Clinical Trials (IRCT) (code: IRCT2016101530309N1). This study was performed in accordance with the ethical standards placed in the 1964 Declaration of Helsinki and its later amendments. Among all patients who were referred to the Isfahan Endocrine and Metabolism Research Center, 60 eligible postmenopausal women (amenorrhea for at least 12 months), aged 45–60, with prediabetes and osteopenia (bone densitometry with a T score of −1.5 to −2.4, diagnosed by dual-energy x-ray absorptiometry (DXA, Hologic 2008 model) and normal serum level of vitamin D were recruited (25 OHD>20 ng/ml). Those patients who had renal failure, unattended the follow-up, received corticosteroid therapy or medications that affect carbohydrate or lipid metabolism including metformin, pioglitazone, statins, beta blockers, anti-inflammatory and ACE inhibitors, were excluded from the study. The subjects were under surveillance throughout the entire study and were followed-up carefully. According to the patients’ declarations, they took no additional medications without informing the research team, and patients who had to use other drugs at any time during the study were excluded. The sample size was determined to be 30 patients in each group based on previous studies, considering 10 people for lost to follow up, with α=0.05, and β=80%. Eventually, 40 people were enrolled for the study. The patients’ enrollment diagram is shown in figure 1.
All participants were given an informed consent and randomized into intervention and placebo groups using block randomization method. The intervention group received 70 mg/week alendronate (Dr. Abidi Company, Iran), and the control group received placebo (produced by the Pharmaceutical faculty of the Isfahan University of Medical Sciences in the same size, shape, and color). The Pharmaceutical faculty allocated codes of A and B to these medications and revealed the codes to the research team after completion of the clinical study. One tablet per week for 12 weeks was prescribed for patients to take in the morning while fasting with a glass of water in the non-supine position for at least an hour after taking the tablet. The patients were randomly assigned to treatment arms on a 1:1 basis to receive alendronate or placebo. The research team members who were recording the data, the physicians examined the patients, and the person who was giving medications to the patients with codes A and B according to the randomization block groups, were all blind to the group allocations. A member of the research team contacted subjects every 2 weeks in order to make sure the use of pills by patients, therefore the included subjects that completed the study protocol had complete adherence to the medications. Two blood samples were collected from each participant at the baseline and at the end of the follow-up period; following 8 hours of overnight fasting to measure the fasting plasma glucose, insulin, HbA1c, and 25 hydroxy vitamin D₃ [25(OH) D₃]. The glucose tolerance test was performed by using 75 g glucose, the blood samples were collected before and after 30, 60, and 120 minutes to measure plasma glucose and insulin. Insulin sensitivity, and resistance were calculated from Matsuda index and homeostasis model assessment of IR (HOMA IR), respectively. The Matsuda index was calculated by: 10,000/square root of (fasting glucose [mg/dl] × fasting insulin [mIU/l]) × [mean glucose [mg/dl] × mean insulin [mIU/l] during oral glucose tolerance test (OGTT)] and HOMA- IR was defined as: (fasting glucose [mmol/ml] × fasting
insulin [mIU/l]/22.5. B cell function was measured from HOMA B, defined as: \(20 \times \text{fasting insulin [mIU/l]} / \left(\text{fasting plasma glucose [mmol/ml]} - 3.5\right)^{23}

Plasma glucose levels were measured by Colorimetric Enzymatic Method using Photometric Single-Point Measurement. The insulin levels were measured by Sandwich chemiluminescent immune assay (DIAPLUS insulin–96 test; Siemens, Germany). Inter-assay coefficients of variations (CVs) were 0.9 for glucose and 1.9 for insulin and their intra-assay CVs were 1.5 and 2.53, respectively. HbA1c was measured using Chromatography Ion Exchange approach. Vitamin D (25 OH D) concentration was assessed by direct competitive chemiluminescent immunoassay (IDS, Boldons, UK).

**Statistical Analysis**

The continuous quantitative variables are reported as means and standard errors (SE) or median (interquartile rang (IQR)). Normality of data was evaluated using the Kolmogorov-Smirnov test and Q-Q plot. Log transformation was used for all positive skewed data including HbA1c, HOMA-IR. Independent samples t-test was used for comparing the quantitative baseline data between two groups. In order to detect within-group differences in the biochemical characteristics, paired samples t–test was used and analysis of covariance (ANCOVA) was performed to determine the differences between two groups, adjusted by baseline values. The changes in glucose and insulin concentrations (obtained during the 2–hour OGTT) were evaluated using repeated measures analysis of variance (ANOVA). All experimental data were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 20, SPSS Inc., Chicago, IL, USA).
Results

This study was carried out between 20\(^{th}\) March 2016 and 21\(^{st}\) December 2016 on women with osteopenia and prediabetes states. A total of 20 participants, 10 patients from placebo–treated (poor compliance, n=10; lost to follow up, n=0) and 10 patients from the 70 mg alendronate–treated (poor compliance, n=5; lost to follow up, n=5) group were excluded. Finally, 60 patients (Placebo–treated group, n = 30; 70 mg alendronate–treated group, n = 30) completed the trial (Figure 1). The excluded participants were compared with those who completed the study on available data for HbA1c and FBS, and no significant differences were detected. Baseline characteristics of patients are presented in Table 1. Participants of both groups were similar according to demographic and anthropometric characteristics, serum measurements and laboratory data including 25OH (D)\(_3\) concentration (P > 0.05) at baseline.

A significant decrease was found in FPG, HbA1c and an increase in Matsuda index (P < 0.001), insulin of 120 minutes, and HOMA-IR (P<0.05) in the alendronate–treated group after intervention (Table 2). At the end of the study, the mean of fasting plasma glucose and HbA1c were statistically different between alendronate– and placebo–treated groups. The same outcomes were obtained between two groups following adjustment of baseline values for biochemical characteristics with ANCOVA. We have also observed statistically significant reductions of FPG (-8.2 (8.63) mg/dl vs. -2.5 (14.26) mg/dl, P=0.002) and HbA1c (-0.2 (0.23) % vs. -0.09 (0.26) %, P=0.015) in alendronate-treated group compared to placebo-treated group during the course of the study.

The mean (SE) of plasma glucose and insulin levels during OGTT in the patients before and after intervention (administration of alendronate and placebo) are shown in figures 2 and 3, respectively. The cubic trend was detected and statistically significant differences in plasma glucose over time at all measured points (30, 60, and 120 minutes during the OGTT) were
observed in 70 mg alendronate–treated group. These changes in alendronate treated patients were significantly higher than placebo-treated group \( [F (1,58) = 4.433, \ p–value = 0.04] \). The changes in plasma insulin concentrations of alendronate-treated patients were statistically insignificant over the time points of OGTT (30, 60, and 120 minutes). The mean of plasma glucose and insulin levels remained unchanged in the placebo-treated participants during OGTT.

**Discussion**

The primary aim of the present RCT study was to investigate the effect of alendronate on plasma glucose, insulin levels, sensitivity and resistance indices during an oral glucose tolerance test (OGTT) among osteopenic prediabetic postmenopausal women. We have found an 8.2 mg/dl decrease in FPG and 0.2% in HbA1c among alendronate-treated group, which remained statistically significant after adjustment for confounding variables in the intervention group, whereas the changes were not significant in the control group. These results suggest that 70 mg/week oral alendronate for 12 weeks can significantly improve FPG and HbA1C levels of prediabetes postmenopausal women and might slow down the rate of progression to diabetes.

The comparison of the results of plasma glucose (OGTT) before and after intervention indicated a statistically significant decrease in plasma glucose in 70 mg alendronate–treated group at all intervals (30, 60, and 120 minutes during the OGTT), as well as significant difference with the placebo group. These results are similar to the observational studies indicating lower incidence of T2DM in patients receiving bisphosphonates\(^{17,18}\) and the present RCT confirmed lower plasma glucose levels in OGTT by alendronate. A cohort study in Denmark\(^{18}\) reported reduced developing rate of T2DM in patients taking medications
(alendronate, etidronate, and raloxifene) for osteoporosis with non-diabetic control subjects and noted a dose–dependent risk reduction for alendronate. More than a year of exposure in individuals older than 60 years without diabetes at the baseline to bisphosphonates (including alendronate, risedronate, etidronate, zoledronate, and ibandronate), reduced the risk of DM (OR= 0.52), compared to general matched unexposed individuals in a retrospective population–based study\textsuperscript{17}. A significantly higher incidence of DM (OR=1.21) is also reported in the control group compared to alendronate-treated osteoporotic patients without DM in a retrospective cohort study\textsuperscript{21}. This incidence rate was statistically significant among patients younger than 65 years and those without hypertension or dyslipidemia\textsuperscript{21}. Although the results of previous studies are in agreement with the present report, our group is specifically included prediabetes and postmenopausal women. We have measured FPG, HbA1C, HOMA-IR index, and Matsuda index and compared two randomized groups with similar baseline characteristics, despite healthy individuals who were chosen for the control groups in previous reports\textsuperscript{17,18,21}.

The present study is the first report on evaluating the effect of alendronate on serum glucose and insulin parameters (FPG, insulin, HOMA–IR, HOMA–B, and Matsuda Index) of patients with osteopenia and prediabetes. Animal studies have shown that insulin signaling in osteoblasts promotes glucose metabolism in a bone resorption–dependent manner\textsuperscript{19,20}, prevent insulin resistance and diabetes, and regulate glucose metabolism through alterations in bone metabolism and fat mass\textsuperscript{24}, suggesting bone as an endocrine regulator for glucose metabolism\textsuperscript{25,26}. In addition, the effect of bisphosphonates on early diabetes-associated bone loss has been confirmed in rodent models\textsuperscript{27,28}. Decarboxylation of osteocalcin produced by osteoblasts due to unknown factors, results in its activation. Osteocalcin interacts with insulin receptor of the pancreas B-cells, leading to altered glucose metabolism\textsuperscript{19} and regulate the expression of the insulin genes and cell proliferation markers in rat models\textsuperscript{24}. We anticipate
that alendronate influence glucose metabolism through the above-mentioned mechanisms, as well as disruption of prenylation of small molecular mass G-proteins and pro-inflammatory cytokines or mutations in gastric inhibitory polypeptide receptors\textsuperscript{29,17}. Further evidence on human have reported improved bone health by bisphosphonates in postmenopausal women with T2DM\textsuperscript{30,31}, but could not confirm the effect of bisphosphonates on osteocalcin and insulin metabolism\textsuperscript{32}, which is in agreement with the present study, indicating no significant difference in plasma insulin levels between 70 mg alendronate–treated group and the control group. We have shown that bisphosphonates may prevent progression of prediabetes to DM in postmenopausal patients.

The major strength of the present study includes designing a well-controlled triple-blind clinical trial, which compares two randomized groups with parallel baseline characteristics, while previous studies had mainly evaluated participants in a retrospective design. We have also investigated the serum variables to assess diabetes–related indices (FPG, Insulin, HOMA–IR, HOMA–B, Matsuda Index), among postmenopausal prediabetic women with a high risk of developing DM and osteoporosis, while previous reports studied the general population.

On the other hand, the present study had some limitations, including the small sample size in each group, which could explain the non-significant differences between groups. We detected no significant differences between available characteristics of excluded patients compared to those who completed the study, however, this comparison was made based on limited data. Although 30 subjects are statistically sufficient to achieve reliable results, it is suggested that future multicentric studies address the same population with a larger sample size.

This article is protected by copyright. All rights reserved.
Administration of 70 mg/week alendronate has significantly reduced FPG, HbA1C, HOMA-IR index, and increased Matsuda index in postmenopausal women with prediabetes and osteopenia, while there was significant difference only in terms of FPG and HbA1c between the two groups at the end of study. This indicates positive effect of alendronate on diabetes related indices, specifically FPG and HbA1c.

Acknowledgements
The authors would like to thank Dr. Massoud Taheri, Radiologist, for his cooperation in encouraging the patients to participate the study, and Dr. Sayed abolfazl mostafavi, pharmacist, for producing the placebo tablets.

Funding: This research was supported by Isfahan Endocrine and Metabolism Research Center.

Compliance with ethical standards
Conflict of interest: The authors declare they have no conflict of interest.

Ethical approval: The study conforms to the principles outlined in the Declaration of Helsinki.

Informed consent: Prior to participation, all participants provided written informed consent to participate in the study and the study was approved by Ethics Committee of the Isfahan University of Medical Sciences (code: Ir.mui.rec.1395.3.292) and registered in the Iranian Registry of Clinical Trials (IRCT) (code: IRCT2016101530309N1).

Disclosure

This research was done in Isfahan Endocrine and Metabolism Research Center and it did not have a sponsor.

References

11. Kanis JA. Assessment of osteoporosis at the primary health care level. WHO Collaborating Centre for Metabolic Bone Diseases. WHO Collaborating Centre for Metabolic Bone Diseases. 2007.


A list of supporting information

Appendix S1: CONSORT 2010 check list of information to include when reporting a randomized trial

Figure legends

Figure 1. Consort Flow diagram for enrollment of participants in the study

Figure 2. The effect of Alendronate and placebo on plasma glucose during oral glucose tolerance test (OGGT) before and after intervention in both groups

*P–value < 0.05 in comparison between before and after treatment by 70 mg Alendronate (cubic–trend) at all measured time points

Figure 3. The effect of Alendronate and placebo on plasma insulin levels during oral glucose tolerance test (OGGT) before and after intervention in both groups
Table 1. Baseline characteristics of 70 mg Alendronate–and Placebo–treated patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Alendronate treated (n=30)</th>
<th>Placebo treated (n=30)</th>
<th>P–value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.5(6.3)</td>
<td>55.3(4.0)</td>
<td>0.406</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.33(1.07)</td>
<td>161.80(1.10)</td>
<td>0.344</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.5(9.3)</td>
<td>68.3(9.4)</td>
<td>0.912</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>26.6(3.1)</td>
<td>26.0(3.0)</td>
<td>0.449</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.0(9.5)</td>
<td>81.5(10.9)</td>
<td>0.346</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>129.7(14.0)</td>
<td>128.3(15.8)</td>
<td>0.731</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>69.4(18.1)</td>
<td>71.0(18.9)</td>
<td>0.728</td>
</tr>
</tbody>
</table>

*Resulted from Independent sample t-test
Table 2. Serum measurements before and 12 weeks after intervention in study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Alendronate treated (n=30)</th>
<th>Placebo treated (n=30)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>P*</td>
</tr>
<tr>
<td><strong>FPG</strong>&lt;sup&gt;b&lt;/sup&gt; (mg/dl)</td>
<td>102.4(1.46) [101.5(96.8-107.2)]</td>
<td>94.2(1.17) [95(90.5-98.2)]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FPI</strong>&lt;sup&gt;a&lt;/sup&gt; (mU/l)</td>
<td>13.87(1.60) [13.2(12.7-14.4)]</td>
<td>11.3(1.05) [11.1(10.5-11.7)]</td>
<td>0.065</td>
</tr>
<tr>
<td>Hb A&lt;sub&gt;1&lt;/sub&gt;C&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>5.60(0.06) [5.55(5.4-5.8)]</td>
<td>5.40 (0.05) [5.35(5.3-5.5)]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>25OH(D)&lt;sub&gt;3&lt;/sub&gt;</strong> (ng/ml)</td>
<td>80.47(9.06) [79.7(78.4-91.7)]</td>
<td>85.17(7.46) [84.5(82.8-86.8)]</td>
<td>0.433</td>
</tr>
<tr>
<td>HOMA–IR</td>
<td>3.57(0.45) [3.39(3.3-3.6)]</td>
<td>2.62(0.24) [2.55(2.3-2.8)]</td>
<td>0.021</td>
</tr>
<tr>
<td>HOMA–B</td>
<td>124.30(12.50) [122.6(11.8-13.2)]</td>
<td>136.3(15.05) [134.6(12.9-13.8)]</td>
<td>0.353</td>
</tr>
<tr>
<td>AUC (glucose)</td>
<td>927.72(47.49) [921.2(915.0-934.3)]</td>
<td>864.86(28.64) [858.6(851.2-862.0)]</td>
<td>0.113</td>
</tr>
<tr>
<td>AUC (insulin)</td>
<td>488.72(75.6) [483.9(73.7-77.4)]</td>
<td>474.24(2.7) [469.8(2.5-2.9)]</td>
<td>0.144</td>
</tr>
<tr>
<td>Matsuda Index</td>
<td>7.7(0.41) [7.57(7.17-7.93)]</td>
<td>9.2(0.41) [9.0(8.7-9.5)]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** BMI, body mass index; AUC, area under curve; 25OH(D)<sub>3</sub>, 25 hydroxy vitamin D3; HOMA–IR, homeostasis model assessment of insulin resistance; HOMA–B, homeostasis model assessment of beta–cell function

Values are shown as means and standard error (SE) and [median(Interquartile rang(IQR)];* Obtained from paired samples test; ** Obtained from ANCOVA after adjusting baseline values.

<sup>a</sup>: Fasting Plasma insulin
<sup>b</sup>: Fasting plasma glucose
<sup>c</sup>: Hemoglobin A1C
Osteopenic patients (n=500)

Excluded (n=420)
- Not meeting inclusion criteria (n=420):
  - Age < 45 years or ≥ 65 years (n=143)
  - Medication affecting carbohydrate or lipid metabolism (n=85)
  - Did not attend screening (n=128)
  - Women not menopause (n=49)
  - Renal failure (n=2)
  - Prednisolone user (n=13)

Randomized (n=80)

Allocation

Allocated to intervention group (n=40)
- Received allocated intervention (n=40)

Allocated to control group (n=40)
- Received allocated intervention (n=40)

Follow-up

Lost to follow-up (did not refer) (n=0)

Discontinued intervention (gastro-intestinal and musculoskeletal adverse effects) (n=10)

Lost to follow-up (did not refer) (n=5)

Discontinued intervention (gastro-intestinal and musculoskeletal adverse effects) (n=5)

Analysis

Analysed (n=30)
- Excluded from analysis (n=0)

Analysed (n=30)
- Excluded from analysis (n=0)