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ORIGINAL ARTICLE

Effects of pioglitazone treatment on blood leptin levels in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: The aim of the present study was to carry out a meta-analysis of randomized controlled trials (RCTs) that investigated the effects of pioglitazone on blood leptin levels in patients with type 2 diabetes.

Materials and Methods: Literature searches were carried out using Medline, the Cochrane Controlled Trials Registry and ClinicalTrials.gov, and RCTs that investigated the effects of pioglitazone on blood leptin levels in patients with type 2 diabetes were selected. Standardized mean differences and 95% confidence intervals were calculated.

Results: A total of 10 RCTs met the eligibility criteria and were included in the meta-analysis. Significantly lower blood leptin levels were observed in the pioglitazone group (standardized mean difference −0.58, 95% confidence interval −1.12 to −0.05%, $P = 0.03$) than in the placebo group. There was no significant difference in blood leptin levels observed between the pioglitazone and oral antidiabetic drug groups (standardized mean difference −0.01, 95% confidence interval −0.20 to 0.19%, $P = 0.93$).

Conclusions: There was a significant difference in blood leptin levels between the pioglitazone and placebo groups. However, relatively few RCTs were included in the study, and there was a high level of statistical heterogeneity; we believe that this could have affected the results.

INTRODUCTION

The association between type 2 diabetes and the future onset of cardiovascular diseases or cardiovascular deaths has been proven.[1–3] Therefore, the prevention of cardiovascular diseases is one of the essential purposes of treatment in patients with type 2 diabetes. Biomarkers that have been pointed out as having an association with cardiovascular disease onset in type 2 diabetes patients include inflammatory cytokines, oxidant stress and coagulation factors.[4–6] Furthermore, another study has reported on a possible relationship between blood leptin levels, which is a hormone that adjusts appetite, and the onset of cardiovascular diseases.[7] Leptin is secreted from adipose tissue, and has effects on the hypothalamus to control food intake.[8] In type 2 diabetes, hyperleptinemia might be a predictive marker of future cardiovascular disease.

Pioglitazone, which improves insulin resistance, is used in the pharmacotherapy of patients with type 2 diabetes. Past research has reported on a meta-analysis studying the effects of pioglitazone use on the aforementioned biomarkers.[9] However, although past studies have reported on randomized controlled trials (RCTs) to study the effects of pioglitazone administration on blood leptin levels, the results have not been consistent. Therefore, the purpose of the present study was to evaluate the effects of pioglitazone on blood leptin levels in patients with type 2 diabetes using meta-analysis.

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METHODS

Study selection

A literature search was carried out using Medline, the Cochrane Controlled Trials Registry and ClinicalTrials.gov (1 July 2016). We used the search strategy of "[thiazolidinediones or pioglitazone] AND [diabetes mellitus or diabetes or NIDDM or non-insulin-dependent or type 2 diabetes mellitus]." We decided to carry out a meta-analysis to measure blood leptin levels under the effect of pioglitazone administration in type 2 diabetes. These studies consisted of those that compared pioglitazone with a placebo or another oral antidiabetic drug (OAD), regardless of diet or exercise therapy. However, in the case of a cross-over trial, we decided to use only the first phase to avoid the carryover effect. Criteria for elimination were insufficient data for meta-analysis, animal tests, gestational diabetes mellitus and overlapping articles. Two authors (SI and RK)

independently evaluated whether each article passed the eligibility criteria of the present study. Any disagreements were resolved by consultation with a third reviewer (KM).

Data extraction and quality assessment

We created a data extraction form to document the characteristics of each study analyzed in this research (i.e., key author's name, publication year, study location, sample size, patient baseline information, basic treatment and treatment duration). We used the mean, standard deviation, standard error and 95% confidence interval (CI) values to describe the outcome (blood leptin levels). If trials compared multiple intervention groups with a single control group within one comparison, the shared control group was split into two groups. Furthermore, two authors (SI and RK) independently used the following criteria to evaluate the quality of the studies analyzed in this research[10–12]: (i) random sequence generation: A = adequate (e.g., referring to a random number table, using a computer random number generator) and B = inadequate or unclear (e.g., date of birth, some rule according to hospital or clinic record number, incompletely described); (ii) allocation concealment: A = adequate (e.g., central allocation, sealed envelopes) and B = inadequate or unclear (e.g., an open allocation schedule, date of birth, incompletely described); (iii) blinding: A = adequate (e.g., blinding of participants and key study personnel ensured), B = exact method unclear and C = non-blinded, inadequate or unclear; and (iv) incomplete outcome data (dropout): A = overall dropout rate of <15% and B = overall dropout rate of >15%, or unclear. We grouped the quality of the studies analyzed in this research under the following three categories: A = low risk of bias, B = moderate risk of bias or C = high risk of bias. Any disagreements were resolved by consultation with a third reviewer (KM).

Statistical analysis

In the present research, we divided the study into two parts: (i) comparison of pioglitazone with a placebo; and (ii) comparison of pioglitazone with another OAD (e.g., metformin, sulfonylurea, α-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors and glinide) to study their respective effects on blood leptin levels. Blood leptin level is a continuous variable and is shown in different units depending on individual studies; therefore, we used standardized mean difference (SMD) and 95% CI for analysis. Treatment efficacy was defined as the difference between groups in the degree of change in blood leptin levels before and after treatment. In cases where data regarding blood leptin levels before treatment were unavailable, treatment efficacy was defined as the intergroup difference in blood leptin levels at the end-point. When only the standard error or *P*-values were reported, standard deviations were calculated according to Altman and Bland.[13] In the absence of supplemental data, standard deviations were calculated from CIs, *t*-values or *P*-values, as described in the Cochrane Handbook.[10] We used the random effects model for analysis, and used I^2 to evaluate statistical heterogeneity. Furthermore, we used the funnel plot to evaluate publication bias. Meta-analyses were carried out using RevMan version 5 (Cochrane Collaboration, http://tech.cochrane.org/revman/download, July 2016). We used the following methods for sensitivity analysis: (i) evaluating with a fixed effect model rather than a random effects model; and (ii) evaluating with low-quality studies removed from the research.

RESULTS

From the literature search, 2,752 reports were extracted, of which 10 RCTs met the eligibility criteria and were included in the meta-analysis (Figure 1).[14–23] The characteristics of the nine RCTs are summarized in Table 1. Participants in controlled trials comparing the pioglitazone and placebo groups had a mean age of 54.8 years, with women accounting for 31.8%. The mean diabetes duration was 5.0 years, and the mean trial duration was 21.0 weeks. Participants in controlled trials comparing the pioglitazone-treated and OAD-treated groups had a mean age of 53.7 years, with women accounting for 48.0%. The mean diabetes duration in these trials was 14.5 years, and the mean trial duration was 16.0 weeks.

Association of pioglitazone with blood levels of leptin

Pioglitazone vs placebo

Five trials compared the pioglitazone and placebo groups.[14–18] The pooled numbers of participants from the pioglitazone and placebo groups were 85 and 85, respectively. Statistical heterogeneity was defined as I^2 = 64% (P = 0.02), and heterogeneity was observed. The pioglitazone group had significantly lower blood leptin levels (SMD −0.58, 95% CI −1.12 to −0.05%, P = 0.03; Figure 2) than the placebo group. Sensitivity analysis using a fixed effect model showed that the pioglitazone group had significantly lower blood leptin levels (SMD −0.64, 95% CI −0.95 to −0.32%, P < 0.001; Figure 3) than the placebo group. However, when low-quality studies[14–17] were removed, there remained only one study in the sensitivity analysis[18] in which there was no significant difference in blood leptin levels observed between the pioglitazone and placebo groups (SMD −0.43, 95% CI −1.10 to 0.24%, P = 0.21; Figure 4).

Pioglitazone vs other OADs (metformin, sulfonylurea or α-glucosidase inhibitors)

There were six trials comparing pioglitazone with OADs (metformin, sulfonylurea or α-glucosidase inhibitors).[16,19–23] The pooled numbers of participants in the pioglitazone and OAD groups were 210 and 203, respectively. Statistical heterogeneity was defined as I^2 = 0% (P = 0.48), and no heterogeneity was observed. There was no significant difference in blood leptin levels observed between the pioglitazone and OAD groups (SMD -0.01, 95% CI −0.20 to 0.19%, P = 0.93; Figure 5). Sensitivity analysis using a fixed effect model showed no significant difference in blood leptin levels between the pioglitazone and OAD groups (SMD -0.01, 95% CI −0.20 to 0.19%, P = 0.93; Figure 6). In the sensitivity analysis carried out with two

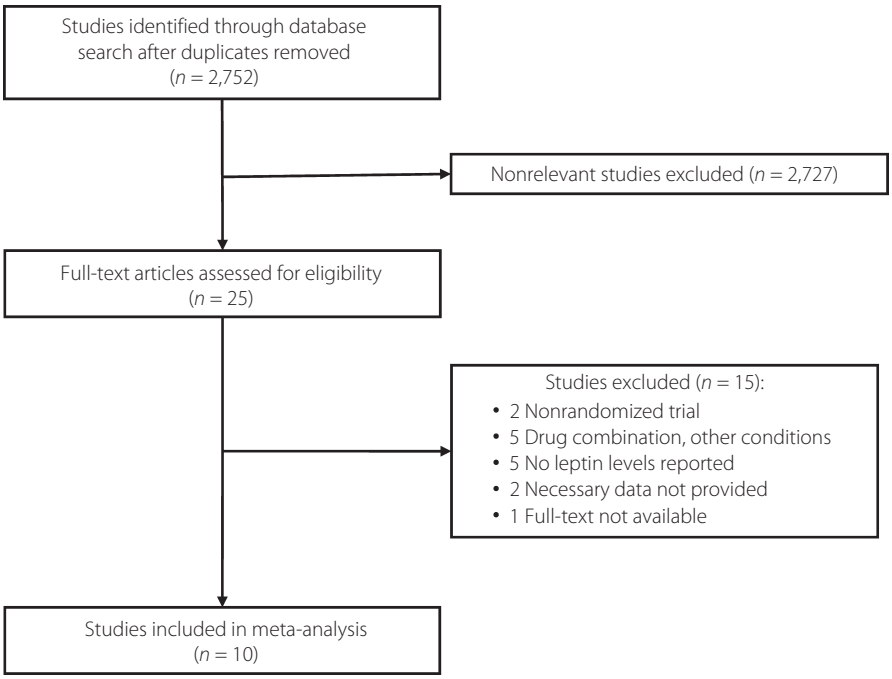

Figure 1 | Study flow diagram.

studies,[19,23] excluding low-quality studies,[16,20–22] there was no significant difference in blood leptin levels between the pioglitazone and OAD groups (SMD −0.03, 95% CI −0.33 to 0.28%, $P = 0.87$; Figure 7).

Evaluation of quality and publication bias

The studies included in the meta-analysis had many qualitative problems. Few studies adequately masked the participants and evaluators, and many studies had a high risk of bias.[15–17,20–22] Overall, there was only one report with a low risk of bias.[23] Funnel plot analyses were not carried out because <10 RCTs were included in the various analyses.[10]

DISCUSSION

The present study showed that the pioglitazone group had significantly lower blood leptin levels than the placebo group. However, there was no significant difference in blood leptin levels between the pioglitazone and OAD groups. Sensitivity analysis (changing to a fixed effect model and excluding low-quality RCTs) showed similar results.

Leptin is a hormone secreted by adipose tissue that circulates in the blood, and suppresses food intake by acting on the hypothalamus to stimulate the satiety center.[8,24] The occurrence of leptin resistance has been highlighted in patients with obesity or diabetes, and in these cases, the effect of leptin decreases.[24] It is believed that leptin resistance can arise from poor leptin transportation in the central nervous system[25] and leptin receptor signal impairment.[26] It is expected that a decrease in leptin activity could promote obesity. It has been reported that in leptin resistance, hyperleptinemia can promote arteriosclerosis.[27] Leptin resistance is associated with metabolic syndrome, as well as insulin resistance (both are cardiovascular risk factors),[28] and it is an independent risk factor for cardiovascular events.[7] In patients with diabetes or obesity, it is believed that hyperleptinemia leads to various poor outcomes.

Pioglitazone acts on the nuclear receptor, peroxisome proliferator-activated receptor-γ, and decreases blood free fatty acids, thereby decreasing fatty acid supply to target organs of insulin, such as skeletal muscles and the liver, and improving insulin resistance. Furthermore, it exerts a hypoglycemic effect by increasing adiponectin, a lipolytic adipokine, and improves insulin sensitivity in peripheral tissues.[29,30] The mechanism underlying the effect of pioglitazone on leptin remains largely unclear. However, it has been reported that the administration of thiazolidinediones decreases leptin gene expression[31–33] and decreases leptin levels in cultured adipocytes.[34] Furthermore, as aforementioned, decreased leptin responsiveness occurs in obesity, because of which enlarged mast cells are not decreased in size; this is another possible mechanism for the attenuated reduction of leptin secretion.[24] It has been reported that glitazones act on adipocytes and promote the downsizing of adipocytes,[35,36] and it is believed that this could affect the drop in leptin levels. In the present study, compared with the placebo group, blood leptin levels in the pioglitazone-treated group were found to be significantly lower; this is consistent with the results of the aforementioned studies. However, the majority of the RCTs included in the meta-analyses had a small sample size, and few RCTs were included. Furthermore, there was a

Table 1 | Characteristics of the pioglitazone interventions included in the present meta-analysis compared with control or other oral antidiabetic drugs

Reference	Year	Region	No. patients	Age (years)	% Women	BMI (kg/m²)	Bodyweight (kg)	Duration of DM (years)	HbA1c (%)	Comparison	Pioglitazone dose (mg/day)	Basic treatment	Duration (weeks)	Leptin (ng/mL)
Pioglitazone vs control														
Tonelli *et al.*[14]	2004	US	9	47	22.2	33.5	NR	NR	10.3	Pioglitazone vs placebo	45	Diet or oral hypoglycemic agents or insulin	21	NR
Nishio *et al.*[15]	2006	Japan	54	66.2	26.9	24.6	NR	NR	7.7	Pioglitazone vs placebo	30	Oral hypoglycemic agents	24	15.6
Jacob *et al.*[16]	2007	US	39	44	27.7	30.7	88.1	5	11.2	Insulin monotherapy vs pioglitazone + insulin	30	Insulin	24	10
Oz O *et al.*[17]	2008	Turkey	24	55.2	48.5	29.1	NR	NR	6.9	Pioglitazone vs placebo	30	Diet	12	17.1
Veleba *et al.*[18]	2015	Czech	35	62	34	32	94	NR	7	Pioglitazone vs placebo	15	Metformin	24	12.6 (median)
Pioglitazone vs other OADs														
Sharma *et al.*[19]	2006	India	30	50.8	40	27.9	71.9	NR	7.7	Pioglitazone vs metformin	30	Diet	12	23.9
Shimizu *et al.*[20]	2006	Japan	30	61.6	63.3	NR	58.1	NR	8.4	Pioglitazone vs voglibose	30	Diet or oral hypoglycemic agents or insulin	12	487.5 (pmol/L)
Jacob *et al.*[16]	2007	US	36	44	27.7	30.7	88.1	5	11.2	Pioglitazone + insulin vs metformin + insulin	30	Insulin	24	10
Taslimi *et al.*[21]	2013	Iran	60	51	50	28.7	76.8	24	8.4	Pioglitazone vs metformin	1000	Diet + exercise	12	9.8
Esteghamati *et al.*[22]	2013	Iran	91	52.3	54.9	29.1	NR	NR	7.9	Pioglitazone vs metformin	30	None	12	19.2 (μg/L)
Maffioli *et al.*[23]	2013	Italy	170	62.8	52.3	30.3	83.5	NR	8.6	Pioglitazone vs glibenclamide	30	Metformin	24	31.6

Unless indicated otherwise, data are shown as mean values. Oral antidiabetic drugs (OADs) include metformin, sulfonylureas or α-glucosidase inhibitors. Leptin levels were determined by radioimmunoassay or enzyme-linked sorbent assay. BMI, body mass index; DM, type 2 diabetes mellitus; HbA1c, hemoglobin A1c; NR, not reported.

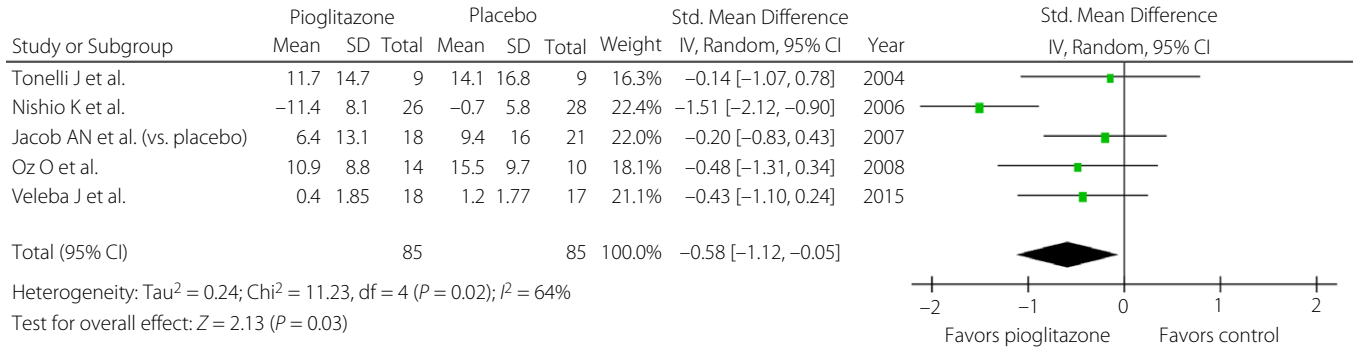

Figure 2 | Forest plot presenting the meta-analysis based on standardized mean differences (SMDs) for the effect of pioglitazone vs placebo on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence intervals (CIs) presented as extending lines. The pooled standardized mean differences with its 95% CI is shown as a diamond.

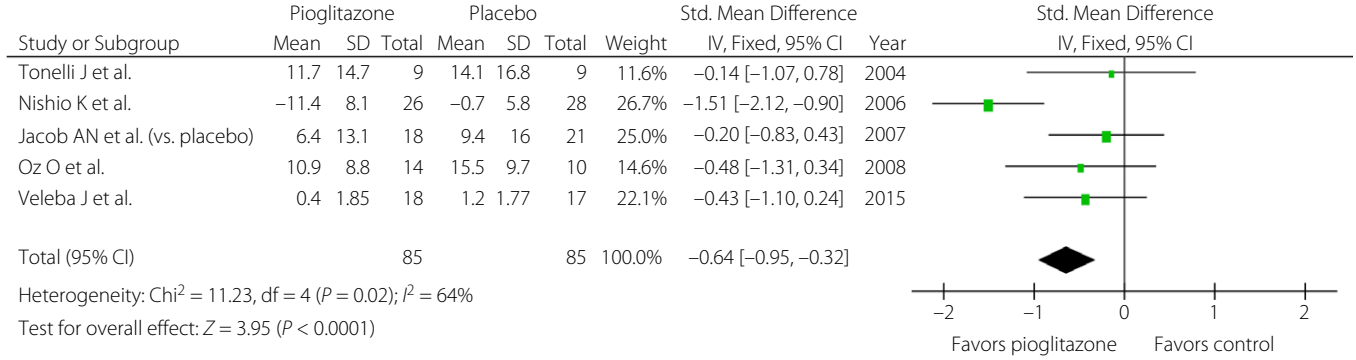

Figure 3 | Sensitivity analysis using a fixed effect model. Forest plot presenting the meta-analysis based on standardized mean differences for the effect of pioglitazone vs placebo on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence intervals (CIs) presented as extending lines. The pooled standardized mean difference with its 95% CI is shown as a diamond.

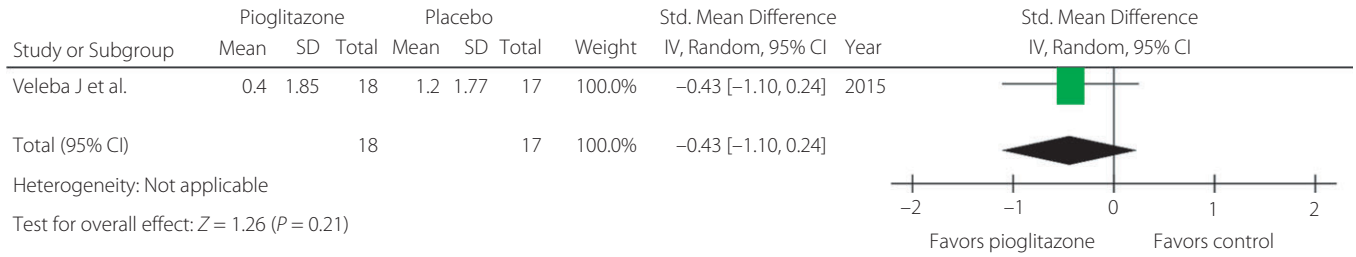

Figure 4 | Sensitivity analysis excluding the low-quality studies. Forest plot presenting the meta-analysis based on standardized mean differences for the effect of pioglitazone vs placebo on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence intervals (CIs) presented as extending lines. The pooled standardized mean difference with its 95% CI is shown as a diamond.

high level of statistical heterogeneity and low-quality studies were also included, which could have affected the validity of the results. Moreover, in most RCTs, leptin levels increased from baseline in the pioglitazone-administrated group. Therefore, we believe that pioglitazone might impede the increase in leptin levels.

There were no statistically significant differences in leptin levels between the pioglitazone and OAD groups. In most RCTs included in the present analysis, metformin was used in the OAD group. It has been reported that the administration of metformin decreases leptin levels,[22] and the underlying mechanism is thought to involve improved insulin

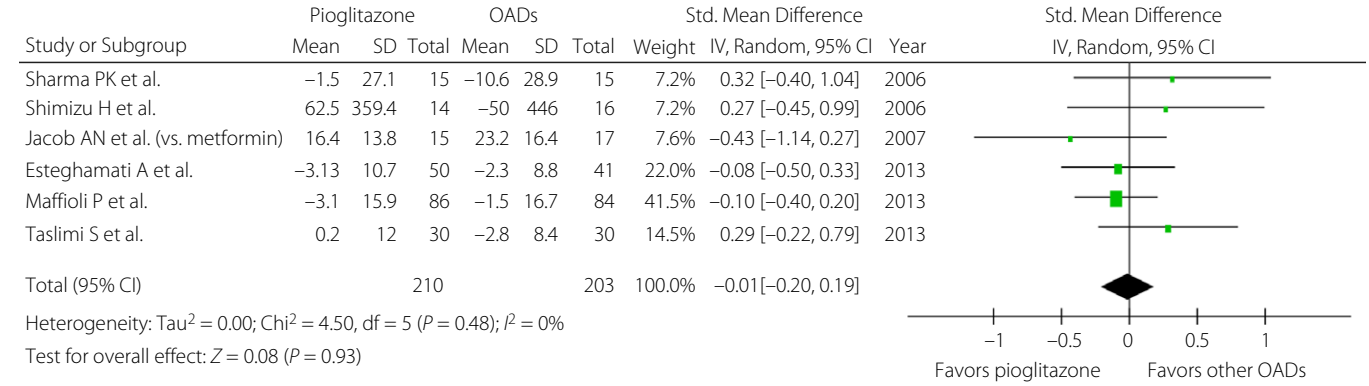

Figure 5 | Forest plot presenting the meta-analysis based on standardized mean differences (SMDs) for the effect of pioglitazone vs oral antidiabetic drugs (OADs [metformin, sulfonylurea or α-glucosidase inhibitors]) on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence interval (CIs) presented as extending lines. The pooled standardized mean difference with its 95% CI is shown as a diamond.

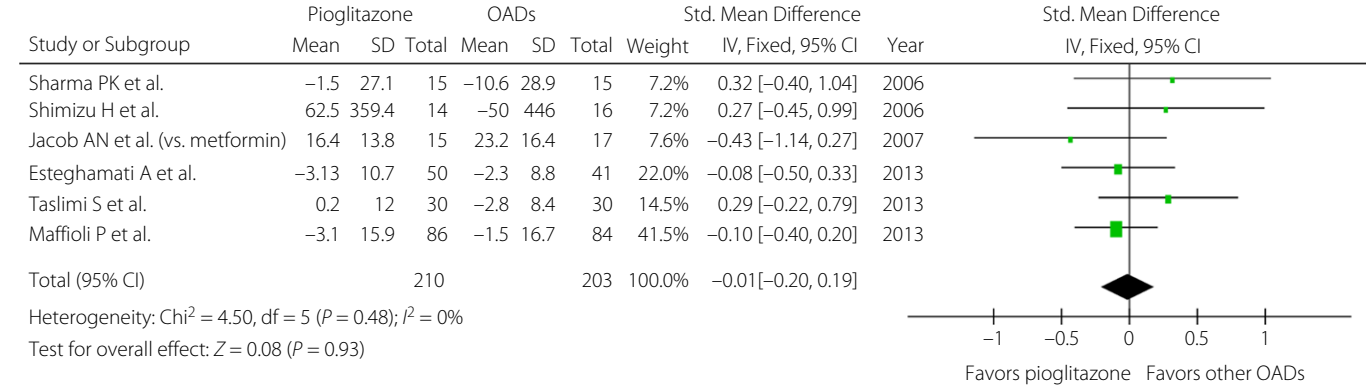

Figure 6 | Sensitivity analysis using a fixed effect model. Forest plot presenting the meta-analysis based on standardized mean differences for the effect of pioglitazone vs oral antidiabetic drugs (OADs [metformin, sulfonylurea or α-glucosidase inhibitors]) on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence intervals (CIs) presented as extending lines. The pooled standardized mean difference with its 95% CI is shown as a diamond.

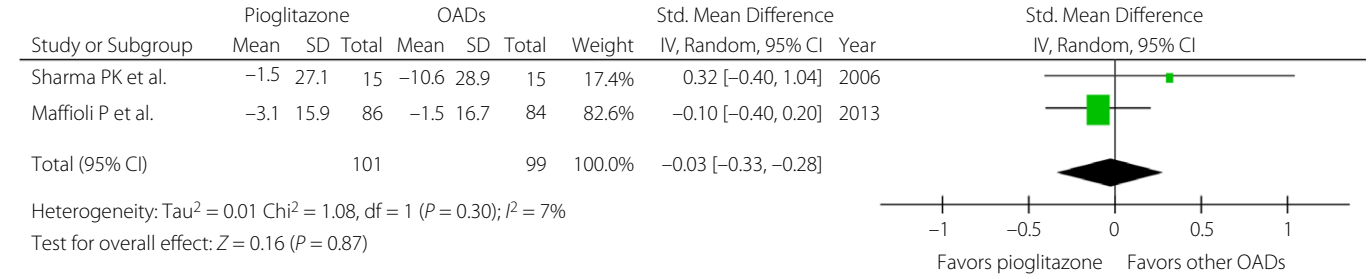

Figure 7 | Sensitivity analysis excluding the low-quality studies. Forest plot presenting the meta-analysis based on standardized mean differences for the effect of pioglitazone vs oral antidiabetic drugs (OADs [metformin, sulfonylurea or α-glucosidase inhibitors]) on leptin. Standardized mean differences in the individual studies are presented as squares with 95% confidence intervals (CIs) presented as extending lines. The pooled standardized mean difference with its 95% CI is shown as a diamond.

resistance[37,38] because of increases in adenosine monosphosphate-activated protein kinase in the central nervous system.[39,40] It has also been suggested that the effect of pioglitazone on blood leptin could differ according to sex.[22] The present study examined men and women combined, and we believe that in comparing the pioglitazone and OAD groups, the effect of pioglitazone on blood leptin could have been masked.

Blood leptin level was a surrogate outcome in the present study. It has been suggested that pioglitazone therapy could decrease the cardiovascular and mortality risks, which are hard end-points.[41,42] To clarify the complex relationship of pioglitazone therapy with these hard end-points because of the change in blood leptin levels, we believe that further studies are required.

The present study had several limitations. First, as aforementioned, there were relatively few RCTs included in the study; therefore, detectability could have been small because of a lack of power. Although a statistically significant difference was observed on comparing the pioglitazone and placebo groups, there was no significant difference in the comparison between the pioglitazone and OAD groups; this is probably because of the aforementioned small sample size. Second, there could have been a publishing bias. Some studies might not have been published, because of which the effect of pioglitazone on leptin could have been overestimated or underestimated. Third, the high level of conceptual heterogeneity could have affected the results. In the RCTs included in the study, there were substantial differences among studies with regard to diabetes duration, age and duration of pioglitazone administration. Consequently, caution should be exercised with the interpretation and generalization of the results. Finally, as mentioned above, a relatively large proportion of analyzed studies were of low quality, and there was a high level of statistical heterogeneity. This could have affected the results.

The present study showed that the pioglitazone-administered group had significantly lower blood leptin levels than the placebo group in patients with type 2 diabetes. However, there was no statistically significant difference observed when compared with the patients treated with OADs. However, the present study had many limitations, warranting caution for the interpretation of its results and the extrapolation of the findings to other populations. Considering the limitations noted above, we believe that further studies are necessary.

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DISCLOSURE

The authors declare no conflict of interest.

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