

# Gender differences in the efficacy of pioglitazone treatment in nonalcoholic fatty liver disease patients with abnormal glucose metabolism

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## Research

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# Abstract

**Background :** Pioglitazone is a promising therapeutic method for nonalcoholic steatohepatitis patients with or without type 2 diabetes. However, there is a remarkable variability in treatment response. We analyzed our previous randomized controlled trial to examine the effects of gender and other factors on the efficacy of pioglitazone treatment in liver fat content in Chinese nonalcoholic fatty liver disease (NAFLD) patients with abnormal glucose metabolism.

**Methods :** This is a secondary post hoc analysis of a previous randomized, parallel controlled, open-label clinical trial (RCT)\* with an original purpose of evaluating the efficacy of berberine and pioglitazone on NAFLD. The per protocol population (n= 184) was randomly divided into three groups: lifestyle intervention (LSI), LSI plus pioglitazone (PGZ) 15mg qd, and LSI plus BBR 0.5g tid, respectively, for 16 weeks. Proton magnetic resonance spectroscopy ( <sup>1</sup> H MRS) was used to assess liver fat content.

**Results :** As compared with LSI, PGZ plus LSI treatment induced further decreased liver fat content in women [-8.26% (-17.18%, -0.65%), p = 0.025], but relatively increased liver fat content in men [9.79% (0.37%, 19.21%), p = 0.046]. There was a significant interaction between gender and efficacy of pioglitazone before ( p = 0.003) and after ( p = 0.011) adjustment for age, smoking, drinking, baseline BMI, BMI change, and treatment adherence.

**Conclusion :** For Chinese NAFLD patients with abnormal glucose metabolism, pioglitazone treatment is recommended for women, but not for men, based on lifestyle interventions.

\* Trial registration: Role of Pioglitazone and Berberine in Treatment of Non-Alcoholic Fatty Liver Disease, NCT00633282. Registered 3 March 2008, <https://register.clinicaltrials.gov>.

## Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of diseases including hepatic steatosis, steatohepatitis, and liver fibrosis. The global incidence of NAFLD is increasing rapidly, and will probably emerge as the leading cause of chronic liver disease among patients with obesity, prediabetes or type 2 diabetes (T2DM) [1]. As NAFLD is closely related to metabolic abnormalities, some experts suggested that "Metabolic associated fatty liver disease (MAFLD)" was used to replace the name of NAFLD [2]. NAFLD patients with T2DM are at an increased risk of progressive liver diseases, as well as extra-hepatic complications including cardiovascular events [3]. Currently, the treatment of NAFLD is mainly lifestyle interventions, and there is no recognized drug with expected efficacy for clinical use. Most of the therapeutic drugs in phase 2b and phase 3 clinical trials did not or barely meet the anticipated liver histological endpoint. Some studies have shown that pioglitazone, vitamin E, liraglutide and Obeticholic acid might be promising drugs [4, 5]. However, vitamin E and Obeticholic acid have some adverse effects such as lipid metabolism disorders, skin itching and their safety of long-term use have not been confirmed [6, 7]. As to liraglutide, requiring injection and with potential risks for pancreatitis and medullary

thyroid cancer<sup>[5]</sup>, its clinical application is limited. Pioglitazone, as a commonly used clinical hypoglycemic drug, has a broader prospect<sup>[8]</sup>.

Pioglitazone, belonging to a class of drugs known as thiazolidinediones (TZDs), was associated with significant histologic improvement in terms of steatosis, inflammation, NAFLD activity score, resolution of NASH and fibrosis in Western NASH patients with or without T2DM<sup>[9, 10]</sup>. Benefits have also been reported in Asian populations<sup>[11, 12]</sup>. Our previous research also showed a significant decrease in liver fat content (LFC) with pioglitazone treatment in NAFLD patients with abnormal glucose metabolism<sup>[13]</sup>. Based on current evidence, 2017 AASLD guidelines proposed that pioglitazone could be used to treat biopsy-proven NASH patients, but it was not recommended for the treatment of common NAFLD patients for its efficacy and safety having not been widely proven<sup>[14]</sup>. Although some studies showed that pioglitazone was effective for NASH, histological improvement of liver did not happen in all patients. For example, only 47% of the patients achieved the primary outcome in the PIVENS trial<sup>[9]</sup>. Therefore, in addition to exploring and developing new NASH drugs, identifying possible factors related to drug response variability is also a feasible direction to treat NAFLD.

Some studies have demonstrated that the baseline NAFLD activity score and exposure index could partly explain the differences in drug response of pioglitazone<sup>[15]</sup>. Elevated levels of plasma adiponectin and endotrophin also might be predictors of the response variation<sup>[16, 17]</sup>. In addition to the efficacy differences of pioglitazone in fatty liver, the efficacy in lowering blood glucose was also affected by some factors. For instance, gender was a major factor affecting the hypoglycemic efficacy of pioglitazone. Among patients with T2DM treated by pioglitazone, women were more likely to respond to treatment (81.8% vs. 30.8%,  $p = 0.0004$ )<sup>[18]</sup>; and had even greater reductions in HbA1c than men<sup>[19]</sup>. Whether there is a gender difference in the efficacy of pioglitazone in treatment of fatty liver has been poorly addressed. The PIVENS study had conducted a subgroup analysis, but gender differences of pioglitazone in liver histology were not found<sup>[9]</sup>. Therefore, in this post hoc analysis, we evaluated the possible influencing factors of pioglitazone's efficacy in the treatment of Chinese NAFLD patients, especially the gender-based difference.

## Materials And Methods

### Study design

The data come from a total of 185 NAFLD patients with impaired glucose regulation (IGR) or T2DM, who participated in a clinical trial (NCT00633282) in department of endocrinology, Zhongshan Hospital, Fudan University from 2008 to 2012, which is a randomized, parallel controlled, open-label clinical trial with three-arm. A detailed description of the RCT has previously been published<sup>[13]</sup>. Briefly, participants were divided into three groups: control group (lifestyle intervention (LSI)), pioglitazone group (LSI + pioglitazone 15mg qd), and berberine group (LSI + berberine 0.5g tid). The treatment lasted for 16 weeks. Safety related events, adherence, pill counts and the serum samples were collected. Inclusion criteria

including: Age was 18-70 years; fatty liver diagnosed by ultrasound; fasting blood glucose  $\geq 5.6$ mmol / L and or 2 hours blood glucose  $\geq 7.8$ mmol / L. Exclusion criteria were: those who have already used hypoglycemic drugs; those with poor glycemic control (HbA1c  $\geq 9.5\%$ ); those who are pregnant, breastfeeding or have severe illness. This trial was approved by the Ethics Committee of Zhongshan Hospital, Fudan University.

### Measurement of liver fat content using $^1\text{H-MRS}$

LFCs were detected by proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) using a 1.5T magnetic resonance (MR) scanner (Siemens Avanto, Erlangen, Germany) equipped for proton spectroscopy acquisitions. Sagittal, coronal, and axial slices covering the whole liver were preliminarily acquired for positioning of the spectroscopy acquisition voxel. Signal intensities of water peak at 4.8ppm (Sw) and the fat peak at 1.4ppm (Sf) were measured and hepatic fat percentage was calculated using the formula  $100 \times \text{Sf} / (\text{Sf} + \text{Sw})$ . The details have been described in the published study <sup>[13]</sup>.

### Statistical methods

R software 3.4.3 was used for statistical analysis. Quantitative data were expressed as mean  $\pm$  SD, or median with the range as required. Categorical variables were expressed as frequency (or percentage). Kolmogorov-Smirnov test was carried out to determine the normality of the continuous variables. Differences between males and females were assessed using the unpaired Student's t test or Mann-Whitney U test for quantitative variables, and the test or Fisher's exact test for qualitative variables. Differences in LFC changes between groups before and after the intervention were expressed as partial regression coefficients (95% confidence interval). Interaction tests were included in the linear regression model as the product of gender and study grouping, and was assessed by Wald test. Model 1 was not adjusted. Model 2 was adjusted for age, smoking, drinking, baseline BMI, BMI changes, and treatment adherence.  $p < 0.05$  was defined as a statistically significant difference.

## Results

### 1. Basic characteristics and intervention results among men and women

Eighty-five male individuals and seventy female individuals were included in the study. Female patients were older than male patients ( $49.62 \pm 10.81$  vs.  $53.47 \pm 7.68$ ,  $p = 0.013$ ). Smoking and drinking rates were significantly higher in male patients than in female patients (both  $p < 0.001$ ). There were no differences about adherence, baseline BMI ( $28.03 \pm 3.46$  vs.  $27.41 \pm 3.95$ ), final BMI ( $26.95 \pm 3.13$  vs.  $26.53 \pm 4.05$ ), baseline LFC ( $33.02 \pm 13.70$  vs.  $36.01 \pm 16.21$ ), final LFC ( $19.65 \pm 12.65$  vs.  $21.76 \pm 15.79$ ), and LFC changes ( $-13.35 \pm 15.41$  vs.  $-14.27 \pm 15.58$ ) between male and female patients (all  $p > 0.05$ ) (Table 1).

Table 1 Basic characteristics and intervention results among men and women

	Male (n=85)	Female (n=70)	Pvalue
Group, n (%)			0.220
LSI	28 (32.94%)	25 (35.71%)	
LSI+PGZ	22 (25.88%)	25 (35.71%)	
LSI+BBR	35 (41.18%)	20 (28.57%)	
Age (age)	49.62 ± 10.81	53.47 ± 7.68	0.013
Smoking, n (%)	23 (27.06%)	2 (2.86%)	<0.001
Drinking, n (%)	25 (29.41%)	1 (1.43%)	<0.001
Treatment adherence (%)	100 (15-100)	100 (32-100)	0.161
Baseline BMI (Kg/m <sup>2</sup> )	28.03 ± 3.46	27.41 ± 3.95	0.301
Final BMI (Kg/m <sup>2</sup> )	26.95 ± 3.13	26.53 ± 4.05	0.466
BMI changes (Kg/m <sup>2</sup> )	-1.08 ± 1.14	-0.88 ± 1.06	0.270
Baseline LFC (%)	33.02 ± 13.70	36.01 ± 16.21	0.215
Final LFC (%)	19.65 ± 12.65	21.76 ± 15.79	0.357
LFC changes (%)	-13.35 ± 15.41	-14.27 ± 15.58	0.714
LSI (n=53)	-12.64 ± 17.78	-8.76 ± 13.49	0.379
LSI+PGZ (n=47)	-9.95 ± 15.18	-15.24 ± 14.54	0.229
LSI+BBR (n=55)	-16.06 ± 13.35	-19.95 ± 17.59	0.359

Data are given as Means (SD) or Median (Min-Max) for continuous variables and percentages for categorical variables; BMI, body mass index; LSI: lifestyle intervention; PGZ: pioglitazone; BBR: berberine; LFC: liver fat content.

## 2. Effects of gender on changes in liver fat content after treatment

LFC stratified by genders were shown in Table 2.

After treatment, the absolute value of LFC was decreased by 11.4% and 12.1% respectively in LSI group and PGZ plus LSI group [13]. It is worth noting that, relative to group LSI, the LFC of group PGZ plus LSI was further decreased in female patients [-8.26% (-17.18%, -0.65%),  $p = 0.025$ ], whereas, was increased in male patients [9.79% (0.37%, 19.21%),  $p = 0.046$ ]. A significant interaction between gender and pioglitazone's efficacy was observed ( $p = 0.003$ ). After adjustment for age, smoking, drinking, baseline BMI, BMI changes, and treatment adherence, the changes of LFC were not significant in males [8.42% (-1.40%, 18.23%),  $p = 0.099$ ], but significant in females [-8.19% (-16.64%, -0.27%),  $p = 0.033$ ]. The interaction test between gender and efficacy remained significant ( $p = 0.011$ ). Compared with LSI, BBR plus LSI group led to further decreased LFC in female patients [-11.88% (-21.61%, -2.14%),  $p = 0.020$ ], while led to no significant changes in male patients [1.50% (-9.38%, 6.38%),  $p = 0.710$ ]. No interaction between gender and efficacy was found in the BBR plus LSI group ( $p = 0.124$ ). Compared with PGZ plus LSI, BBR plus LSI intervention was associated with relatively increased LFC in males [-11.29% (-18.99%, -3.58%),  $p = 0.007$ ], and no significant changes in females [-3.61% (-13.61%, 6.38%),  $p = 0.483$ ]. No interaction between gender and efficacy was found between PGZ plus LSI and BBR plus LSI intervention ( $p = 0.222$ ).

Table 2 Interaction between changes of LFC and gender among three groups

	Model 1			Model 2		
	Changes of LFC (%) $\beta$ (95%CI)	<i>P</i> value	Interaction test <i>P</i> value	Changes of LFC (%) $\beta$ (95%CI)	<i>P</i> value	Interaction test <i>P</i> value
PGZ vs LSI						
male	9.79 (0.37, 19.21)	0.046	0.003	8.42 (-1.40, 18.23)	0.099	0.011
female	-8.26 (-17.18, -0.65)	0.025		-8.19 (-16.64, -0.27)	0.033	
BBR vs LSI						
male	-1.50 (-9.38, 6.38)	0.710	0.124	1.34 (-7.40, 10.07)	0.766	0.355
female	-11.88 (-21.61, -2.14)	0.020		-11.59 (-22.35, -0.83)	0.040	
BBR vs PGZ						
male	-11.29 (-18.99, -3.58)	0.007	0.222	-8.17 (-16.85, 0.51)	0.073	0.169
female	-3.61 (-13.61, 6.38)	0.483		-1.26 (-11.79, 9.28)	0.817	

Data are showed as median with the interquartile range; LFC: liver fat content; Model 1 was not adjusted; model 2 was adjusted for age, smoking, drinking, baseline BMI, BMI changes, and treatment adherence.

## Discussion

To the best of our knowledge, it is the first study to demonstrate that gender is an independent factor affecting the pioglitazone's efficacy on LFC in Chinese NALFD patients with abnormal glucose metabolism. That means, relative to lifestyle intervention, prescribing pioglitazone further reduces LFC in women, but leads to an opposite result in men. This study suggests that in women with NAFLD and abnormal glucose metabolism, prescribing pioglitazone based on lifestyle intervention will be more appropriate.

Previous studies have suggested that there were sex differences of TZDs in treating diabetes or obese patients. TZDs were more effective in women than in men in glycemic control and lipids improvement. Clinical Practice Research Datalink (CPRD) found that male sex and lower BMI were associated with a lesser response with TZDs (both  $p < 0.001$ ) [20]. A Diabetes Outcome Progression Trial (ADOPT) and Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycemia in Diabetes (RECORD) found that non-obese males had a more significant overall HbA1c reduction with sulfonylureas than with TZDs ( $p < 0.001$ ); in contrast, obese females had a greater HbA1c reduction with TZDs than with sulfonylureas ( $p < 0.001$ ) [20]. Similarly, triglycerides decreased significantly in women not in men with pioglitazone treatment ( $p = 0.015$ ) [21]. Our previous study showed that after pioglitazone treatment, women experienced a greater drop in blood glucose and insulin (Supplement data 1). On the contrary, for overweight or obese individuals, pioglitazone intervention combined with energy-restricted diet and resistance training for 16 weeks, abdominal visceral fat was significantly reduced in men but was not in women [22].

However, little research has reported the gender differences of pioglitazone on NAFLD. The PIVENS study did a subgroup analysis, but no significant difference of NASH improvement was found between men and women (30% vs. 36%,  $p = 0.64$ ), the similar result was observed with vitamin E treatment (42% vs. 44%,  $p = 0.49$ ) [9]. Other medical agents for NASH like liraglutide, omega-3 fatty acids and Obeticholic acid, have not been reported to show sex-based differences in liver histologic improvement. This study

reports that pioglitazone has gender differences in treatment of fatty liver, and pioglitazone is more favorable for female patients. The differences between Eastern and Western NAFLD populations might be related to racial differences. Future clinical trials should focus more on sex differences in drug efficacy.

Sex differences in the effects of pioglitazone are possibly related to sex hormones, which exert different effects on metabolism in men and women. Pioglitazone decreased testosterone levels (total content and active ingredient) in men with diabetes [23], and in women, pioglitazone decreased androgen levels of polycystic ovary syndrome (PCOS) [24]. Androgen levels are strongly associated with NAFLD. A Korean study found that after adjusting for confounding factors such as age, smoking, BMI, diabetes and exercise, reduced testosterone levels in men were associated with an increased risk of NAFLD (lowest testosterone: OR = 5.12,  $p = 0.0004$ ) [25]. Consistent results were reported in another study from Germany (OR = 3.39,  $p < 0.001$ ) [26]. In addition, the total body fat and visceral fat of hypogonadal and normal gonadal men were decreased after testosterone supplementation [27]. For female patients, elevated circulating free testosterone levels increased the prevalence of NAFLD [28]. Drugs, that lower circulating testosterone levels such as metformin or TZDs, can improve the metabolic features of PCOS [29]. There is an interesting paradox that, in men, testosterone deficiency increases visceral fat content and insulin resistance; while in women, high androgen levels increase insulin resistance and visceral fat [30]. At the animal model, the testosterone levels of db/db mice were lower than that of the wild type mice, and exogenous testosterone replacement alleviated fatty liver of db/db mice [31]. Estrogen, another sex hormone, is one of the protective factors of NAFLD by suppressing lipid accumulation, inflammation and fibrosis [32]. To date, the effects of TZDs on estrogen levels have not been reported in humans, other than a few vitro experiments showed that TZDs could inhibit estrogen synthesis [33]. Taken together, the effects of TZDs on sex hormones and the effects of sex hormones on metabolism are both complicated. The gender differences in LFC observed in our study might be explained by the effects of androgen. Large-scale and well-designed studies are needed to assess this issue further.

Gender differences in pioglitazone action might be also related to pharmacokinetics. The clearance rate of pioglitazone in female mice was slower than that in male mice. After single oral administration or continuous oral administration, the blood active metabolic concentrations in female mice were higher than that in male mice (1.5-3 times) [34]. CYP2C8 is a critical enzyme in the metabolism of pioglitazone, and carrying the CYP2C8\*3 allele will accelerate the metabolism of pioglitazone [35]. A population study showed that NASH patients with the CYP2C8\*3 allele had less improvement in liver fibrosis after pioglitazone intervention ( $p = 0.006$ ) [36]. However, it has not been reported whether there are gender differences in the expression of CYP2C8 in humans, and only one study found that the mRNA and protein levels of CYP2C8 in the liver of white individuals were independent of gender [37]. In this study, we did not measure the metabolites of pioglitazone, whether pharmacokinetic participated in the gender differences in LFC cannot be confirmed.

This study has several limitations. Firstly, as this is a post hoc analysis, residual confounding cannot be eliminated entirely. We would like to underscore that our findings serve as hypothesis-generating. Secondly, this study did not detect the pathological changes of liver or NAFLD fibrosis score, and whether there are gender differences in liver histology remains unknown. Further trials using liver histology as the main observation outcome are necessary to evaluate the gender-specific differences in fatty liver of pioglitazone therapy. Thirdly, the sample size is relatively small, and larger-scale clinical trials should be conducted to confirm this provocative finding. In the end, the effects of pioglitazone on sex hormone levels could not be verified because sex hormone levels were not measured.

### Perspectives and Significance

This study suggests that, when selecting an optimal therapeutic strategy for NAFLD in clinical practice, factors affecting the efficacy of drugs should be more carefully considered, then to tailor individualized treatment for patients.

## Conclusions

This study found that gender differences existed in the treatment regimentation of Chinese NAFLD patients with abnormal glucose metabolism, pioglitazone treatment is recommended for women, but not for men, based on lifestyle interventions.

## Declarations

### Ethics approval and consent to participate

This trial was approved by the Ethics Committee of Zhongshan Hospital, Fudan University.

### Consent for publication

Not applicable.

### Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

XG and HMY designed the experiments. LW, WYW, XXC and MFX performed the experiments. JG and HMY analyzed and interpreted the data. LW, HMY and JG wrote the manuscript. All authors read and approved the final manuscript.

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