Journal of Diabetes and Endocrinology Vol. 2(1), pp. 8-13, January 2011
Available online at http://www.academicjournals.org/JDE
ISSN 2141-2685 ©2011 Academic Journals

Full Length Research Paper

Significant correlation of hepatic ISI with BMI/BW after short term pioglitazone therapy via triglyceride metabolism in Type 2 Diabetes Mellitus

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Accepted 2 December, 2010

Analysis of pharmacological effect of pioglitazone on hepatic insulin sensitivity index (hISI), peripheral resistance (IR), body mass index (BMI), body weight (BW) and lipids in type II diabetes mellitus. Patients were treated with 30 mg of pioglitazone (PIO) daily and investigated for BW, BMI, FBS, fasting insulin (FI) and triglycerides (TG). hISI and IR were calculated by McAuley (McA), HOMA & QUICKI indices ISI equation. There was no significant difference in BMI, BW and TG after 3 months. There was a significant reduction in FI (37.58 ± 6.09 to 15.37 ± 3.28 mU/L), IR by McA (4.68 ± 0.25 to 6.18 ± 0.31) HOMA and QUICKI (17.51 ± 3.36 to 5.41 ± 1.57 & 0.27 ± 0.0 to 0.34 ± 0.01, p > 0.001). No significant correlation was observed between BMI or BW with IR indices before, but significant correlation developed between BMI with FI (r = 0.4, p > 0.05) and McA (r = 0.48, p = 0. 02) after 3 months. The reduction of hISI was significant and found a substantial positive association between hISI with BMI. Correlations between hISI with HOMA, QUICKI and McA were significant but no significant correlation was detected between TG, HOMA or QUICKI with BMI or BW before or after therapy in our study cohort. There was an improvement of both hepatic and peripheral insulin sensitivity with three months of PIO. Significant correlations between BMI vs. McA and FI but not with HOMA or QUICKI can be related to inclusion of TG in McA's equation but not in other indices. Reduction of both hepatic and peripheral IR suggests effects of PIO on fat clearance from liver. We propose that reduction of IR is related to the TG metabolism possibly by clearance of VLDL-TGs and activation of lipoprotein lipase in plasma by PIO.

Key words: McAuley, insulin resistance, HOMA, QUICKI, fasting insulin, type 2 diabetes.

INTRODUCTION

Incidence of type II diabetes is reaching epidemic proportions around 59 million of population, particularly in south Asian region. Type II diabetes is characterized by the presence of insulin resistance (IR) and relative insulin deficiency: Early diagnosis is important for the management strategies of type 2 diabetes mellitus (DM) (Grundy, 1998; Wickelgren, 1998; Hettihewa et al., 2005). The euglycaemic insulin clamp and the intravenous glucose tolerance tests are gold standard methods for measurement of insulin resistance in research, but they are cumbersome in clinical practice and are difficult to perform in population based research studies. Therefore indirect indices; McAuley, HOMA and QUICKI were used for assessment of IR in our study (Hettihewa et al., 2005; McAuley et al., 2001; Bergman et al., 1985). These indirect indices are used by most of the medical scientist to evaluate IR in clinical set up because these vales are accepted after several research confirmations.

The accumulation of visceral fat is particularly assumed to play an important role in the etiology of IR notably by the over exposure of the liver to free fatty acids

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(DeFronzo and Ferrannini 1991), which results in insulin resistance and hyperinsulinemia (Grundy, 1998; Wickelgren, 1998; Yoshinori et al., 1987). Peroxisome proliferator-activated receptor-γ(PPAR-γ) agonists, improve insulin sensitivity and lipemia partly through enhancing adipose tissue proliferation and capacity for lipid retention (Yoshinori et al., 1987; Magalie et al., 2004) Identification of correlation of γ(PPAR-γ) agonists with obesity is necessary to develop new therapeutic strategies using PPAR-γ and dietary recommendations. This study was planned to determine the relationships of -PPAR-γ with obesity and fat indices which will be useful in selecting the most suitable clinical indications of -γ(PPAR-γ agnosit for type 2 DM.

Objectives

Our objective was to determine the effect of PIO therapy on relationships of IR with obesity in adult type 2 diabetic population.

MATERIALS AND METHODS

The protocol for this study was approved by ethical committee of the Faculty of Medicine, University of Ruhuna. 42 patients with type 2 diabetes were randomly selected when there is fasting blood sugar (FBS) > 7 mmol/L (126 mg/dl) and in an occasion when they are symptomatic in DM or in two occasions when they are asymptomatic in DM. All patients were given verbal and written information about the study prior to providing written consent and invited for written feedback of individual participation at the end of the study. Clinical history including age, sex, drugs, smoking, alcohol consumption, level of physical exercise, previous history and family history of diabetes, dyslipidaemia, coronary artery disease and peripheral vascular disease were obtained by a trained medical officer using a questionnaire. Exclusion criteria were: age outside the range of 30 to 65 years, hypothyroidism, liver, kidney or heart failure and neoplasm. Patients were given 30 mg of PIO daily and investigations were repeated at monthly interval during 3 months. Height and weight were determined with the subjects wearing light clothing without shoes. Each participant's weight and height and BMI were recorded. After 12 h of overnight fasting, 3 ml of blood is collected to a sterile centrifuge tubes under strict sterile venipucture. The plasma was separated immediately using centrifugation at 4000 rpm for a period of 10 min. FBS was assessed by spectrophotometric analysis by commercial kit at wave length 450 (Kit diagnostica–Merck). FI was measured by ELISA reader (insulin commercial kit-diagnostic– automation). TG levels were measured enzymatically by colorimetric tests (commercial kit-from LABKIT P and T diagnostics). McAuley described a method for measurement of insulin resistance, which correlates with euglycemic clamp technique and it was used as an index of IR (McAuley et al., 2001). It was calculated as follows.

McA = exp [2.63-0.28 (insulin in mU/L) – 0.31 (triglycerides in mmol/L)]

HOMA = insulin (μU/m) x [glucose (mmol/L)/22.5]

$$\text{QUICKI} = \frac{1}{(\log \text{insulin} + \log \text{glycaemia in mg/dL})}$$

Subjects with McAuley (McAuley et al., 2001) ≤ 5.8 and FI ≥ 12 mu/L (McAuley et al., 2001; Hettihawa et al., 2006; Berger and Moller, 2002; Auwerx, 1996) has been considered as insulin resistant in diabetic population. Patients were considered as insulin resistant when McA ≤ 5.8, HOMA ≥ 2.6 and QUICKI ≤ 0.33 (McAuley et al., 2001). Hepatic ISI was calculated by estimated from the FPG and FPI as follows (Yoshinori et al., 1987)

$$\frac{k}{\text{FPG} \times \text{FPI}}$$

This equation (Yoshinori et al. 1987) is mathematically equivalent to the reduced formula of the homeostasis model assessment (HOMA), where k = 22.5 x 18, and the hISI correlates closely with that measured directly with tritiated glucose (Yoshinori et al., 1987, 2002). The product of basal hepatic glucose production (measured with tritiated glucose) and the FI concentration provides a direct measure of hepatic IR under postabsorptive conditions, whereas the inverse provides a measure of hepatic insulin sensitivity (Yoshinori et al., 1987, 2002).

Statistical analysis

For the descriptive statistics after having checked the normality of distribution of the variables using the Kolmogorov-Smirnov test, the usual central and dispersion methods were used: average, SD, and 95% CI. Minimum number of patients was statistically decided by using the equation for sample calculation using alfa and beta error and standard deviation. Power were carried out based on the results of the current study, comparing changes in FI, IR, BW and BMI in 3 month of PIO allowing declaration of a difference before and after in same treatment group, at a significance level $\alpha = 0.05$, with power of 80%. The statistical significance of differences between the means was evaluated using the paired Student's T-test in the case of normal distribution of variables. The Kolmogorov-Smirnov test was used when at least in one of the data sets the normal distribution was excluded. Correlation between two variables was studied with the Spearman rank-order. All statistical analyses were performed using Microcal origin for windows software 4.1(2005) and Microsoft Excel whenever applicable.

RESULTS

Baseline characteristics and changes in insulin resistance in our study group

The study cohort included 42 patients with mean age 45.83 ± 1.82. Female to male ratio of patients was 7:5. Table 1 shows the significant difference in mean values of FI, McA, HOMA and QUICKI indices after 3 months of PIO. Though there was a reduction of TG it was not statistically significant. Table 1

Statistically significant correlation between BMI with McA and FI after 3 months of PIO therapy

Our results show that there is no significant difference in changes of BMI, TG and BW after 3 months of PIO therapy (Table 1). In contrast, there was a significant reduction in FI, IR by McA, HOMA and QUICKI indices at the end of treatment ($p<0.001$, Table 1). There was no

Table 1. Statistically significant correlation between BMI with McA and FI after 3 months of PIO therapy.

Basic characteristic	Baseline	3 month after pioglitazone therapy	Level of significance
Age		45.83 ± 1.82	
BW (kg)	58.78 ± 2	59.08 ± 2	p> 0.05, p = 0.42
BMI (kg/m^2)	23.95 ± 0.82	24.08 ± 0.85	p>0.05, p = 0.37
TG (mmol/L)	1.82 ±0.08	1.70 ± 0.05	p<0.001*
FI (mU/L)	37.58 ± 6.09	16.58 ± 3.62	p<0.001*
McAuley	4.84 ± 0.27	6.26 ± 0.28	p<0.001*
HOMA	17.50 ± 3.36	5.40 ± 1.57	p<0.001*
QUICKI	0.27 ± 0.00	0.34 ± 0.01	p<0.001*

Table 2. Correlation of BMI with HOMA and QUICKI after 3 months of PIO Before and after the therapy.

Parameters	Before the therapy	After the therapy
BMI vs HOMA	r = - 0.02, p = 0.9	r = 0.22, p >0.05
BMI vs QUICKI	r = - 0.23, p = 0.28	r = -0.37, p >0.05
BW vs HOMA	r = - 0.01, p = 1.0	r = 0.13, p >0.05
BW vs QUICKI	r = - 0.17, p = 0.4	r = -0.30, p >0.05

significant correlation between BMI and BW with McA, HOMA, QUICKI or FI before the therapy (p>0.05). But there was significant correlation between BMI with FI (r = -0.4, p < 0.05) and McA (r = -0.50, p < 0.02) after with 3 months of PIO therapy (Figure 1). There was no significant correlation between BW with either of McA, HOMA, QUICKI or FI after PIO (data not shown).

Correlation of BMI with HOMA and QUICKI after 3 months of PIO

We extended our study to evaluate the correlation with other IR indices because of the observation based on significant correlation between BMI with McA or FI. Our results showed that there was a significant difference in IR by HOMA and QUICKI values before and after the treatment. This further confirmed that reduction of IR in our participants with PIO is significant. Next, we investigated to analyze correlations between HOMA, QUICKI with BMI or BW. Although there was no statistically significant correlation between HOMA and QUIKI with either BMI or BW (Table 2).

Considering significant correlation between BMI with McA but not with HOMA or QUICKI, There is a scientific prediction to see a possibility of involvement of TG metabolism in improvement of IR. Therefore we further investigated to see any correlation with TG in our study cohort. Although there was reduction in clinical values in investigations in TG levels it was not scientifically significant. In addition to that we could not find any significant correlation between TG with any of the above parameters in our study group.

Correlation between TG and other biochemical and clinical parameters of the study group with the pioglitazone treatment

Statistically significant correlation of BMI with hepatic ISI after 3 months of PIO therapy

We further extended our study to see the effects of PIO on hISI in our study cohort. The reduction of hISI with the treatment of 15 mg of PIO was statistically significant (Figure 1). There was a significant reduction in mean hISI after 3 months of PIO in our patients (0.15 ± 0.03 to 0.43 ± 0.05, p<0.05, Figure 2B). There was a significant negative correlation between hISI with BMI (Figure 2) after the PIO therapy (r, - 0.44, p<0.03). Correlation between hISI with HOMA, QUICKI and McA also significant (p<0.001, data are not shown).

DISCUSSION

In light of the well-documented relationship between obesity and IR the treatment Grundy, 1998; Louise, et al., 2004) effects of PIO appear to be paradoxical in that their insulin-sensitizing effects occur in the presence of an increase in BW and whole-body adiposity. Therefore goal of this study was to identify effects of PIO on IR and the possible mechanism on lipid in the process of improvement of IR in diabetic patients. Recent study had demonstrated that the PIO induced weight gain is associated with an increase in subcutaneous adipose tissue and a concomitant decrease in visceral fat content (Yoshinori et al., 1987). Increase in BW in our study despite the improved insulin sensitivity can be explained by this fat redistribution due to remodeling of abdominal fat tissue (Yoshinori et al., 1987). Another previous study shows that there was a dose-dependent increase in BW and BMI after 24 weeks in the pioglitazone-treated groups (Berger and Moller, 2002). The seemingly paradoxical relationship between weight gain and

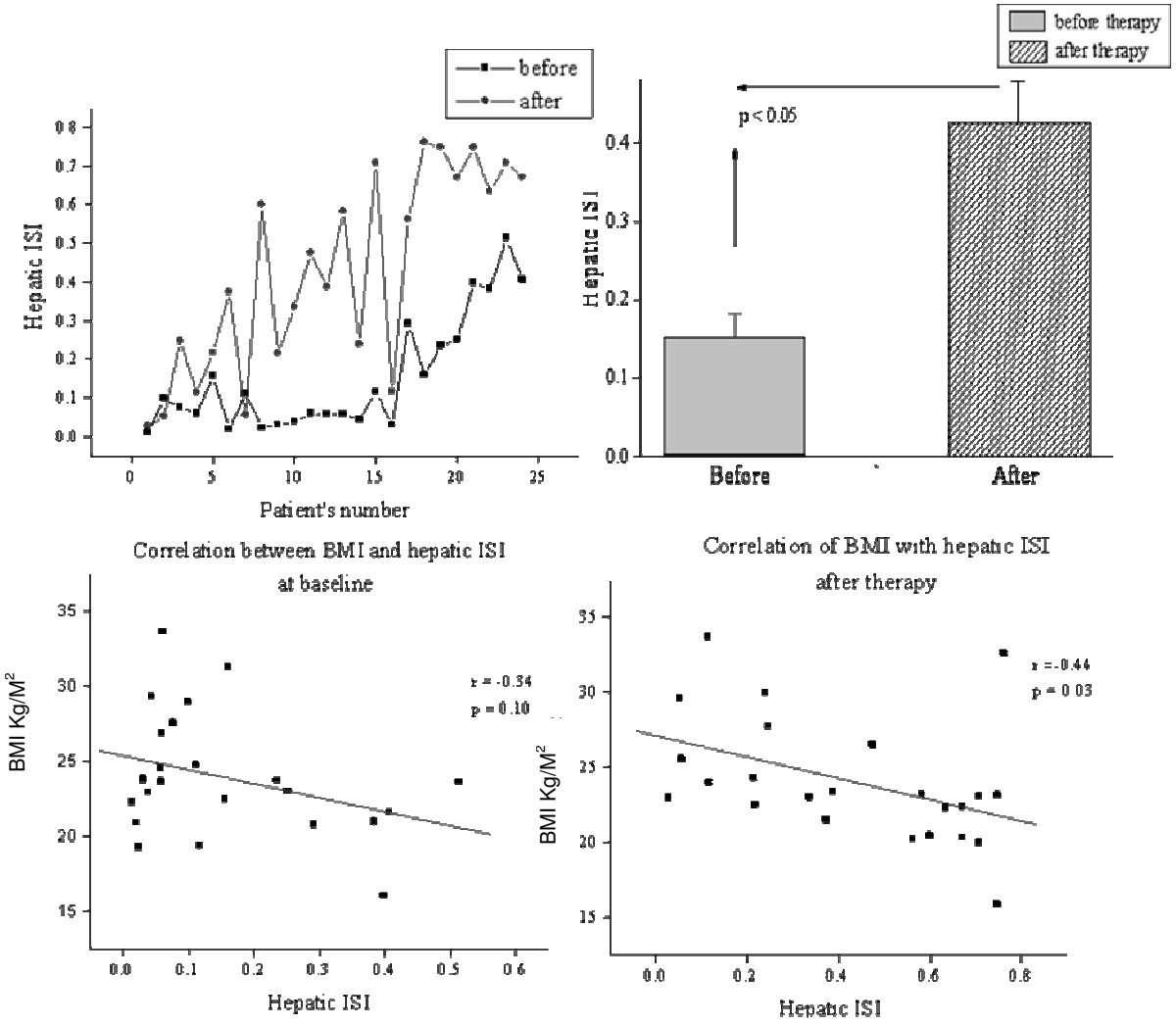

Figure 1. Correlation between changes in BW, BMI with mean FI and McA with the 15 mg of PIO treatment in patients with type 2 diabetes.

improved glucose homeostasis/insulin sensitivity most likely is explained by the basic cellular mechanism of action of the thiazolidinediones, which exert their effects through the PPAR-γ. PPAR-γ activation also induces key enzymes involved in lipogenesis in newly formed adipocytes (Wickelgren 1998).

Our patients, who were insulin resistant, have become insulin sensitive after three months of PIO. In addition, there was significant correlation between BMI and McA as well as with FI levels after PIO. Significant correlations between BMI vs McA and FI but not with HOMA or QUICKI indicate the feasible mechanism of reducing IR by PIO possibly by interference with TG metabolism. Our results are supported with previous results showing PPARγ agonists improve insulin sensitivity mainly through adipose tissue remodeling, increased capacity for lipid uptake/retention, and altered adipocytokine secretion pattern (Yoshinori et al., 1987; Kazunori et al., 2005). Kazunori et al also shows PIO reduces TG by decreasing secretion of both VLDL, TGs and VLDL apoB via lipoprotein lipase activation, by improving adipose tissue sensitivity to insulin and also reduction of plasma insulin and hepatic lipogenesis (Kazunori, et al., 2005). They did not observe any significant difference in total cholesterol and LDL levels with PIO (Kazunori et al., 2005). Increased visceral fat is associated with IR (Kazunori et al., 2005), and reduction in visceral fat would be expected to lead to an enhancement in insulin sensitivity (Randle et al., 1963). Because thiazolidinedione treatment consistently reduces plasma free fatty acid levels (Randle et al., 1963), this may provide another explanation for the improvement in insulin sensitivity despite weight gain. Considering above reports our data suggest that there may be a common metabolic pathway for both reduction of IR and plasma TG levels possible via increase of lipoprotein lipase activity.

Insignificant correlation between BMI with HOMA or QUICKI can be due to exclusion of TG levels in HOMA

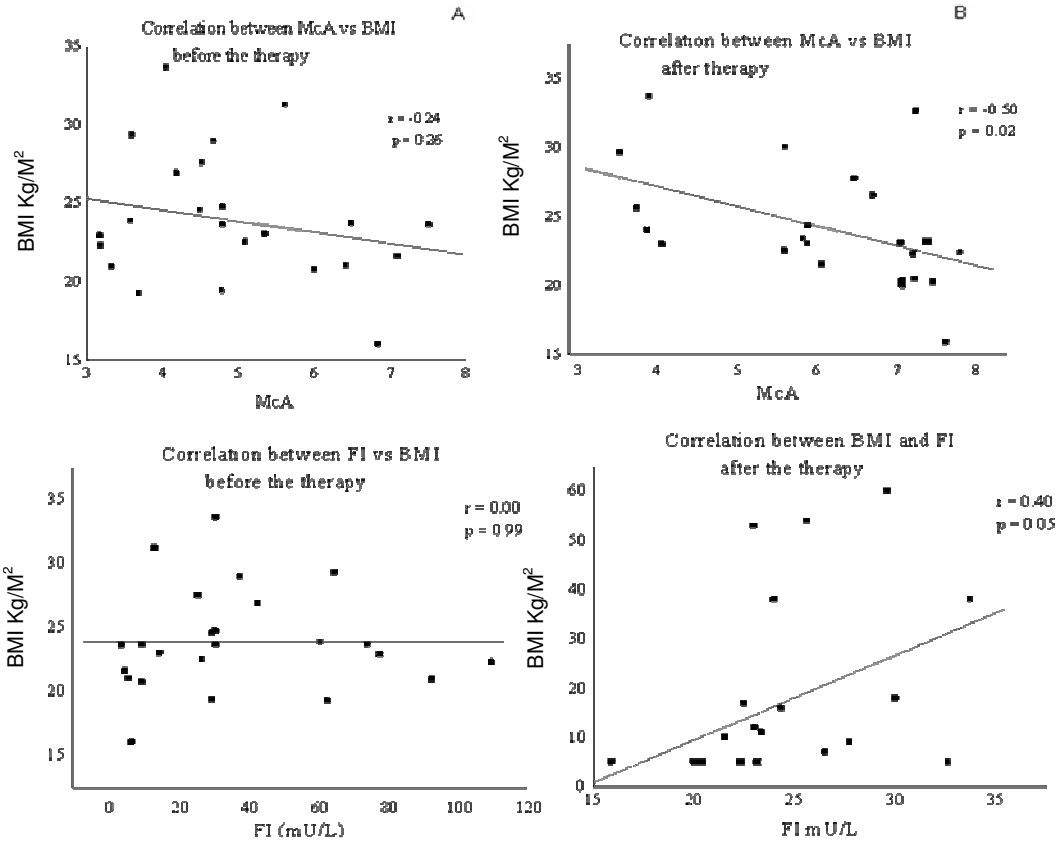

Figure 2. A - Changes of hISI index in our study cohort after 15 mg of PIO therapy. The changes in data are statistically significant ($p<0.05$). B shows the difference in mean values of hISI index in our study cohort ($p<0.05$). There was a statistically significant correlation between BMI and hISI index after PIO therapy ($p<0.05$).

and QUICKI equations. Further, McA was identified as method of detecting IR when confronted with minimal model approximation of the metabolism of glucose (MMAMG) with very high sensitivity and specificity values (McAuley et al., 2001; Bergman et al., 1985). In contrast, another study shows evidence in all participants (black and white adolescent girls), during 10 years, changes in BMI were positively correlated with changes in insulin ($r = 0.26$, $P < 0.0001$) as well as in HOMA insulin resistance ($r = 0.24$, $P < 0.0001$) (Auwerx, 1996). This finding concurs with our results to explain development of correlation between BMI with IR indices after the PIO therapy. Although we studied patients with 15 mg of PIO we would not comment on the effects of high doses of 30 or 45 mg of PIO on correlation of IR with BMI or hepatic ISI. But Yoshinori et al says PIO improves glycemic control through the dose-dependent enhancement of ß-cell function and improved whole-body and hepatic insulin sensitivity (Kazunori et al., 2005). We also found that PIO treatment causes significant increment of hepatic ISI in diabetic patients and it has significant correlations with BMI, McA, HOMA and QUICKI indices. Our results are compatible with Yoshinori Miyazaki et al. showing that hepatic ISI increased in the 15-, 30-, and 45-mg/day pioglitazone groups (Yoshinori et al., 2002) ($P < 0.05$–0.01). Because basal hepatic glucose production is closely correlated with FBS, the inverse of the product of FBS and FI provides an index of hepatic insulin sensitivity (Raskin et al., 2000). It can be concluded that PIO decreases FBS levels through improvements in hepatic/ whole-body insulin sensitivity and in ß-cell function in type 2 diabetic patients.

ACKNOWLEDGEMENTS

We gratefully acknowledge Mrs. N. Samaranayake and Mrs. A.G. Punyalatha for their assistance in the laboratory work, in the Department of Pharmacology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

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