

## Correlation between BMI and insulin resistance in type 2 diabetes mellitus patients on pioglitazone treatment

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### Abstract

We investigated the kinetic effect of pioglitazone on changes of body mass index (BMI), body weight (BW), lipids and insulin resistance (IR) in patients with type 2 diabetes mellitus.

**Materials and method:** 24 patients with type 2 diabetes were randomly selected using fasting blood glucose (FBS) > 7 mmol/L (126 mg/dL) in one occasion if the patient is symptomatic, or in two occasions if the patient is asymptomatic. Patients were treated with 15 mg of pioglitazone (PIO) daily and investigated for BW, BMI, FBS, fasting insulin (FI) & triglycerides (TG). IR was calculated by McAuley (McA), HOMA & QUICKI indices at baseline and repeated after 3 months.

**Results:** Mean age was 45.83 ± 1.82 years. There was no significant difference of BMI (23.95 ± 0.82 kg/m<sup>2</sup> to 24.08 ± 0.85 kg/m<sup>2</sup>), BW (58.78 ± 2.00 kg to 59.08 ± 2.00 kg) and TG (1.82 ± 0.08 mmol/L to 1.7 ± 0.05 mmol/L) after 3 months (mean ± SE, p>0.05). There was a significant reduction in FI (37.58 ± 6.09 to 15.37 ± 3.28 mU/L) and IR by McA (4.68 ± 0.25 to 6.18 ± 0.31) with PIO treatment (p<0.001). Reduction of IR by HOMA and QUICKI indices were also significant (17.51 ± 3.36 to 5.41 ± 1.57 and 0.27 ± 0.0 to 0.34 ± 0.01, p>0.001 respectively) after therapy. There was a reduction of TG levels in our participants but it is not statistically significant. No significant correlation was observed between BMI or BW with any of the IR indices before the therapy but significant correlation developed later between BMI with FI (r = 0.4, p>0.05) and McA (r = 0.48, p = 0.02) after 3 months. The reduction of hepatic insulin sensitivity index (hepatic ISI) was

significant and found a substantial positive association between hepatic ISI with BMI after the PIO therapy. Correlation between hepatic ISI with HOMA, QUICKI and McA also significant but no significant correlation was detected between TG, HOMA or QUICKI with BMI or BW before or after therapy in our study cohort.

**Conclusions:** There was an improvement of both hepatic and peripheral insulin sensitivity with three months of PIO. In addition, 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. Therefore we propose that reduction of IR is related to the TG metabolic pathway possibly by clearance of VLDL-TGs and activation of lipoprotein lipase in plasma by PIO.

### Introduction

Incidence of type 2 diabetes is reaching epidemic proportions globally, particularly in south Asian region. Type 2 diabetes is characterised by presence of IR and relative insulin deficiency, hence early identification is important for the management strategies of DM [1-3]. 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 [3-5].

The accumulation of visceral fat is particularly assumed to play an important role in the aetiology of IR notably by the overexposure of the liver to free fatty acids [6], which results in insulin resistance and hyperinsulinaemia [1,2,7]. Peroxisome proliferator-activated receptor-(PPAR-) agonists, improve insulin sensitivity and lipaemia partly through enhancing adipose tissue proliferation and capacity for lipid retention [7,8]. Identification of correlation of (PPAR-) agonists with the obesity hence the BMI is necessary to develop public health policy and dietary and physical activity recommendations that are both comprehensive and effective in reversing the current trend.

### Objectives

Our objective is to determine the effect of PIO therapy on kinetic changes of IR and obesity in adult type 2 diabetic population and correlation of IR with obesity hence BMI or BW or TGs.

### Materials and method

The protocol for this study was approved by Ethical Committee of Faculty of Medicine, University of Ruhuna. 24 patients with type 2 diabetes were randomly selected when there is FBS >7 mmol/L (126 mg/dL) in one occasion when they are symptomatic or two occasions when they are asymptomatic. All patients were given verbal and written information about the study prior to providing written consent and invited for verbal and written feedback of individual results at the end of the study. Clinical history including age, sex, drugs, smoking, alcohol consumption level of physical exercise, previous history & family history of diabetes, dyslipidaemia, coronary artery disease and peripheral vascular disease were obtained. Exclusion criteria were; age outside the range of 30-65 years, hypothyroidism, liver, kidney or heart failure and neoplasm. Patients were given 15 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 were

recorded and BMI was calculated using height (m) and weight (kg). After 12 hours of overnight fasting, blood samples were collected and deposited in dry tubes. The plasma was separated immediately using centrifugation at 4000 rpm for a period of 10 minutes. FBS was assessed by absorbance method (diagnostica – Merck). FI was measured by ELISA (diagnostic – automation). TG levels were measured enzymatically by colorimetric tests (LABKIT). McAuley described a method for measurement of insulin resistance (McA), which correlates with estimates of IR measured by the euglycemic clamp technique, was used as an index of IR [4]. It was calculated as follows.

$$\text{McA} = \exp [2.63 - 0.28 (\text{insulin in mU/L}) - 0.31 (\text{triglycerides in mmol/L})]$$

$$\text{HOMA} = \frac{\text{insulin (U/m)} \times [\text{glucose (mmol/L)}]}{22.5}$$

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

Subjects with McAuley 5.8 [4] and FI 12mu/L [4, 10-12] has been considered as insulin resistant in diabetic population. Patients were considered as insulin resistant when  $\text{McA} \geq 5.8$ ,  $\text{HOMA} \geq 2.6$  and  $\text{QUICKI} \leq 0.33$  [4]. Hepatic ISI was calculated by FPG and FPI as follows [7].

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

This equation [7] is mathematically equivalent to the reduced formula of the homeostasis model assessment (HOMA), where  $k = 22.5 \times 18$ , and the hepatic ISI correlates closely with that measured directly with tritiated glucose [7, 13]. 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 [7, 13].

### Statistical analysis

For the descriptive statistics after having checked the normality of the variables using the Kolmogorov-Smirnov test, the usual central and dispersion methods were used: average, SD, and

95% CI. Power and sample size calculations 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 = 0.05, with power of 80%. The statistical significance of differences between the means were evaluated using the paired Student's T-test in the case of normal distribution of data sets, and using the Kolmogorov-Smirnov test 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 4.1 (2005) and Microsoft Excel whenever applicable.

## Results

### 1.1 Baseline characteristics and changes in insulin resistance in our study group

The study cohort included 24 patients with mean age range from 45.83 ± 1.82. Female to male ratio 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. Overall, these data support the conclusion that PIO treatment significantly increased insulin sensitivity in these patients.

**Table 1 - Baseline basic characteristics and characteristics after three months of pioglitazone treatment**

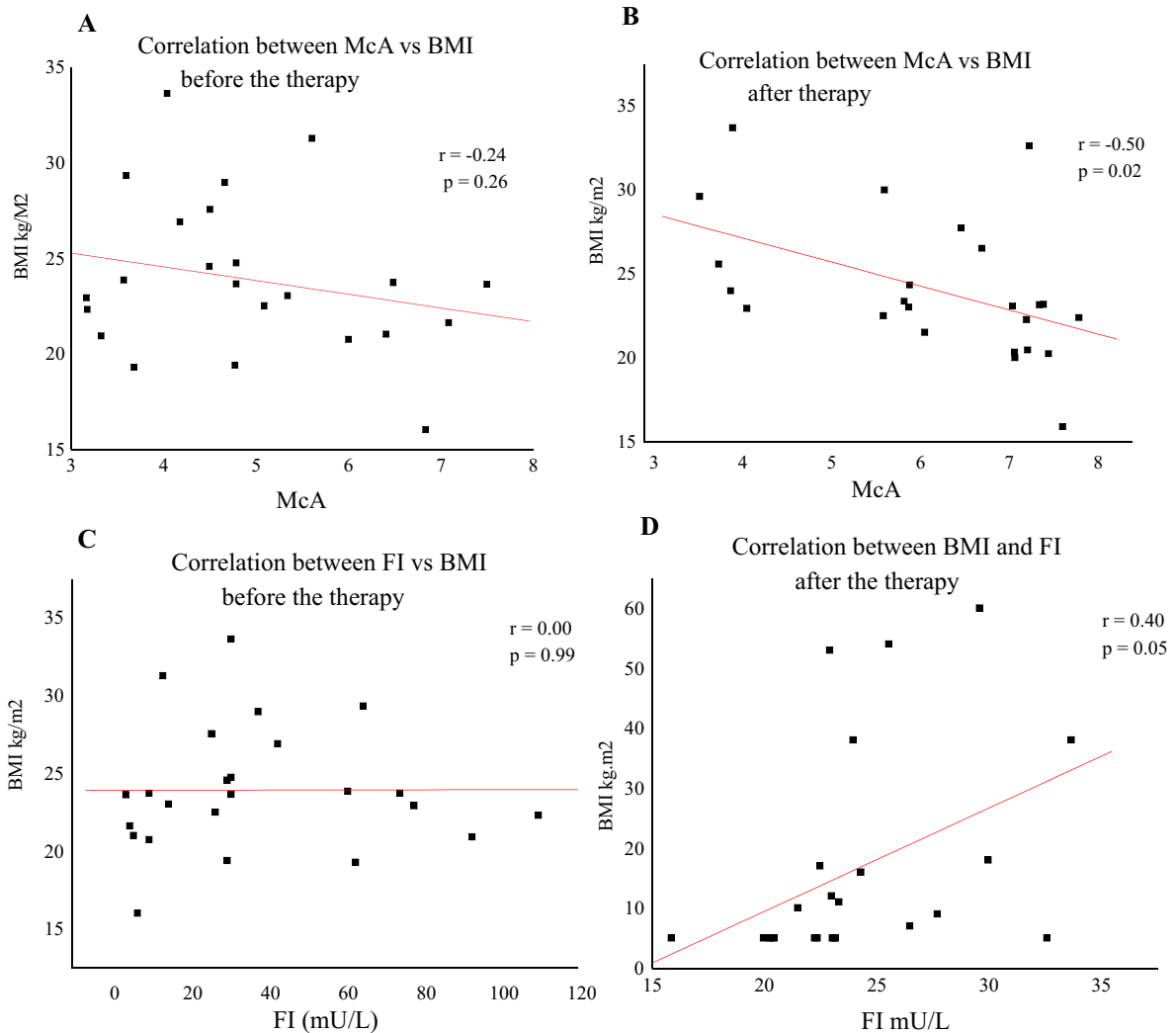
Data are mean ± SEM \*  $P < 0.001$  vs baseline

<i>Basic characteristics</i>	<i>Baseline</i>	<i>3 month</i>	<i>Level of significance</i>
Age	45.83 ± 1.82		
BW ( Kg)	58.78 ± 2	59.08 ± 2	P=0.42
BMI ( Kg/m <sup>2</sup> )	23.95 ± 0.82	24.08 ± 0.85	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*

### 1.2 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 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 ( $r = 0.48$ ,  $p = 0.02$ ) after with 3 months of PIO therapy (fig 1). There was no significant correlation between BW with either of McA, HOMA, QUICKI or FI after PIO (data not shown).



**Figure 1 - Changes of correlation of fasting insulin (FI) and IR by McAuley index (McA) with BMI after 3 months of PIO therapy.** The Pearson's correlation coefficient and associated *P* value are shown.

### 1.3 Correlation of BMI with HOMA and QUICKI after 3 months of PIO.

Observation of significant correlation between BMI with McA or FI, we extended our study to evaluate correlation with others indirect indices as well. We found that difference in IR by HOMA and QUICKI after PIO therapy also statistically significant. This further confirmed that reduction of IR in our participants with PIO. Next, we investigated to see any correlation between HOMA, QUICKI with BMI or BW. Although there was an improvement of correlation between HOMA and QUICKI with either BMI or BW, it was not statistically significant (table 2).

**Table 2 – Correlation of BMI or body weight with HOMA or QUICKI indices**

Parameters	Before the therapy	After the therapy
BMI vs HOMA	$r = -0.02, p = 0.9$	$r = 0.22, p = 0.3$
BMI vs QUICKI	$r = -0.23, p = 0.28$	$r = -0.37, p = 0.07$
BW vs HOMA	$r = -0.01, p = 1.0$	$r = 0.13, p = 0.54$
BW vs QUICKI	$r = -0.17, p = 0.4$	$r = -0.30, p = 0.14$

Considering significant correlation between BMI with McA but not with HOMA or QUICKI, we thought that possibility of involvement of TG metabolism in improvement of IR despite increment of BMI. Therefore we further investigated to see any correlation with TG in our study cohort.

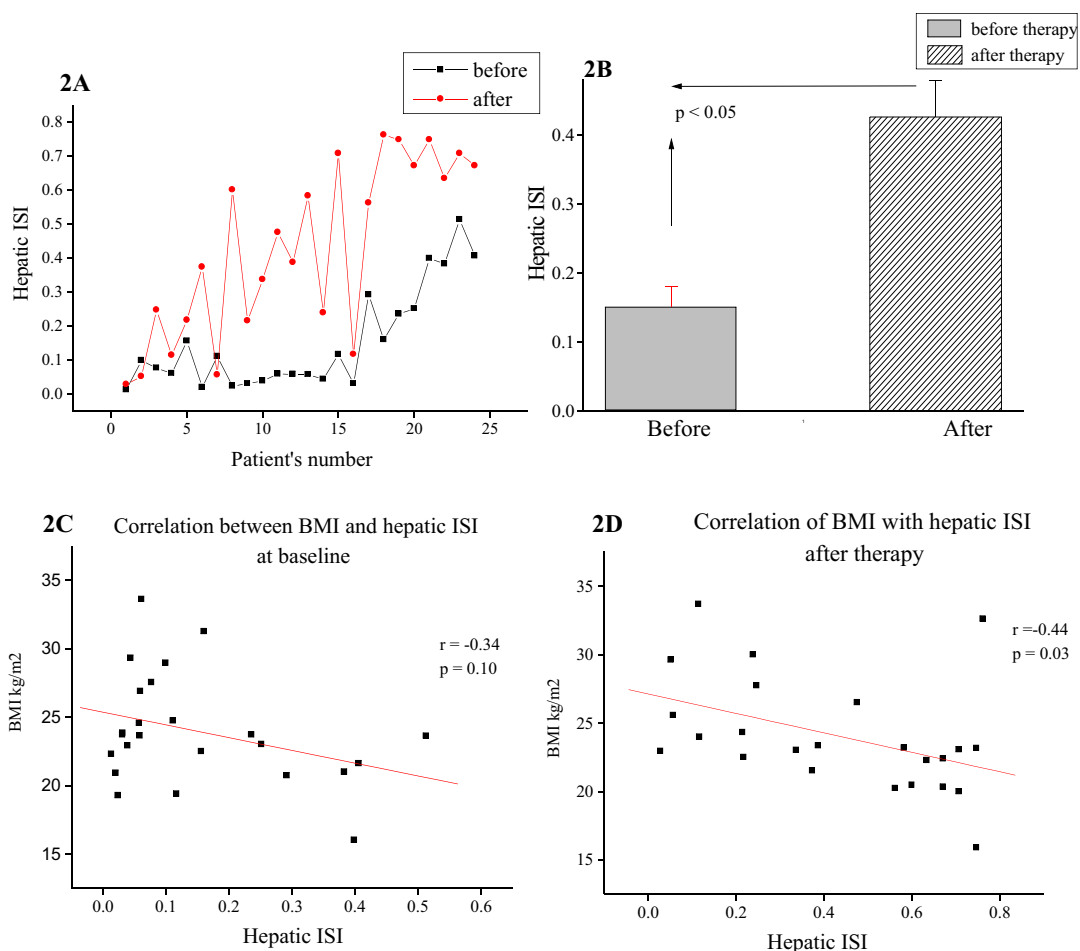
Correlation coefficient was calculated between TG with BMI, BW, FI, McA, HOMA and QUICKI. Although there was clinical reduction (but not statistically significant) in TG levels, we could not find any significant correlation between TG with any of the above parameters in our study group (table 3).

**Table 3 - Correlation between TG and other biochemical and clinical parameters of the study group with the pioglitazone treatment.**

Parameter	Before		After	
	r - value	p - value	r - value	p - value
TG vs BW	0.11	0.60	-0.08	0.72
TG vs BMI	0.09	0.66	0.07	0.73
TG vs FI	0.09	0.64	-0.04	0.86
TG vs McA	-0.12	0.56	-0.07	0.98
TG vs HOMA	0.21	0.32	-0.34	0.22
TG vs QUICKI	-0.01	0.95	0.26	0.21

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

Observed results in peripheral IR with the PIO, we further extended our study to see any effects of PIO on hepatic ISI in our study cohort. The reduction of hepatic ISI with the treatment of 15mg of PIO was statistically significant (figure 2A). There was a significant reduction in mean hepatic ISI after 3 months of PIO in our patients (0.15 0.03 to 0.43 0.05,  $p < 0.05$ , fig 2B). Pearson's correlation coefficient was used to investigate the correlations. There was a substantial positive association between hepatic ISI with BMI (figure 2C & 2D) after the PIO therapy. Correlation between hepatic ISI with HOMA, QUICKI and McA also significant ( $p < 0.001$ , data are not shown).



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

## Discussion

In light of the well-documented relationship between obesity and IR the treatment 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 the possible mechanism by PIO on interference on lipid in the process of improvement of IR in diabetic patients.

Recent studies have demonstrated that the PIO induced weight gain is associated with an increase in subcutaneous adipose tissue and a concomitant decrease in visceral fat content [7]. 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 [7]. Another previous study shows that there was a dose-dependent increase in BW and BMI after 24 weeks in the pioglitazone-treated groups [11]. The seemingly paradoxical relationship between weight gain and 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-. PPAR- activation also induces key enzymes involved in lipogenesis in newly formed adipocytes [2].

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 PPAR agonists improve insulin sensitivity mainly through adipose tissue remodeling, increased capacity for lipid uptake/retention, and altered adipocytokine secretion pattern [14, 16]. Kazunori N. *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 [16]. They did not observe any significant difference in total cholesterol and LDL levels with PIO [16]. Increased visceral fat is associated with IR [16], and reduction in visceral fat would be expected to lead to an enhancement in insulin sensitivity [17]. Because thiazolidinedione treatment consistently reduces plasma free fatty acid levels [17], 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 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 [4, 5]. 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 ( $r = 0.24, P < 0.0001$ ) [12]. 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 mg 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  $\beta$ -cell function and improved whole-body and hepatic insulin sensitivity [16]. 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 [14] ( $P < 0.05-0.01$ ). 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 [18]. It can be

concluded that PIO decreases FBS levels through improvements in hepatic/whole-body insulin sensitivity and in  $\beta$ -cell function in type 2 diabetic patients.

### Acknowledgements

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

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