

# PIOGLITAZONE VERSUS METFORMIN IN TWO RAT MODELS OF GLUCOSE INTOLERANCE AND DIABETES

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## ABSTRACT

Insulin resistance has been implicated in the pathogenesis of type 2 diabetes. High fat diets cause insulin resistance. Both metformin and pioglitazone are considered “insulin sensitizers” and used as antihyperglycemic agents for type 2 diabetes treatment. The aim of this study is to Compare pioglitazone and metformin effects on carbohydrate metabolism and insulin sensitivity in diabetic and glucose intolerant rats on high fat diet. Male albino rats were randomized to seven groups. The 1<sup>st</sup> group received high carbohydrate diet (control). The 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups received high sunflower oil diets for 6 weeks and either left untreated, or given pioglitazone or metformin during the last 3 weeks. The 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> groups were made diabetic by STZ injection on day 15 of the 6 weeks-high fat diet regimen. They were either left untreated, or given pioglitazone or metformin during the last 3 weeks. High-fat diet induced glucose intolerance; represented by increase of serum glucose associated with increase in liver glucose-6-phosphatase and decreases in liver glucose-6-phosphate dehydrogenase and glucokinase activities. No significant differences were observed between pioglitazone and metformin. In diabetic rats, both pioglitazone and metformin decreased elevated serum glucose by ~30%. Only metformin increased hepatic glycogen, and normalized glucose-6-phosphatase activity. On the other hand, pioglitazone normalized elevated renal glycogen content and increased glucose-6-phosphate dehydrogenase activity. High sunflower oil diet impaired glucose tolerance. Pioglitazone and metformin had comparable effects on estimates of carbohydrate metabolism and insulin sensitivity in high-fat fed rats, but different effects in diabetic rats.

**Keywords:** Pioglitazone – metformin – carbohydrate metabolism – high fat diet – type 2 diabetes.

## INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorders affecting almost 6% of the world's population. According to report of the International Diabetes Federation in 2001, the number of diabetic patients will reach 300 million in 2025. More than 97% of these patients will have type II diabetes (Adeghate *et al.*, 2006). In Egypt, one study estimated the combined prevalence of diagnosed and undiagnosed diabetes in the Egyptian population  $\geq 20$  years of age to be 9.3% with a gradient increase from rural (4.9%) to urban areas from lower (13.5%) to higher (20%) socioeconomic standard (Herman *et al.*, 1997).

Insulin resistance, defined as a state of reduced responsiveness to normal circulating levels of insulin, plays a major role in the development of type 2 diabetes. While there is a genetic component involved in developing insulin resistance, onset appears to be triggered by lifestyle. Obesity, along with physical inactivity, can account for approximately 50% of the variability in the insulin mediated glucose disposal in healthy, non-diabetic, normotensive individuals (Reaven *et al.*, 2004). High saturated fat, high calorie, processed

carbohydrate and low fiber diets increase the incidence of insulin resistance (McAuley and Mann, 2006).

Pioglitazone and metformin are extensively used in Egypt and worldwide to treat patients with type II diabetes. Both drugs are considered to be insulin “sensitizers”. However, the full mechanism of action of those two drugs is still unraveled and further investigations remain in necessity to compare their clinical efficacy in different models of glucose intolerance.

Pioglitazone, a thiazolidinedione (TZD) insulin sensitizer, is a peroxisome proliferator activated receptor gamma (PPAR- $\gamma$ ) agonist. It increases insulin sensitivity by regulating the expression of a variety of genes involved in carbohydrate and lipid metabolism, increases GLUT-4 and glucokinase activity, decreases phosphoenol pyruvate carboxykinase (PEPCK) expression, and decreases production by fat cell of several mediators that may cause insulin resistance, such as tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ) and resistin (Cheng and Fantus, 2005; Tjokropawiro, 2006). Pioglitazone increases hepatic and peripheral insulin sensitivity, thereby inhibiting gluconeogenesis and increasing peripheral and splanchnic glucose uptake (Waugh *et al.*, 2006). The prediabetic treatment with

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pioglitazone, despite significant weight gain, completely prevents the development of diabetes and enhances  $\beta$  cell function with preservation of islet cell changes in rats (Choi *et al.*, 2007).

Metformin is the only available biguanide in the market. The mechanisms of metformin-mediated improvement of insulin sensitivity have remained obscure, despite multiple pathways of action being proposed, including a decrease of hepatic glucose production, an increase of peripheral glucose utilization and a reduction of intestinal glucose absorption. It has been documented that metformin activates 5'AMP activated protein kinase (AMPK) in hepatocytes, thereby reducing activity of acetyl CoA carboxylase and lowering expression of lipogenic transcription factor as well as inhibiting hepatic gluconeogenesis (Cheng *et al.*, 2006). The Diabetes Prevention Program (DPP) found that metformin decreased new diagnosis of type 2 diabetes by 31% with more pronounced reduction in young under 45 years by 44% and in the obese with body mass index (BMI)>35 by 53% (Ashcroft, 2006).

Both pioglitazone and metformin appear to have additional effects in ameliorating oxidative stress and inflammation; rendering them attractive tools for prevention of insulin resistance and diabetes (Molavi *et al.*, 2007).

To our knowledge, very few studies are available that directly compare the effects of pioglitazone and metformin on different pathways of carbohydrate metabolism in experimental models of glucose intolerance and insulin resistance. Reports about the comparison between the two drugs with regard to efficacy of glycemic control as related to modulation of metabolism are scarce as well. For the objectives of adding information to these unraveled areas, we studied the effects of pioglitazone and metformin monotherapy on key enzymes of HMP (hexose monophosphate) shunt, gluconeogenesis and glycolysis in liver, as well as on hepatic and renal glycogen contents in experimental models of glucose intolerance and diabetes. Estimates did not pick major differences between the effects of the two drugs. Some distinctions are displayed in the results.

## MATERIALS AND METHODS

### Chemicals

Pioglitazone HCl was provided by the raw materials department of NODCAR (National Organization of Drug Control and Research, Cairo, Egypt). Metformin HCl was kindly provided by CID pharmaceuticals, Cairo, Egypt. Streptozotocin, Glucose-6-phosphate sodium salt, glucokinase, NADP and ATP were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All

the other used chemicals were of the highest analytical grades commercially available.

### Animals

Male Wistar albino rats weighing 170–190 g, purchased from National Research Centre, Cairo, Egypt, were used in the study. The rats were kept in controlled environment and fed ad libitum throughout the study. Study protocols were approved by the local ethics committee in the NODCAR.

### Diets

Rats were randomly assigned to either one of two diet regimens; control rats (n=9) were fed high carbohydrate diet (20% Kcal protein, 10% fat, 70% carbohydrate), all the rest of rats (n=70) were fed high-fat diet (20% Kcal protein, 60% fat, 20% carbohydrate). Diets used in this study (outlined in table 1) are given ad libitum for 6 weeks. Food was withdrawn 5 hours before blood sampling on day 42. Rats had free access to water throughout the study. Rats were weighed at the beginning of the study and then weekly till the end of the study.

**Table 1:** Composition of diets used in this study

	High Carbo- hydrate Diet	High Fat Diet
	<i>g/kg Diet</i>	
Casein	188	254
Cornstarch	438	169
Sucrose	219	85
Wheat bran/cellulose	38	51
Sunflower oil	41	339
Gelatin	14	19
Salt mix	50	65
Vitamin mix	9.7	13
Vitamin E acetate (500 IU/g)	--	0.31
DL-Methionine /L- cystine	2.3	3
	<i>%kcal</i>	
Carbohydrate	69	20
Fat	10	59
Protein	21	21
Caloric value (kcal/g)	3.82	5.20

### Experimental design

Rats were randomly divided into 7 groups. The first group (n=9) received high carbohydrate diet for 6 weeks (42 days) and left untreated (control group). All the other 6 groups received high-fat diet continuously for 6 weeks. Among the 6 groups, following 3 weeks of high-fat diet ingestion, three groups (each n=8) were treated with either pioglitazone (2.7 mg/kg/day, suspended in distilled water and given by oral tube) (HF Pio), metformin (180 mg/kg/day, dissolved in distilled water and given by oral

tube) (HF Met), or no drug (HF) for 3 weeks. The other 3 groups were rendered diabetic by intraperitoneal injection of streptozotocin (50 mg/kg) freshly prepared in 0.1M citrate buffer at pH 4.5 on day 15 and allowed to drink oral glucose 5% w/v overnight. Minimal dose of insulin (1unit/rat) was given for each rat on 2<sup>nd</sup> and 3<sup>rd</sup> day after streptozotocin administration (Wohaieb and Godin, 1987). Induction of diabetes was confirmed on day 21 when serum glucose level was at least 250 mg/dl (Seki *et al.*, 2004). Diabetic rats were then randomly divided into three groups, diabetic untreated (STZ-HF) (n=7) and diabetic treated with either pioglitazone (STZ-HF Pio) (n=6) or metformin (STZ-HF Met) (n=6) with the same protocol as that of high-fat fed rats (table 2).

#### Blood sampling and tissue collection

On day 42 blood samples were collected in plastic centrifuge tubes and allowed to clot at 4°C for 30 minutes. Serum was then separated by centrifugation at 3000 rpm for 20 minutes. On day 43, rats were killed. Livers and kidneys were separated, washed with ice-cold saline, plotted dry with filter paper, weighed and prepared at once for studying enzyme activities and glycogen contents.

#### Enzyme activities and glycogen content

One hundred mg fresh liver tissue was immediately homogenized in 4 ml ice cold EDTA/physiological saline solution and centrifuged at 15000 rpm for 20 minutes at 1.5°C. 0.5 ml of the clear supernatant was used for glucose-6-phosphate dehydrogenase assay as described by Löhr and Waller (Löhr and Waller, 1974). One unit of glucose-6-phosphate dehydrogenase is defined as the amount of enzyme needed to convert 1  $\mu$ mol of glucose-6-phosphate per minute to 6-phosphogluconate at 25°C. For glucose-6-phosphatase assay, another 100 mg fresh liver tissue was similarly homogenized in 4 ml ice cooled citrate buffer and centrifuged at 15000 rpm for 30 minutes at 1.5°C. 0.1 ml of the clear supernatant was used for glucose-6-phosphatase assay as described by Taussky and Shorr (Taussky and Shorr, 1953). One unit of glucose-6-phosphatase is defined as micro moles of inorganic phosphate liberated per minute per gram fresh liver tissue at 37°C. One gram fresh liver tissue was homogenized with 9 ml Tris-KCl-EDTA buffer and centrifuged at 15000 rpm for 1 hour at 1.5°C. 0.025 ml of the clear

supernatant was used for glucokinase assay according to the method of Jamdar and Greengard (Jamdar and Greengard, 1970). One unit of glucokinase is defined as micro moles of NADP/NADPH formed per minute per gram fresh liver tissue at 37°C. Two hundred fifty mg fresh liver tissue was digested with 5 ml 30% KOH and 0.5 ml of the digested tissue was used for glycogen precipitation using 3 ml absolute ethanol. Glycogen was then isolated by centrifugation for 15 minutes at 4000 rpm and determined according to Montgomery method (Montgomery, 1975). The same above procedure was used for renal glycogen determination but using 2 ml of tissue digest instead of 0.5 ml.

#### Blood glucose, triglycerides and insulin

Blood glucose and triglycerides levels were determined in the sera using UDI (United Diagnostic Industry) glucose and triglycerides enzymatic kits, respectively, whereas serum insulin was estimated using rat insulin ELISA kit purchased from Shibayagi, Japan.

#### Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 10 software and Microsoft Excel. All values were presented as means  $\pm$  S.E. (standard error). Comparisons among groups were made by application of two-way analysis of variance ANOVA followed by one way ANOVA and LSD post hoc analysis. Differences were considered statistically significant if  $p < 0.05$ .

## RESULTS

#### Experiments on high fat-fed rats

As shown in table 3, HF rats did not show significant differences in body, liver and kidney weights when compared with control rats. Similarly, serum triglycerides and insulin were not significantly affected. On the other hand, serum glucose level was significantly elevated in HF rats by 18%. This elevation was associated with 18% increase in glucose-6-phosphatase activity, 31% decrease in glucose-6-phosphate dehydrogenase activity, and 46% decrease in glucokinase activity as compared with control rats. Moreover, liver glycogen content was reduced by 34%, though not significant.

**Table 2:** Assignments of the study groups to drugs and diets.

Days/Groups	Control	HF	HF Pio	HF Met	STZ-HF	STZ-HF Pio	STZ-HF Met
0→14	x	+	+	+	+	+	+
15	x	+	+	+	+ S	+ S	+ S
16→20	x	+	+	+	+	+	+
21→42	x	+	+ $\Delta$	+ $\bullet$	+	+ $\Delta$	+ $\bullet$

x = High carbohydrate diet, + = High fat diet,  $\Delta$  = Pioglitazone (2.7mg/kg/day),  $\bullet$  = Metformin (180 mg/kg/day), S = Streptozotocin (50 mg/kg)

Oral administration of pioglitazone (2.7 mg/kg/day) for 21 days was able to significantly decrease body weight and glucose-6-phosphatase activity by 12% and 22%, respectively as compared to HF group. Meanwhile, glucose-6-phosphate dehydrogenase activity was increased by 29%. Other parameters were affected by pioglitazone administration but not significantly under the conditions of the experiment. We were not able to detect significant differences between results of the aforementioned pioglitazone group and the results of oral administration of metformin (180 mg/kg/day) for 21 days.

#### Experiments on high fat-fed STZ diabetic rats

As compared to control rats, STZ-HF rats showed significant decreases in body weight, serum insulin, and liver glycogen by 17%, 51% and 85%, respectively. These were associated with significant increases in kidney weight by 42%, renal glycogen by 92% and serum glucose by 314%. Serum triglycerides were not altered significantly. With regard to liver carbohydrate metabolizing enzymes, significant increase in glucose-6-phosphatase activity by 22% and decreases in glucose-6-phosphate dehydrogenase and glucokinase activities by 47% and 65%, respectively were observed in STZ-HF rats as compared to control animals (table 3).

Oral administration of pioglitazone (2.7 mg/kg/day) and metformin (180 mg/kg/day) significantly reduced blood

glucose level by 32% and 29%, respectively. This decrease was associated with significant decrease in kidney glycogen by 52% and significant increase in glucose-6-phosphate dehydrogenase activity by 20% in the pioglitazone group. In the metformin group, however, blood glucose level decrease was associated with significant increase in liver glycogen by 125% and significant decrease in glucose-6-phosphatase activity by 29%. The significant differences between the two treatments were elicited in liver and kidney glycogen contents and activities of glucose-6-phosphate dehydrogenase and glucose-6-phosphatase.

#### DISCUSSION

The main objective of our study is to compare the effects of pioglitazone and metformin on carbohydrate metabolism and insulin sensitivity in prediabetic and diabetic states. We are unaware of similar study that focuses on the similarities and differences between the two drugs on carbohydrate metabolism. Although it is commonly stated that thiazolidinediones lower glucose concentration primarily by increasing glucose uptake and metformin by decreasing glucose production, the data supporting these statements are scarce and often contradictory (Inzucchi, 2002; Kerpichnikov *et al.*, 2002). In vitro and animal studies have identified multiple potential targets for these drugs (Basu *et al.*, 2008).

**Table 3:** The effects of administration of pioglitazone and metformin for 21 days on study parameters in high fat-fed and STZ diabetic rats. Data are presented as mean  $\pm$  S.E.

	Parameters	Control n=9	HF n=8	HF Pio n=8	HF Met n=8	STZ-HF n=7	STZ-HF Pio, n=6	STZ-HF Met n=6
	Body wt (g)	258.1 $\pm$ 3.45	253.4 $\pm$ 6.34	223.6 $\pm$ 4.13 <sup>a, b</sup>	240.8 $\pm$ 9.13 <sup>a</sup>	216.0 $\pm$ 11.2 <sup>a, b</sup>	224.3 $\pm$ 11.7 <sup>a</sup>	201.8 $\pm$ 10.2 <sup>a</sup>
Serum	Glucose (mg/dl)	98.27 $\pm$ 2.95	116.1 $\pm$ 6.06 <sup>a</sup>	106.8 $\pm$ 3.71	120.2 $\pm$ 4.65 <sup>a</sup>	406.9 $\pm$ 19.6 <sup>a, b</sup>	278.6 $\pm$ 29.0 <sup>a, c</sup>	290.0 $\pm$ 10.6 <sup>a, c</sup>
	Triglycerides (mg/dl)	142.9 $\pm$ 9.56	149.2 $\pm$ 9.94	142.0 $\pm$ 5.33	136.3 $\pm$ 6.2	147.6 $\pm$ 22.5	114.9 $\pm$ 15.6	171.9 $\pm$ 23.8
	Insulin (ng/ml)	2.07 $\pm$ 0.14	2.01 $\pm$ 0.16	1.32 $\pm$ 0.30	2.29 $\pm$ 0.58	1.00 $\pm$ 0.10 <sup>a, b</sup>	1.14 $\pm$ 0.47	2.77 $\pm$ 0.97 <sup>c, d</sup>
Kidney	Wt/100g body wt	0.62 $\pm$ 0.017	0.59 $\pm$ 0.027	0.64 $\pm$ 0.016	0.6 $\pm$ 0.007	0.88 $\pm$ 0.06 <sup>a, b</sup>	0.85 $\pm$ 0.06 <sup>a</sup>	0.94 $\pm$ 0.06 <sup>a</sup>
	Glycogen (mg/g)	0.85 $\pm$ 0.058	0.73 $\pm$ 0.086	0.62 $\pm$ 0.057 <sup>a</sup>	0.61 $\pm$ 0.027 <sup>a</sup>	1.64 $\pm$ 0.16 <sup>a, b</sup>	0.79 $\pm$ 0.14 <sup>c</sup>	1.96 $\pm$ 0.32 <sup>a, c, d</sup>
Liver	wt/100g body wt	3.05 $\pm$ 0.12	3.14 $\pm$ 0.16	2.89 $\pm$ 0.08	3.07 $\pm$ 0.12	3.48 $\pm$ 0.19	3.61 $\pm$ 0.2 <sup>a</sup>	3.83 $\pm$ 0.28 <sup>a</sup>
	glycogen (mg/g)	19.07 $\pm$ 3.33	12.65 $\pm$ 1.86	7.03 $\pm$ 0.56 <sup>a</sup>	6.15 $\pm$ 2.04 <sup>a</sup>	2.93 $\pm$ 0.45 <sup>a, b</sup>	3.55 $\pm$ 0.53 <sup>a</sup>	6.62 $\pm$ 1.44 <sup>a, c, d</sup>
	Glucose-6-phosphatase (unit/g)	0.21 $\pm$ 0.01	0.25 $\pm$ 0.01 <sup>a</sup>	0.20 $\pm$ 0.023 <sup>b</sup>	0.19 $\pm$ 0.018 <sup>b</sup>	0.26 $\pm$ 0.012 <sup>a</sup>	0.257 $\pm$ 0.021 <sup>a</sup>	0.21 $\pm$ 0.009 <sup>c, d</sup>
	Glucose-6-phosphate dehydrogenase unit/g)	2.98 $\pm$ 0.073	2.06 $\pm$ 0.14 <sup>a</sup>	2.65 $\pm$ 0.073 <sup>a, b</sup>	2.6 $\pm$ 0.11 <sup>a, b</sup>	1.59 $\pm$ 0.076 <sup>a, b</sup>	1.91 $\pm$ 0.13 <sup>a, c</sup>	1.754 $\pm$ 0.12 <sup>a</sup>
	Glucokinase (unit/g)	0.054 $\pm$ 0.003	0.029 $\pm$ 0.002 <sup>a</sup>	0.029 $\pm$ 0.001 <sup>a</sup>	0.034 $\pm$ 0.004 <sup>a</sup>	0.019 $\pm$ 0.003 <sup>a, b</sup>	0.027 $\pm$ 0.003 <sup>a</sup>	0.022 $\pm$ 0.003 <sup>a</sup>

<sup>a</sup> Significance from control at p<0.05

<sup>b</sup> Significance from high fat-fed rats for groups HF Pio, HF Met, STZ-HF at p<0.05

<sup>c</sup> Significance from STZ-HF diabetic rats for groups STZ-HF Pio and STZ-HF Met at p<0.05

<sup>d</sup> Significance from STZ-HF Pio for group STZ-HF Met at p<0.05.

Moreover, the results have not always been consistent in human.

For these reasons, we tested the drugs in two models of impaired glucose tolerance; one is induced by high-fat diet only (prediabetic state) and the other is induced by high-fat diet associated with impaired insulin secretion (diabetic state).

The model of high-fat diet-induced glucose intolerance had been extensively used by others and was shown to impair carbohydrate metabolism, increase hepatic glucose production and induce insulin resistance (Wilkes *et al.*, 1998; Mithieux *et al.*, 2002; Henriksen *et al.*, 2008). It is generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised  $\beta$ -cell function (Buettner *et al.*, 2006). Sunflower oil, which is used in this study to induce glucose intolerance, is very similar in composition to safflower (carthame) oil that has been used in previous studies for that purpose (Wilkes *et al.*, 1998; Mithieux *et al.*, 2002). Sunflower oil is very abundant in Egypt and represents the main oil used in daily diets.

It was previously reported that rats fed HF diets for 3 weeks exhibited moderate hepatic insulin resistance as compared with rats fed HF diets for 6 weeks (Mithieux *et al.*, 2002). The 6 weeks model was thus used in this study. Results demonstrated that the 6 week sunflower-rich diet increased hepatic glucose output through several mechanisms; significant reduction in hepatic glycogen deposition, stimulation of the gluconeogenic glucose-6-phosphatase activity and decreased hepatic glucose utilization through reducing the activities of the HMP shunt enzyme glucose-6-phosphate dehydrogenase and glycolytic enzyme glucokinase in liver.

The molecular mechanisms behind high-fat diet-induced glucose intolerance are still not fully revealed. However, several mechanisms were postulated. One study correlated the increased glucose intolerance caused by high-fat diet to the elevated levels of plasma NEFA (nonesterified fatty acids) (Wang *et al.*, 2002). Others referred to the elevated circulating leptins, PPAR-gamma genotype susceptibility, increased fatty acid oxidation in muscles, and deficiency in muscle mitochondria (Ahren and Scheurink, 1998; Kadowaki *et al.*, 2003; Bringolf *et al.*, 2005; Hancock *et al.*, 2008). Özela *et al.* indicated that neither defects in insulin receptor function nor elevated membrane glycoprotein PC-1 activities are involved in the development of insulin resistance in rats with high-fat feeding, and the insulin resistance induced with high-fat feeding is likely due to postreceptor defects in skeletal muscle (Özela *et al.*, 1996). The door is still open for more investigations to come.

Impairment of insulin production by streptozotocin added to the results of high-fat diet several modifications. Compared to the HF-group, STZ-HF animals experienced lower body weights, hepatic glycogen contents, serum insulin and activities of glucose-6-phosphate dehydrogenase and glucokinase. Meanwhile higher renal weight, renal glycogen content, and serum glucose were observed. This pattern well complies with the reported biochemical changes in experimental model of type II diabetes.

Doses chosen for pioglitazone and metformin in this study are in harmony with their therapeutic doses in human, being equivalent to 30 mg/day for pioglitazone and 2000 mg/day for metformin. Drugs were administered for 3 weeks to study their effects when given as chronic treatments, and were administered concomitantly with diet to study their effects under uncontrolled diet conditions.

In presence of high-fat diet, administration of pioglitazone or metformin caused almost similar biochemical changes, but with few exceptions. Both drugs did not show significant effects in this model with regard to liver and kidney weights, serum glucose and insulin levels, and glucokinase activity. On the other hand, they significantly improved glucose-6-phosphate dehydrogenase activity and normalized glucose-6-phosphatase activity. These findings are consistent with the findings of Sugiyama *et al.* for pioglitazone effect on glucose-6-phosphatase activity and oppose their findings for pioglitazone on glucokinase activity, which showed increased hepatic glucokinase activity by pioglitazone. This may be attributed to the differences in the insulin resistance model used, as Sugiyama *et al.* used genetically obese Wistar rats (Sugiyama *et al.*, 1990). For metformin, these findings are consistent with those of Mithieux *et al.* (2002). The shift of glucose-6-phosphate flux to HMP shunt, mediated by reduction of glucose-6-phosphatase and increase in glucose-6-phosphate dehydrogenase, is also consistent with the findings of Kletzien *et al.* (1992) for pioglitazone and Mithieux *et al.* (2002) for metformin (Kletzien *et al.*, 1992; Mithieux *et al.*, 2002).

However, it was noticeable that the decrease of glucose-6-phosphatase activity was not accompanied by stimulation of hepatic glycogenesis. Rather, a remarkable decrease in liver glycogen was observed for both drugs as compared to normal and untreated HF animals. These data contradict the findings of Mithieux *et al.* for metformin and that of Sugiyama *et al.* for pioglitazone (Mithieux *et al.*, 2002; Sugiyama *et al.*, 1990). In the former study liver glycogen content was dramatically increased by 3-5 times by concomitant administration of 50 mg/kg/day metformin with high-fat diet for 6 weeks. In the second study 0.3-3 mg/kg/d for 7 days pioglitazone enhanced insulin-stimulated glycogen synthesis in genetically-obese hyperglycemic rats. Meanwhile, our results are consistent with the findings of Radziuk and Pye who reported the

decrease of both gluconeogenesis and glycogen synthesis by metformin and with those of Otto *et al.* who reported inhibition of glycogen synthesis by metformin in cultured rat hepatocytes (Radziuk and Pye, 2001; Otto *et al.*, 2003).

Contradictory data for the metabolic effects of pioglitazone and metformin are not uncommon in literature. One of the observed differences between pioglitazone and metformin is the more pronounced reduction in body weight of rats on pioglitazone therapy. These rats showed reduced rate of body weight gain from the beginning of the study.

To compare the effects of pioglitazone and metformin in the diabetic state, rats were fed high-fat diet before STZ injection and this feeding was continued throughout the study. High-fat feeding induced insulin resistance and STZ injection induced partial  $\beta$  cell destruction and insulin deficiency, giving a diabetic model similar to the pathophysiologic changes in type 2 diabetic human, being preceded by insulin resistance state before  $\beta$  cell failure and appearance of overt diabetes (Cheng and Fantus, 2005).

With regard to the effects of pioglitazone and metformin on diabetic rats, both drugs succeeded to reduce serum glucose level elevated by STZ by ~30 %. This complies with the findings of Pavo *et al.* who reported comparable improvement in glycemic control by pioglitazone and metformin in patients with type II diabetes (Pavo *et al.*, 2003).

The decrease of glycogen content in liver (insulin dependent tissue) and the increase in kidneys (insulin independent tissue) in STZ animals well complies with previous reports (Gad *et al.*, 2006). Only pioglitazone was able to reduce elevated renal glycogen content in STZ-HF rats. On the other hand, only metformin increased hepatic glycogen content. These observations are similar to the findings of Okine *et al.* who observed an increase in hepatic glycogen content of STZ induced diabetic mice after metformin administration (Okine *et al.*, 2005). This contradicts our findings of metformin effect on hepatic glycogen content of HF rats, suggesting that the inhibitory effect of metformin on glycogen synthesis is lost under hyperglycemic conditions.

Previous studies have shown that STZ diabetic rats exhibited severe loss of body weight associated with a significant increase in kidney weight as well as kidney weight to body weight ratio (Gad *et al.*, 2006). These alterations were also evident in our study. Reduced body weight of STZ-HF rats was further reduced by metformin, though not significant, and was slightly increased by pioglitazone. This is consistent with the well known properties of metformin and pioglitazone concerning their

effect on body weight, where metformin is known to decrease or at least not to increase body weight and pioglitazone is known to increase body weight via water retention (Cheng and Fantus, 2005). The increase in kidney weight in STZ-HF Met group is correlated with the increased renal glycogen.

STZ-HF rats showed increased glucose-6-phosphatase activity associated with reduced activities of both glucose-6-phosphate dehydrogenase and glucokinase activities. This shift is mainly induced by the high glucagon/insulin ratio in STZ animals. Similar to its effect in HF rats, metformin was able in STZ-HF rats to normalize glucose-6-phosphatase activity. This is consistent with the findings of Heishi *et al.*, who found reduction of glucose-6-phosphatase gene expression associated with reduction of glucose-6-phosphatase activity in livers of obese diabetic db/db mice (Heishi *et al.*, 2006). However, the insignificant effect of metformin on glucose-6-phosphate dehydrogenase activity contradicts the findings of Ashokkumar *et al.* that demonstrated the ability of metformin to restore glucose-6-phosphate dehydrogenase activity almost to control levels. However, this experiment was done in neonatal STZ induced diabetic rats, where a single 100 mg/kg STZ injection was given to 2 days old rats (Ashokkumar *et al.*, 2005). On the other hand, pioglitazone was able in STZ-HF rats to increase glucose-6-phosphate dehydrogenase activity, similar to its effect on HF rats.

Finally, our salient conclusions of this study are:

- 1) High-sunflower oil diet impairs glucose tolerance and disrupts carbohydrate metabolism via decreasing hepatic glycogen content and impairing activities of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and glucokinase.
- 2) STZ induced diabetes caused marked elevation in serum glucose level associated with marked decrease in serum insulin level and body weight, impairment of carbohydrate metabolism as demonstrated by dramatic decrease in hepatic glycogen content, increase in renal glycogen content and impairment of activities of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and glucokinase.
- 3) In high-fat diet rats, metformin and pioglitazone had almost similar effects; both activated glucose-6-phosphate flux to HMP shunt, mediated by reduction of glucose-6-phosphatase and increase in glucose-6-phosphate dehydrogenase activities.
- 4) In diabetic high-fat diet rats, metformin and pioglitazone equally depressed elevated serum glucose level by ~30%. Pioglitazone therapy was associated with a decrease in renal glycogen and an increase in glucose-6-phosphate dehydrogenase activity. On the other hand, metformin therapy was associated with an increase in hepatic glycogen and normalization of glucose-6-phosphatase activity.

Further comparative studies between pioglitazone and metformin are recommended on the metabolic, cellular and molecular levels to show whether or not pioglitazone has any added favorable actions than the cheaper, safer, and early-used metformin.

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