Amelioration of High Fructose Diet-Induced Insulin Resistance, Hyperuricemia, and Liver Oxidative Stress by Combined Use of Selective Agonists of PPAR- α and PPAR- γ in Rats

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Keywords

Fenofibrate · High fructose diet · Insulin resistance · Metabolic syndrome · Pioglitazone · Uric acid

Abstract

Background: The use of high-fructose (Fr) corn sweeteners and sucrose in manufactured food has markedly increased recently. This excessive Fr intake has been proposed in the etiology of the metabolic syndrome, which shows an increasing prevalence throughout the world. **Objective:** In this study, we questioned whether fenofibrate (FF), a peroxisome proliferator-activated receptor (PPAR)- α agonist, and pioglitazone (PG), a PPAR- γ agonist, might be effective in ameliorating the metabolic syndrome in a rat model. **Materials and Methods:** The metabolic syndrome was induced by feeding rats a high-Fr (60%) diet for 10 weeks. The rats were divided into 5 groups: control group, fed a normal rat chow; Fr + vehicle group; Fr + FF group; Fr + PG group; and Fr + (FF + PG) group (treated with both drugs). Drug or vehicle treatment was given daily for 6 weeks (from weeks 5 to 10). Thereafter, blood and liver samples were obtained for biochemical studies. **Results:** Rats fed a high-Fr diet devel-

oped hyperglycemia, hyperinsulinemia, hyperuricemia, hypertriglyceridemia, and hypercholesterolemia, and had increased serum alanine aminotransferase, hepatic tumor necrosis factor- α , and malondialdehyde levels but decreases in both glutathione content and superoxide dismutase activity. Rat treatment with FF and/or PG attenuated these alterations. The improvement was greater with the combined treatment than with either drug alone, and normalization of insulin sensitivity was observed only in rats treated with the combination therapy. **Conclusion:** Acting on the 2 main PPAR subfamilies, the combination of FF and PG provides a more efficacious therapy for modulating the changes in serum insulin, uric acid, and lipids, as well as the accompanying hepatic inflammation and oxidative stress that characterize the Fr-induced metabolic syndrome.

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Introduction

During the past 2 decades, dietary consumption of fructose (Fr) has increased several folds above the amount present in natural foods because of the use of high-Fr corn

sweeteners and sucrose in manufactured food [1, 2]. This excessive intake of Fr has been proposed in the etiology of the metabolic syndrome whose prevalence is dramatically increasing throughout the world in Western and developing countries [3, 4]. In addition, oral ingestion of Fr, unlike glucose, results in an increase in serum uric acid, which may contribute to the pathogenesis of the metabolic syndrome [5].

The metabolic syndrome is a group of common pathologies including hyperglycemia, hyperinsulinemia, insulin resistance (IR), and dyslipidemia manifested essentially by elevated serum triglycerides (TG) and total cholesterol levels concomitant with reduced high-density lipoprotein-cholesterol, which increase the risk of type 2 diabetes mellitus and cardiovascular disease [6–8]. Although high-Fr intake has been linked to excessive weight gain, there is consensus that obesity is not a primary requisite for the diagnosis of the metabolic syndrome [9]. In fact, the metabolic syndrome carries a greater predictive value for cardiovascular morbidity and mortality than its components [10]. Thus, it is crucial to identify therapeutic approaches for preventing and treating this serious health problem.

Therapeutically, important questions about the metabolic syndrome remain to be addressed, including whether the syndrome is a condition that represents a clear indication for certain drug therapies. An example of such a therapy is the class of the dual peroxisome proliferator-activated receptor (PPAR) agonists. These agents target PPAR- α and PPAR- γ and were empirically discovered because of their abilities to improve insulin sensitivity and dyslipidemia [11]. Taken together, simultaneous activation of PPAR- α and PPAR- γ could be a potential therapeutic option to attenuate hyperlipidemia and hyperglycemia in the metabolic syndrome. However, previous studies have demonstrated that concomitant activation of PPAR- α and PPAR- γ using PPAR- α/γ dual agonists such as tesaglitazar and mura-glitazar may be associated with cardiovascular risks and carcinogenicity due, most probably, to the unbalanced supratherapeutic activation of PPAR- α and PPAR- γ [11, 12].

Therefore, the present study was undertaken to evaluate the effects of a selective agonist for each receptor type using fenofibrate (FF), a commonly used PPAR- α agonist [13], and pioglitazone (PG), a commonly used PPAR- γ agonist [14], given either alone or in combination, on the serum and hepatic pathogenetic alterations that occur with the metabolic syndrome induced by high-Fr diet in a rat model.

Materials and Methods

Animals

Thirty adult male albino rats matched for age (3–4 months old) and weight (120–150 g) were used in this study. Rats were housed 2/ cage under good sanitary conditions and normal humidity, with free access to food and water during the experimental period (10 weeks). The rats were maintained in the Animal Research Facility of the Medical Research Institute, Alexandria University, under standard conditions of temperature ($25 \pm 1^\circ\text{C}$) with a 12-h light/dark cycle and allowed 1 week of acclimatization prior to study start.

Induction of IR (Metabolic Syndrome)

The metabolic syndrome was induced by feeding rats with a high (60%)-Fr diet for 10 weeks [15]. This diet was prepared by adding 600 g Fr to 400 g standard rat chow. Control rats were fed standard rat chow without Fr. The standard diet contained 46% complex carbohydrates, composed mainly of corn starch, whereas the Fr diet contained 60% Fr as the main carbohydrate. The caloric contents of these diets are 3.1 and 3.6 kcal/g, respectively [5, 15].

Experimental Design

- The rats, in this study, were divided into 5 groups with 6 rats each.
- Group I (control group): the rats in this group had free access to standard rat chow and tap water throughout the study. These rats were given the vehicle (3 ml/kg carboxymethyl cellulose [CMC] as a 0.5% suspension) orally by gavage, starting at the end of the 4th week and continued daily for the last 6 weeks of the experimental period.
- The rats of the following groups were fed the high-Fr diet and received daily drug (or vehicle) treatment orally by gavage starting at the end of the 4th week and continued for the last 6 weeks of the experimental period:
- Group II (Fr group, Fr-fed rats): the rats of this group were treated with the vehicle as in group I.
- Group III (Fr + FF group): the Fr-fed rats of this group were treated with FF (Medizen Pharmaceutical Co., Alexandria, Egypt) at 09.00 a.m. at a dose of 100 mg/kg/day [16] in 3 mL/kg CMC.
- Group IV (Fr + PG group): the Fr-fed rats of this group were treated with PG (Amriya Pharmaceutical Co., Alexandria, Egypt) at 10.00 a.m. at a dose of 15 mg/kg/day [17] in 3 mL/kg CMC.
- Group V (Fr+ [FF + PG] group): the Fr-fed rats of this group were treated with both FF (100 mg/kg/day) and PG (15 mg/kg/day) as in groups III and IV.

Before the beginning of drug (or vehicle) treatment, a blood sample was obtained from the tail vein of each rat to determine the blood glucose level. Body weight was measured daily, and the net percentage increase in body weight over the period of the study (10 weeks) was calculated in each rat. At the end of the experimental period, the rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.), and a midline incision was made. Blood was collected from the descending aorta, left 60 min to clot, and serum was separated by centrifugation at 5,000 rpm for 5 min and stored at -20°C until used for biochemical analysis. Immediately after blood collection, livers were excised, washed with ice-cold saline, blotted dry, and preserved at -80°C until assayed for hepatic tissue biochemical parameters.

Table 1. Initial (before treatment) and final (6 weeks after daily drug or vehicle treatment) fasting blood glucose levels and percent increase in body weight (BW) of rats fed a high-fructose diet (Fr) and treated daily with fenofibrate (FF), pioglitazone (PG), or both drugs

Experimental groups	Fasting blood glucose levels, mg/dL		Increase in BW, %
	initial (week 4)	final (week 10)	
Control	99.67±0.95	99.83±2.36	121.61±3.91
Fr	117.67±4.42 ^a	122.67±2.49 ^a	117.37±3.56
Fr + FF	118.00±3.31 ^a	105.00±3.06 ^{b, c}	116.28±3.36
Fr + PG	123.50±5.09 ^a	102.60±2.38 ^{b, c}	117.67±5.53
Fr + (FF + PG)	117.00±2.71 ^a	103.67±3.29 ^{b, c}	106.17±2.22

Increase in BW (%) = (final BW – initial BW) × 100/initial BW. Results are expressed as means ± SEM ($n = 6$ rats per group). ^a $p < 0.05$ vs. the corresponding control group, ^b $p < 0.01$ vs. the corresponding initial glucose level (paired t test), ^c $p < 0.001$ vs. the corresponding Fr group.

Blood Biochemical Analyses

Fresh blood samples were used for estimating fasting blood glucose levels using an automatic blood glucose meter (Super Glucocard, Japan). Fasting serum insulin levels were determined using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Sun Red Biological Technology Co., Shanghai, China). IR was assessed by calculating the HOMA (homeostasis model assessment)-IR index using the following formula [18]:

$$\text{HOMA-IR} = [\text{fasting blood glucose (mg/dL)} \times \text{fasting insulin } (\mu\text{IU/mL})] / 405.$$

Insulin sensitivity was assessed by calculating the quantitative insulin sensitivity check index (QUICKI) using the following formula [19]:

$$\text{QUICKI} = 1 / [\log \text{fasting glucose (mg/dL)} + \log \text{fasting insulin } (\mu\text{IU/mL})].$$

Serum alanine aminotransferase (ALT) activity and TG, total cholesterol, and uric acid levels were measured using standard diagnostic kits (Biodiagnostic Co., Cairo, Egypt).

Hepatic Tissue Biochemical Analyses

A known weight of liver tissue was homogenized with 0.05 M potassium phosphate buffer (pH 7.5) to prepare a 10% (w/v) homogenate. After centrifugation of the homogenate at 4,000 rpm for 15 min, the supernatant was removed. The reduced glutathione (GSH) content was determined by a method based on the reduction of 5,5'-dithiobis-2-nitrobenzoic acid with GSH, as described previously [20], and lipid peroxidation was estimated by the thiobarbituric acid reaction by measuring the malondialdehyde (MDA) level [21]. Another portion of liver tissue was homogenized in 0.1 M potassium phosphate buffer (pH 7.4) and centrifuged, and then the clear supernatant was removed. For the superoxide dismutase (SOD) assay, the activity of the enzyme was determined using a method based on the inhibition of phenazine methosulfate-mediated reduction in nitroblue tetrazolium dye with SOD [22], and the tumor necrosis factor (TNF)- α level was determined using a rat TNF- α ELISA kit (Sun Red Biological Technology Co., Shanghai, China).

Statistical Analysis

Results are expressed as means ± SEM. Differences among the experimental groups, in terms of the studied parameters, were assessed with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA). The paired t test was used to compare initial and final fasting blood glucose levels. Correlations between variables were tested by computing the correlation coefficient (r , Pearson's test). Differences with $p < 0.05$ were considered significant.

Results

Effect of a High-Fr Diet and Drug Treatment on Blood Glucose Levels and Body Weight Gain

Fr feeding for 4 weeks caused a significant increase in blood glucose levels compared to control diet feeding (Table 1). Daily administration of FF, PG, or FF + PG to Fr-fed rats, starting at the beginning of week 5 and continued for 6 weeks, reversed the changes in blood glucose levels compared to the vehicle-treated Fr-fed rats. The pairwise analysis of data showed normalization of blood glucose levels in all drug-treated groups compared to rats fed control diet. As shown in Table 1, no significant difference was observed among the 5 experimental groups with respect to the gain in body weight regardless of the type of diet or treatment given.

Effect of Drug Treatment on Serum Uric Acid and Insulin Levels, IR, and Insulin Sensitivity in High Fr Diet-Fed Rats

As shown in Table 2, rats fed a high-Fr diet showed significantly higher serum uric acid and insulin levels than control diet-fed rats. Daily administration of FF or

Table 2. Effects of treatment with fenofibrate (FF), pioglitazone (PG), or both drugs (FF + PG) on serum uric acid and insulin levels, insulin resistance (IR), and insulin sensitivity in rats fed a high-fructose (Fr) diet

Experimental groups	Serum uric acid, mg/dL	Serum insulin, μ IU/mL	HOMA-IR	QUICKI
Control	1.59 $\pm$ 0.25	5.57 $\pm$ 0.52	1.41 $\pm$ 0.12	0.364 $\pm$ 0.004
Fr	4.06 $\pm$ 0.43 ^a	11.98 $\pm$ 0.37 ^a	3.29 $\pm$ 0.20 ^a	0.320 $\pm$ 0.003 ^a
Fr + FF	1.67 $\pm$ 0.12 ^b	8.67 $\pm$ 0.60 ^{a, b}	2.38 $\pm$ 0.19 ^{a, b}	0.337 $\pm$ 0.005 ^{a, b}
Fr + PG	2.55 $\pm$ 0.32 ^b	8.20 $\pm$ 0.20 ^{a, b}	2.16 $\pm$ 0.10 ^{a, b}	0.341 $\pm$ 0.002 ^{a, b}
Fr + (FF + PG)	1.22 $\pm$ 0.26 ^{b, c}	6.38 $\pm$ 0.23 ^{b-d}	1.63 $\pm$ 0.07 ^{b, d}	0.350 $\pm$ 0.002 ^{b, d}

Results are expressed as means $\pm$ SEM ($n = 6$ rats per group). HOMA-IR, homeostasis model assessment-IR; QUICKI, quantitative insulin sensitivity check index. ^a $p < 0.05$ vs. the control group, ^b $p < 0.05$ vs. the Fr group, ^c $p < 0.05$ vs. the Fr + PG group, ^d $p < 0.05$ vs. the Fr + FF.

Fig. 1. Correlation between serum uric acid level and serum insulin level (**a**) and serum triglyceride level (**b**) in rats of all experimental groups.

Fig. 2. Serum triglyceride (a) and total cholesterol (b) levels in rats fed control or high-fructose (Fr) diet with daily treatment with fenofibrate (FF), pioglitazone (PG), or both drugs (FF + PG). Results are expressed as means $\pm$ SEM ($n = 6$ rats per group) and analyzed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ compared to the control group; # $p < 0.05$ compared to the Fr group; \$ $p < 0.05$ compared to the Fr + FF group; + $p < 0.05$ compared to the Fr + PG group.

PG alone to Fr-fed rats for 6 weeks reversed the changes in serum uric acid levels and partially, but significantly, reduced the increase in serum insulin levels compared to the vehicle-treated Fr-fed group. Treatment of Fr-fed rats simultaneously with both FF and PG resulted in normalization of the serum levels of both uric acid and insulin compared to control diet-fed rats. As shown in Figure 1a, a positive correlation between serum uric acid and insulin levels was observed in this study using the combined results from all rats ($r = 0.733$, $p < 0.001$).

At the end of the experimental period (10 weeks), high Fr-fed rats had a significantly higher HOMA-IR score (IR index) and a significantly lower QUICKI score (an insulin sensitivity index) than control diet-fed rats (Table 2). Daily administration of FF or PG during the last 6 weeks of the experimental period significantly attenuated these changes in Fr-fed rats. Normalization of HOMA-IR and

QUICKI scores was observed only in Fr-fed rats treated with FF + PG as compared to control diet-fed rats.

Effect of Drug Treatment on Serum TG and Total Cholesterol in High Fr-Fed Rats

Serum TG and total cholesterol levels were significantly increased in high Fr-fed rats compared to control diet-fed rats (Fig. 2). Treatment with FF, PG or their combination during weeks 5–10 of the Fr feeding period abolished the elevation in the TG level (Fig. 2a). This decrease in serum TG levels correlated directly with the reduction in serum uric acid levels ($r = 0.735$, $p < 0.001$, Fig. 1b). Treatment of high Fr-fed rats with FF or FF + PG resulted also in a significant decrease in serum total cholesterol levels compared to vehicle-treated Fr-fed rats (Fig. 2b). Treatment of rats with PG alone had no effect on the changes in serum total cholesterol induced by high-Fr feeding.

Table 3. Effects of treatment with fenofibrate (FF), pioglitazone (PG), or both drugs (FF + PG) on serum alanine aminotransferase (ALT) activity and liver tissue tumor necrosis factor (TNF)- α and oxidative stress markers in rats fed a high-fructose (Fr) diet

Experimental groups	Serum	Liver tissue			
	ALT, U/mL	TNF- α , ng/g tissue	MDA, nmol/g tissue	GSH, μ mol/g tissue	SOD, U/g tissue
Control	58.67 $\pm$ 2.56	2.28 $\pm$ 0.19	15.59 $\pm$ 1.5	410.33 $\pm$ 16.20	798.33 $\pm$ 38.98
Fr	76.00 $\pm$ 2.13 ^a	4.46 $\pm$ 0.45 ^a	33.47 $\pm$ 4.13 ^a	113.67 $\pm$ 20.05 ^a	214.11 $\pm$ 29.13 ^a
Fr + FF	62.67 $\pm$ 2.51 ^b	2.45 $\pm$ 0.10 ^b	15.35 $\pm$ 1.72 ^b	520.67 $\pm$ 80.13 ^b	787.00 $\pm$ 27.15 ^b
Fr + PG	65.17 $\pm$ 3.05 ^b	3.14 $\pm$ 0.11 ^b	16.55 $\pm$ 1.81 ^b	210.00 $\pm$ 38.22 ^{a, c}	506.61 $\pm$ 27.18 ^{a-c}
Fr + (FF + PG)	59.67 $\pm$ 2.38 ^b	2.70 $\pm$ 0.12 ^b	17.71 $\pm$ 1.51 ^b	418.33 $\pm$ 53.03 ^{b, d}	766.38 $\pm$ 47.70 ^{b, d}

Results are expressed as means $\pm$ SEM ($n = 6$ rats per group). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase. ^a $p < 0.05$ vs. the control group, ^b $p < 0.05$ vs. the Fr group, ^c $p < 0.05$ vs. the Fr + FF group, ^d $p < 0.05$ vs. the Fr + PG group.

Effect of Drug Treatment on Serum ALT and Hepatic Tissue TNF- α and Oxidative Stress Markers in High Fr-Fed Rats

Compared to control diet-fed rats, high Fr-fed rats showed a significant increase in serum ALT activity and hepatic tissue TNF- α and MDA levels (Table 3). Normalization of these parameters was observed at the end of the experimental period in high Fr-fed rats treated with FF, PG, or FF + PG. As shown in Figure 3, the reduction in hepatic TNF- α levels correlated directly with the decrease in serum uric acid ($r = 0.635$, $p < 0.001$; Fig. 3a), hepatic MDA ($r = 0.675$, $p < 0.001$; Fig. 3b), and the HOMA-IR score levels ($r = 0.703$, $p < 0.001$, Fig. 3c). Additionally, the hepatic GSH content and SOD activity were significantly lower in the vehicle-treated high Fr-fed rats than in control diet-fed rats (Table 3). These changes were prevented in high Fr-fed rats treated with FF or FF + PG, while treatment with PG alone failed to produce a similar significant effect on hepatic GSH content but significantly attenuated the decrease in hepatic SOD activity compared to the vehicle-treated high Fr-fed rats.

Discussion

The high Fr diet used in this study resulted in several metabolic disturbances including hyperglycemia, hyperinsulinemia, IR, hyperuricemia, hypertriglyceridemia, and hypercholesterolemia together with evident hepatic oxidative stress in rats. These changes correspond with the diagnostic criteria of the human metabolic syndrome [2, 8, 23]. Clinically, the epidemics of this syndrome often

parallel high Fr intake [24]. Fr, unlike glucose, does not directly stimulate insulin secretion, possibly due to the absence of the Fr transporter (GLUT5) on pancreatic β -cells [1]. However, chronic exposure to Fr can indirectly create compensatory hyperinsulinemia with IR due to a downregulation of insulin receptors with lower expression of insulin receptor mRNA in the liver, adipose tissue, and skeletal muscles [25, 26]. On the other hand, the hypertriglyceridemia and hypercholesterolemia, observed in high Fr-fed rats, may be attributed to several mechanisms. These include enhanced hepatic lipogenesis, overproduction and impaired peripheral catabolism of very low-density lipoprotein, increased gene expression of acetyl CoA-carboxylase and fatty acid synthase, and decreased TG clearance due to a reduction in lipoprotein lipase activity in endothelial cells [27].

Also, our results demonstrated that the association of elevated serum uric acid levels with hyperinsulinemia, hypertriglyceridemia, and elevated hepatic TNF- α expression was strong. The mechanism by which excess Fr intake raises serum uric acid has been previously studied. Fr enters hepatocytes, where it is rapidly phosphorylated by fructokinase to Fr-1-phosphate [28]. During this reaction, ATP donates phosphate with the generation of ADP, which is further metabolized to uric acid in the liver resulting in hyperuricemia. In this regard, it has been suggested that uric acid may have a role in the pathogenesis of the Fr-mediated metabolic syndrome [29]. Compatible with this concept, it has been reported that lowering the serum uric acid level with the use of xanthine oxidase inhibitors or uricosuric agents could alleviate or prevent some of the features of the metabolic

Fig. 3. Correlation between hepatic tissue tumor necrosis factor (TNF)- α level and serum uric acid level (**a**); hepatic tissue malondialdehyde (MDA) level (**b**); and homeostasis model assessment-insulin resistance (HOMA-IR) score (**c**) in rats of all experimental groups.

syndrome, particularly hyperinsulinemia and hypertriglyceridemia in rats [5, 30]. On the other hand, some other reports on the metabolic syndrome consider hyperuricemia to be a consequence of elevated serum insulin levels with enhanced renal reabsorption of uric acid in the metabolic syndrome [29, 31]. This interpretation is supported by the finding that thiazolidinediones, which improve insulin sensitivity and lower insulin levels, also reduce elevated serum uric acid levels in diabetic patients [32, 33]. Consistent with this observation is the finding, in our study, that in Fr-fed rats improving insulin sensitivity with PG treatment reduced serum uric acid levels and improved other features of the metabolic syndrome. In addition, our results showed that normalization of serum TG levels with FF also reduced elevated serum uric acid levels in Fr-fed rats. One explanation for these results is the possibility that the de novo increase in purine synthesis in Fr-fed rats may be pathogenetically linked to hepatic fatty acid synthesis, resulting in TG overproduction [34]. However, to fully determine whether uric acid is a result or an initiator of the metabolic syndrome, further studies are needed.

Inflammation has been reported to have a role in pathogenesis of IR and the metabolic syndrome [35]. Our study showed a significant elevation in the hepatic level of TNF- α , a proinflammatory cytokine, with a significant increase in serum ALT activity, an indicator of liver dysfunction, in Fr-fed rats compared to control diet-fed rats. Elevated hepatic TNF- α has been shown to contribute to the progression of low-grade hepatic inflammation, fibrosis, and cirrhosis, elicit a direct negative effect on the insulin signaling pathway by altering tyrosine and serine phosphorylation of insulin receptor substrate, and to induce liver IR [36, 37].

Moreover, there is evidence that oxidative stress plays a role in the pathophysiology of the metabolic syndrome. In our study, high-Fr feeding of rats resulted in a significant increase in lipid peroxidation and decrease in antioxidant defenses (GSH and SOD) in the liver. These results are consistent with earlier studies that had shown attenuation of oxidative stress in Fr-fed rats treated with S-methyl-L-cysteine and other agents with antioxidant properties [15, 23]. High Fr intake may induce oxidative stress through different pathways, including a decrease in Cu-Zn SOD [38] and mRNA expression of catalase [39], and increases in NADPH oxidase activity [40], xanthine oxidase activity [41], and the production of TNF- α , which increases the release of reactive oxygen species (ROS) and mitochondrial permeability [42]. Clearly, depletion of antioxidant defenses may hinder ROS inactivation, in-

crease lipid peroxidation, and augment ROS-mediated tissue injury [43].

A major finding, in our study, was that FF treatment offered multiple benefits in the metabolic syndrome induced by Fr. Treatment of Fr-fed rats with FF normalized elevated serum levels of glucose, uric acid, TG, total cholesterol, and ALT activity. FF treatment also resulted in a significant decrease in both IR and elevated serum insulin levels. In addition, FF treatment significantly reduced the elevated hepatic TNF- α and MDA levels and significantly increased the reduced GSH level and SOD activity in the liver of Fr-fed rats compared to control diet-fed rats.

The lipid-lowering effects of FF observed in our study are in accordance with the results of previous studies and could be attributed to PPAR- α activation in tissues with a high capacity for fatty acid oxidation, particularly the liver [44–46]. This action results in a decrease in plasma TG concentration by increasing lipoprotein lipase activity and accelerating fatty acid β -oxidation. Also, down-regulation of the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a rate-limiting enzyme in cholesterol biosynthesis, occurs upon PPAR- α activation with a subsequent decrease in plasma cholesterol levels [45]. In addition, we demonstrated that FF treatment of our Fr-fed rats favorably modulated the imbalanced liver tissue oxidant/antioxidant status. This antioxidant effect of FF may, at least in part, depend on increased levels of antioxidant enzymes, such as SOD, through PPAR- α activation, and on its ability to inhibit superoxide anion generation by leukocytes [44, 47]. The beneficial effects of FF observed in our study were also, at least partially, attributable to its anti-inflammatory effect manifested by a significant decrease in the hepatic level of TNF- α in Fr-fed rats as compared to control diet-fed rats. This finding is in accordance with the results of earlier studies in which FF treatment in patients with atherosclerosis and hyperlipoproteinemia resulted in a reduction in plasma cytokine concentrations [48].

Similarly, PG treatment, in this study, improved insulin sensitivity and reduced elevated blood glucose, insulin, uric acid, TG, and total cholesterol levels, as well as ALT activity in Fr-fed rats. It also reduced elevated hepatic TNF- α and MDA levels and improved hepatic SOD activity. The decrease in elevated blood glucose levels by PG is attributable to PPAR- γ activation, which leads to enhanced insulin sensitization and glucose metabolism by increasing the expression of the glucose transporter GLUT-4 [33, 49]. Previous studies have shown that IR may lead to fatty acid changes in the liver, and the excess

of free fatty acids in the IR state is directly toxic to the liver cells with a resultant increase in serum transaminases such as ALT [50, 51]. PG can beneficially affect these changes and improve liver function tests through improving insulin sensitivity and an anti-inflammatory effect [50, 52]. In addition, PPAR- γ agonists have been shown to inhibit gene expression for tissue TNF- α that has been associated with IR, at least in part, through the inhibition of signaling from insulin receptors [52]. The reduction in the elevated hepatic TNF- α level by PG may be a mechanism by which PG could ameliorate the hepatic low-grade inflammation associated with the metabolic syndrome [53].

Our findings also indicate that PG has antioxidant properties that can attenuate ROS production and improve antioxidant defense mechanisms. Consequently, it was not surprising that the level of hepatic tissue MDA, a biological marker of oxidative stress-induced injury [43], was reduced in Fr-fed rats by PG treatment in our study. This observation parallels the finding of a previous study which reported that PG had ameliorative effects on the oxidative stress associated with alloxan-induced diabetes in rats [54]. The mechanisms that may be involved in the antioxidant effect of PG include preservation of SOD expression and upregulation of mitochondrial uncoupling protein-2 which results in depolarization of the mitochondrial inner membrane potential and reduced superoxide anion generation [55, 56].

Compatible with the above findings regarding the effects of FF and PG, it had previously been shown that the treatment with both drugs improved nonalcoholic steatohepatitis-related disturbances in serum glucose, insulin, and TG by modulating hepatic and adipose tissue expression of genes responsible for IR and fatty acid synthesis in rats given 10% Fr in drinking water for 12 weeks [57]. With this low concentration of Fr, pronounced alterations commonly associated with the metabolic syndrome, such as hyperuricemia, hypercholesterolemia, and changes in hepatic antioxidant defense mechanisms, may be absent [23, 58, 59]. These criteria were evaluated in our study, together with other metabolic disturbances, in a model of the metabolic syndrome in rats fed a high-Fr (60%) diet for 10 weeks and treated with FF and/or PG from weeks 5 to 10 of the experimental period. Thus, our study has expanded the spectrum of the effects of both drugs to include their ameliorative effects on the high Fr-induced metabolic syndrome in rats.

Finally, it is worth mentioning that an important finding of this study is that, in Fr-fed rats, combination treat-

ment with FF and PG was: (1) superior to the treatment with either drug alone in normalizing serum insulin levels, (2) more effective than treatment with FF alone in restoring insulin sensitivity, and (3) more effective than treatment with PG alone in reducing elevated serum uric acid and total cholesterol levels and improving the decreased hepatic GSH level and SOD activity. Thus, our results imply that a more efficacious therapy for ameliorating the serum and hepatic biochemical alterations that characterize the Fr-induced metabolic syndrome may be achieved by the combination of FF and PG.

Conclusions

The need to develop an effective pharmacological treatment for the Fr-induced metabolic syndrome remains an elusive target. To our knowledge, this study is the first reported work evaluating the efficacy of the combination treatment with FF and PG in the Fr-induced metabolic syndrome. The results of our study demonstrated that the treatment of Fr-fed rats with both FF and PG exhibited a higher anti-metabolic syndrome efficacy than treatment with either drug alone. Thus, acting on the 2 main PPAR subfamilies, a combination of FF, a PPAR- α agonist, and PG, a PPAR- γ agonist, may provide beneficial, potentiating effects in order to modulate serum and hepatic biochemical alterations associated with the Fr-induced metabolic syndrome.

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Statement of Ethics

This study was approved by the Animal Care and Use Committee of the Medical Research Institute, Alexandria University (Alexandria, Egypt). All animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* [60].

Disclosure Statement

The authors declare that they have no conflicts of interest regarding this study.

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Author Contributions

M.M.F. conceived the idea of this work and made its design. E.H.A. and W.F.E. carried out the laboratory work. M.M.F. analyzed and interpreted the data of the experiments, and wrote the article. All authors read and approved the article before submission.

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