

**DIFFERENTIAL EFFECTS OF PEROXISOME PROLIFERATOR-
ACTIVATOR RECEPTOR (PPAR) ALPHA AND GAMMA AGONISTS ON
BODY WEIGHT AND ADIPOSE DEPOTS IN FRUCTOSE FED WISTAR
RATS**

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Abstract

The aim of this study was to investigate the effect of fenofibrate (PPAR-alpha agonist) and rosiglitazone (PPAR-gamma agonist) on body weight and adipose depots in an experimental model of the metabolic syndrome.

Metabolic syndrome was induced in 48 male Wistar rats by adding a fructose in drinking water (10% solution) for 12 weeks. During the last 4 weeks, 16 rats were treated with fenofibrate (100 mg/kg/day), 16 rats were treated with rosiglitazone (5 mg/kg/day) by intragastric tube, while the remaining 16 did not receive any medication (fructose group). Another control group of 16 rats consumed standard rat chow and water for 12 weeks.

Chronic fructose administration for 12 weeks significantly increased the body weight ($p < 0.05$), as well as the weight of the measured fat pads: perirenal ($p < 0.001$) and epididymal ($p < 0.001$) as representatives of the visceral adipose depots and the inguinal pads ($p < 0.05$) as a representative of the subcutaneous adipose depots compared to the control group. This was accompanied with a decrease of the subcutaneous/visceral fat ratio. Treatment with fenofibrate over the final 4 weeks significantly decreased the body weight ($p < 0.001$) and the weight of the epididymal, perirenal and inguinal fat pads ($p < 0.001$ for all parameters), without changes of the subcutaneous/visceral fat ratio.

On the other hand, rosiglitazone promoted weight gain. Treatment with this PPAR-gamma agonist significantly decreased the weight of the epididymal ($p < 0.01$) and perirenal ($p < 0.05$) pads, but increased the weight of the inguinal fat pads ($p < 0.001$) compared to the fructose group, which led to an increase of the subcutaneous/visceral fat ratio.

This study indicates that treatment with the PPAR-alpha agonist fenofibrate decreases body weight and reduces the fat depots, whereas PPAR-gamma agonist promotes weight gain and a body fat redistribution from visceral towards subcutaneous depots in an animal nutritive model of the metabolic syndrome.

Key words: fructose, fenofibrate, rosiglitazone, PPAR, metabolic syndrome, body weight.

Introduction

The metabolic syndrome is a constellation of signs and symptoms which increase a patient's risk of developing heart disease and diabetes mellitus. The metabolic syndrome includes central obesity, hypertension, hyperglycemia, and dyslipidemia. Diagnosing the metabolic syndrome in a patient identifies areas that can be addressed and with appropriate treatment can lower the risk of diabetes and cardiovascular disease [1, 2]. Therefore, the pharmacological treatment of the metabolic syndrome should be focused on regulation of the insulin resistance and reduction of the cardiovascular risk factors [3, 4].

The fibrates (fenofibrate, clofibrate, etc.) are in clinical use for treatment of dyslipidemia for more than 50 years, but recently it was shown that their hypolipidemic action is mainly due to the activation of the nuclear peroxisome proliferator activated receptor- alpha (PPAR-alpha). These receptors are located in the metabolically active organs (liver, heart, skeletal muscles) and regulate genes that have important role in the intracellular lipid metabolism. Several studies suggest that these compounds affect a broader spectrum of processes (such as inflammation, insulin resistance, endothelial function) and that beside their officially approved indications, they could have a potential role in the treatment of other metabolic and vascular diseases [5, 6, 7].

Thiazolidinediones (pioglitazone and rosiglitazone) are insulin-sensitizing drugs that provide effective approach for treating type 2 diabetes. They elicit their action through activating the peroxisome proliferator-activated receptor gamma (PPAR-gamma). Many in vitro and in vivo studies have confirmed that treatment with thiazolidinediones affects factors involved in insulin signal pathways, glucose transport, lipid metabolism and adipocytokines secretion. Therefore, beside their current indication (manifest diabetes mellitus- type 2), PPAR-gamma agonists could be used in the treatment of the metabolic syndrome [8, 9].

In this study, a nutritive experimental model of the metabolic syndrome was used, that was achieved by chronic fructose administration (as a 10% solution in the drinking water) in Wistar rats for a period of 8 weeks. The rats developed hyperinsulinemia, impaired glucose tolerance, hypertriglyceridemia, increased free fatty acid levels, and hypertension and decreased HDL-levels [10]. The aim of present study was to evaluate the effects of fenofibrate (PPAR-alpha agonist) and rosiglitazone (PPAR-gamma agonist) on body weight and adipose tissue depots in fructose-fed Wistar rats.

Material and methods

Male Wistar rats (200±25 g) were kept at the experimental stable of the Institute of Preclinical and Clinical Pharmacology and Toxicology. The animals were housed in standard cages (four rats/cage) and maintained under controlled room temperature and humidity with 12/12-hour light-dark cycle. Rats were fed a standard commercial chow and had a free access to drinking water. All performed procedures were in accordance to the principles for care and use of laboratory animals [11].

The rats were divided into 4 groups:

- Group 1 (n=16): represents a control group, and consumed standard rat chow and drinking water in a period of 12 weeks.
- Group 2 (n=16): consumed fructose as a 10% solution in the drinking water for a period of 12 weeks.
- Group 3 (n=16): consumed fructose as a 10% solution in the drinking water for a period of 12 weeks + rosiglitazone (ALKALOID AD, R. Macedonia) in a dose of 5 mg/kg/day by intragastric tube in the last 4 weeks.
- Group 4 (n=16): consumed fructose as a 10% solution in the drinking water for a period of 12 weeks + fenofibrate (LEK, R. Slovenia) in a dose of 100 mg/kg/day by intragastric tube in the last 4 weeks.

Fructose solution was prepared fresh daily during the 12 weeks, by dissolving fructose (ADM Corn Processing) in the drinking water.

Body weight was measured at the beginning of the study (week 0), after 8 weeks of fructose diet and weekly during the treatment with the study medications.

At the end of the study (week 12), the animals were sacrificed. Dissection and measurement of the epididymal and perirenal fat pads (as representatives of the intraabdominal fat tissue) and of the inguinal fat pads (as representatives of the subcutaneous fat tissue) was performed. The differences in the adipose tissue distribution was further evaluated through calculation of the subcutaneous/ intraabdominal fat tissue ratio (inguinal fat pads weight/ epididymal + perirenal fat pads weight).

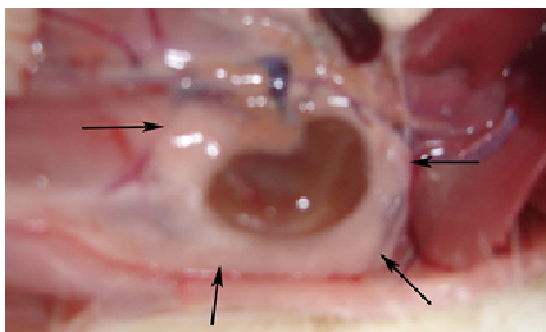


Figure 1. Dissection of perirenal fat pads



Figure 2. Dissection of epididymal fat pads

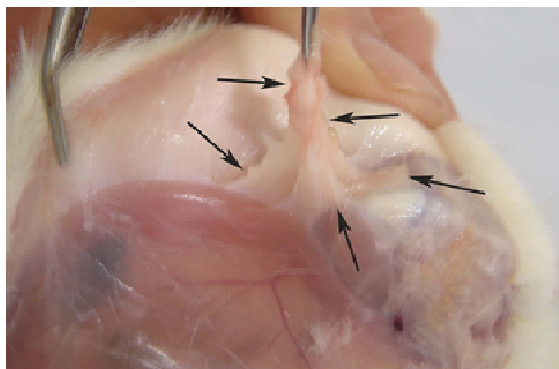


Figure 3. Dissection of inguinal fat pads

Statistical evaluation

The data are shown tabular and graphically and are evaluated with the statistical programmes Statistica for Windows 8.0 and KINETICA 4.2 (Innaphase corporation, USA).

The differences between the determined time-points, as well as the differences between the groups were analysed with the Student *t* test for dependent and independent samples, respectively. Values for $p < 0.05$ were considered as statistically significant.

Results

Changes of the body weight in different experimental groups during the study are presented in Table 1.

The increase of the body weight in the fructose group of animals was bigger than the control group that consumed water, after 8 weeks ($p < 0.05$), as well as after 12 weeks ($p < 0.05$). The 4-week treatment with rosiglitazone induced an additional increase of the body weight compared to the values measured at the beginning of the study ($p < 0.001$), but this increase was not statistically different ($p = 0.27$) compared to the fructose group.

Table 1. Body weight (g) in different experimental groups during the study.

	Week 0	Week 8	Week 9	Week 10	Week 11	Week 12
Control group (H₂O)	205±13	236±16	243±17	247±17	253±19	258±16
Fructose group	202±10	248±17	253±14	259±16	263±14	269±15
Fructose+ Rosiglitazone ROSI	200±13	244±18	254±16	263±16	270±17	275±15
Fructose+ Fenofibrate	201±24	242±32	234±33	222±35	216±36	214±32

The monotherapy with fenofibrate induced a significant decrease of the body weight compared to the values measured at the beginning of the study ($p < 0.001$), as well as compared to the fructose group ($p < 0.001$). At the end of the study, this experimental group was characterized with the greatest body weight decrease (Figure 4).

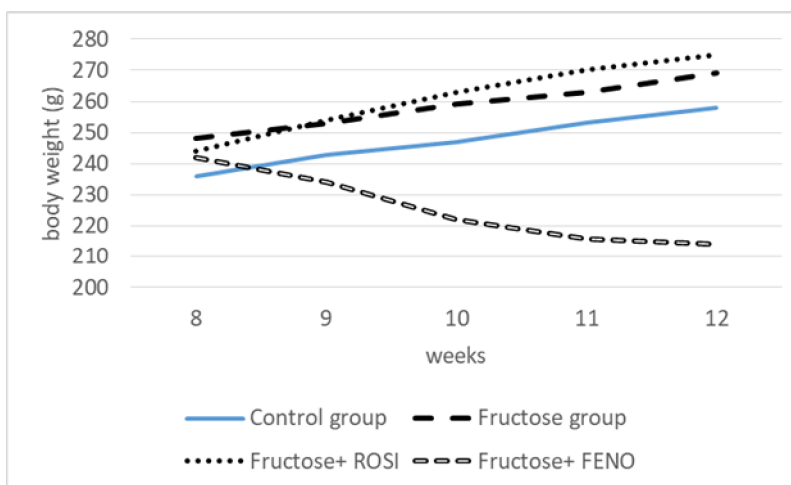


Figure 4. Changes of the body weight in different experimental groups before (week 8) and after 4-week treatment with the investigational medicines (week 12).

The measured fat pads are presented in grams and as a percentage of the total body mass in Table 2.

Table 2. Epididymal, perirenal and inguinal fat pads in different experimental groups at the end of the study (week 12).

	Epididymal fat pads (g)	% body weight	Perirenal fat pads (g)	% body weight	Inguinal fat pads (g)	% body weight
Control group (H₂O)	0.98±0.28	0.38	1.06±0.23	0.41	1.26±0.18	0.47
Fructose group	1.34±0.17	0.50	1.42±0.17	0.53	1.49±0.23	0.55
Fructose+ Rosiglitazone	1.13±0.18	0.41	1.23±0.16	0.45	1.93±0.40	0.70
Fructose+ Fenofibrate	0.76±0.18	0.35	0.85±0.28	0.39	0.95±0.39	0.44

Adding fructose in the drinking water caused an increase of all three fat pads: epididymal ($p<0.001$), perirenal ($p<0.001$) and inguinal ($p<0.05$) compared to the control group of animals that consumed tap water.

Treatment with the PPAR-gamma agonist rosiglitazone significantly decreased the weight of the epididymal ($p<0.01$) and perirenal ($p<0.05$) pads, but increased the weight of the inguinal fat pads ($p<0.001$) compared to the fructose group, which led to an increase of the subcutaneous/visceral fat ratio.

Treatment with the PPAR-alpha agonist fenofibrate over the final 4 weeks significantly decreased the body weight ($p<0.001$) and the weight of the epididymal, perirenal and inguinal fat pads ($p<0.001$ for all parameters).

The average total weight of the examined fat pads in the control group was 3.3 grams, and the subcutaneous/ intraabdominal fat tissue ratio was 0.61. Fructose administration induced an increase of the total weight (4.25 grams), without any significant changes of the subcutaneous/ intraabdominal fat tissue ratio. Treatment with fenofibrate uniformly reduced all measured fat pads (total weight= 2.56 grams), without changes of the subcutaneous/visceral fat ratio.

On the other hand, rosiglitazone treatment did not significantly increase the total fat pad weight (4.29 g) compared to the fructose group, but induced a significant increase of the subcutaneous/visceral fat ratio (1.17), which indicates body fat redistribution from visceral towards subcutaneous depots (Figure 5).

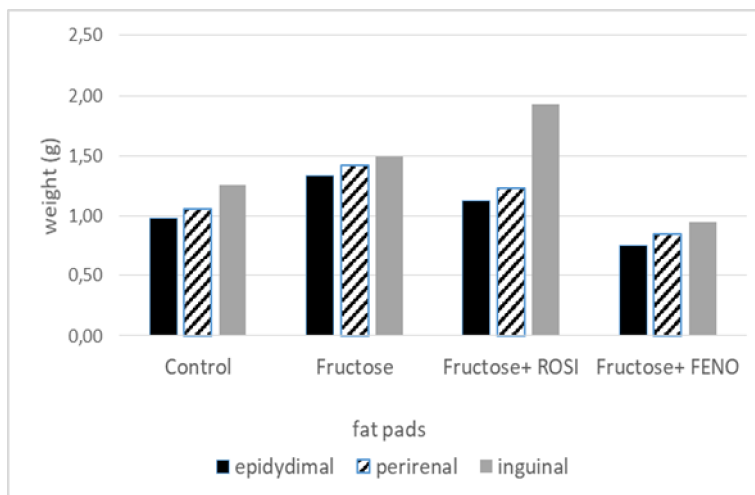


Figure 5. Adipose tissue distribution in different experimental groups at the end of the study (week 12).

Discussion

Human metabolism has evolved to efficiently convert chemical energy obtained through the consumption of food into thermal and chemical energy. Our body's metabolic pathways have developed to provide energy to tissues in times of physical threat and survival, or to efficiently conserve energy in times of food deprivation. Today, westernized societies have an abundance of food (food security) and many individuals have little need to perform physical activity. This combination has led to excessive nutrient storage, placing significant stress on our metabolic pathways, and leading to an increase in the prevalence of disease stemming from metabolic dysfunction [2; 12].

The metabolic syndrome, which probably develops as a consequence of the insulin resistance, is characterized with impaired glucose tolerance, hyperinsulinemia, dyslipidaemia and hypertension. These metabolic disturbances are often accompanied with increased body weight and abdominal (central, visceral) obesity. In the present study, the chronic fructose administration in drinking water (10% solution) over a period of 8 weeks induced a metabolic syndrome in the experimental animals [13] that was also characterized with increased body mass, as well as increase of all three measured fat pads (epididymal, perirenal and inguinal) compared to the control group of animals that consumed tap water. The used nutritive model of the metabolic syndrome very much resembles the metabolic syndrome which is commonly found in the human population.

The treatment with the PPAR-alpha agonist fenofibrate induced body weight reduction, as well as reduction of the measured fat pads in fructose-fed rats. Several other studies point that fibrates, beside their well-established lipid-lowering effects, have beneficial metabolic effects in reducing the serum insulin levels and

improving the insulin sensitivity in peripheral organs [14-17]. In spontaneously hypertensive rats, treatment with bezafibrate did not change the mean arterial pressure, but it reduced the insulin resistance [18, 19]. The insulin resistance is closely linked to the fat accumulation in the intraabdominal depots. Several studies report a marked improvement of the insulin sensitivity after surgical removal of the intraabdominal fat pads, diet or pharmacological interventions [20-23].

The 4-week treatment with the PPAR-gamma agonist rosiglitazone promoted weight gain in the experimental animals. The increase of the body weight is well-known and established adverse effect during treatment with the PPAR-gamma agonists, and in the mechanisms of its development several components are implicated: decrease of serum insulin and leptin concentrations (that function as a satiety signals in the central nervous system), enlargement of the adipose depots, increase of the plasma volume etc. [24, 25]. Parallel to the body weight increase, the rosiglitazone treatment induced an increase of the average weight of the measured fat pads, which at first sight might look like a contradictory change that occurs simultaneously with amelioration of the insulin resistance [13]. However, these quantitative changes should be evaluated in the context of changes of the body fat distribution. In the present study, the PPAR-gamma activation induced a fat redistribution from intraabdominal towards the subcutaneous depots.

The results from Laplante et al. [26, 27] explain that the depot-specific effects of the PPAR-gamma agonists are due to differences in the expression of genes that are responsible for modulation of different aspects of the lipid metabolism. The PPAR-gamma activation enhances the capacity for lipid transport and esterification in the subcutaneous depots in a bigger extent than in the visceral fat depots. On the other hand, the genes responsible for fatty acids oxidation and thermogenesis are more expressed in the visceral than in the subcutaneous depots after treatment with this group of drugs. The precise molecular mechanisms that are responsible for the observed differences in the lipid metabolism induced by PPAR-gamma agonist remain to be further elucidated. Additionally, the results from several clinical studies performed in diabetic patients show that PPAR-gamma agonists have tendency to stimulate fat accumulation in the subcutaneous area, while reducing the liver fat deposits, as well as the intramyocellular lipid accumulation [28-32].

Conclusion

This study indicates that treatment with the PPAR-alpha agonist fenofibrate decreases body weight and reduces all measured fat depots, whereas PPAR-gamma agonist rosiglitazone promotes weight gain and a body fat redistribution from visceral towards subcutaneous depots in an animal nutritive model of the metabolic syndrome.

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