

FLUOXETINE INFLUENCE ON BODY WEIGHT, SERUM ADIPONECTIN LEVEL AND ADIPOSE TISSUE TRIGLYCERIDES LEVELS IN RATS

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Abstract

The effects of fluoxetine on metabolic parameters are poorly understood. Adiponectin is an adipocyte hormone inversely correlated with body fat percentage in adults. The aim of this study was to investigate the hormonal changes and metabolic parameters of the perirenal adipose tissue of rats. Two doses (6 mg/kgbw and 12 mg/kgbw) of oral fluoxetine were administered, and dose- and sex-dependent body weight changes were observed. In male rats a weight gain was observed in the control group, instead in the treated groups the high doses of fluoxetine partially blocked this effect. In treated female rats no body weight changes were observed compared to the control group. The perirenal adipose tissue triglyceride content reduction with a simultaneous elevation of plasma adiponectin level suggests that fluoxetine had important peripheral effects.

Rezumat

Efectul fluoxetinei asupra parametrilor metabolici nu este pe deplin elucidat. Adiponectina este un hormon adipocitar al cărui nivel plasmatic, la adulţi se află în corelaţie inversă cu adipozitatea. Scopul studiului a fost cercetarea unor modificări metabolice şi hormonale ale ţesutului adipos perirenal la şobolani, după administrare orală de doze diferite de fluoxetină (6 mg/kgc şi 12 mg/kgc). În acest studiu experimental am observat modificări ale masei corporale dependente de sex şi de doza medicaţiei utilizate. La animale de sex masculin s-a observat o creştere în greutate în cazul lotului martor, în schimb la lotul tratat cu fluoxetină în doze mari această creştere a fost parţial inhibată. La animalele de sex feminin tratate, nu s-a observat modificarea masei corporale faţă de lotul martor. Reducerea conţinutului de trigliceride a ţesutului adipos perirenal în paralel cu creşterea nivelului de adiponectină sugerează un important efect periferic al fluoxetinei.

Keywords: fluoxetine, body weight, perirenal adipose tissue triglyceride content, adiponectin

Introduction

Epidemiologic evidences showed that obesity correlated with depression, and depressed adults have a 37% increased risk of developing type 2 diabetes [31]. Fluoxetine is the most widely prescribed selective serotonin reuptake inhibitor (SSRI) antidepressant. Although with limited cardiovascular toxicity [12, 22], SSRI's were reported to be associated with sexual dysfunction [1, 23] and weight changes [6, 15, 28, 30]. Tricyclic antidepressants (TCAs) and perhaps mono-amine oxidase inhibitors (MAOIs) may be more likely to cause weight gain than the selective serotonin reuptake inhibitors (SSRIs), while mirtazapine may be placed between the SSRIs and the TCAs in terms of relative risk of weight gain [27]. Bupropion may be the only modern agent that

reduced the body weight [17, 18]. Regarding fluoxetine, initial studies showed weight loss, but long-term follow up revealed that the weight reducing effect is associated with a gain on body weight developing over time [28].

However, the effects of fluoxetine on metabolic parameters are poorly understood. Adiponectin is an adipocyte hormone involved in regulating glucose levels as well as fatty acid breakdown. Levels of this hormone are inversely correlated with body fat percentage in adults [32], but the clinical role on weight change is not fully understood.

The aim of this study was to investigate the hormonal changes and metabolic parameters of the perirenal adipose tissue of the rats after fluoxetine administration.

Materials and Methods

Animals

Forty-two Wistar rats (21 female, 21 male), 50 weeks age, weighing between 240 - 310 g provided by the Biobase of University of Medicine and Pharmacy of Tîrgu Mureş, Romania, were used for the present study. All experimental procedures were conducted according to the institutional and national guidelines for laboratory animals, respecting the Directive 2010/63/EU, and also with the approval of the University of Medicine and Pharmacy of Tîrgu Mureş's Ethics Committee. For housing, standard cages were used with three or four animals in each. Food and water were available *ad libitum*; the light-dark cycle was 12 hours assured by natural light. The temperature and

relative humidity of the rooms were $22 \pm 2^\circ\text{C}$ and 30 - 60%, respectively.

Chemicals

Fluoxetine hydrochloride salt was kindly supplied by SC VIM Spectrum SRL, Tîrgu Mureş, Romania.

Experimental protocol

Male and female animals, each sex separately, were randomly divided in three groups, each group consisted of seven animals ($n = 7$). The resulting 6 groups, according to the substance and dosage they were receiving (see Table I), were as follows: 2 control groups (7 male and 7 female animals) and 4 experimental groups (2 male groups and 2 female groups) receiving low (6 mg/kgbw) and high (12 mg/kgbw) doses of fluoxetine (FLX).

Table I
Experimental protocol

Group	Drug	Dose	Volume of administration	Route	Duration
Male control ($n = 7$)	Distilled water	-	0.5 mL	Oral by gavage	42 days
Male 6 mg/kgbw FLX ($n = 7$)	Fluoxetine	6 mg/kgbw	0.5 mL	Oral by gavage	42 days
Male 12 mg/kgbw FLX ($n = 7$)	Fluoxetine	12 mg/kgbw	0.5 mL	Oral by gavage	42 days
Female control ($n = 7$)	Distilled water	-	0.5 mL	Oral by gavage	42 days
Female 6 mg/kgbw FLX ($n = 7$)	Fluoxetine	6 mg/kgbw	0.5 mL	Oral by gavage	42 days
Female 12 mg/kgbw FLX ($n = 7$)	Fluoxetine	12 mg/kgbw	0.5 mL	Oral by gavage	42 days

Doses were chosen considering literature data on antidepressants [9]. The drug was dissolved in distilled water and a final concentration of 7 mg/mL solution was obtained. After grouping the animals, their initial weight was registered, and the drug was administered by gavage. Due to the long half time (15 h) of norfluoxetine [9], the active metabolite of fluoxetine, the medication was administered once daily at 8.00 a.m. Weekly changes of body weight (BW) were followed and evaluated during the experiment. After the treatment period, animals were sacrificed with an overdose of anesthetics (ketamine 300 mg/kgbw and xylazine 30 mg/kgbw administered intra-peritoneally). 3 - 5 mL blood was collected by cardiac puncture, and the whole blood was immediately placed into a centrifuge tube without anticoagulant. After 30 minutes, the clotted blood was centrifuged at $3.000 \times g$ for 15 minutes at $4^\circ\text{C} \pm 2^\circ\text{C}$. Serum samples were transferred into separate tubes and stored at -20°C until analysis. Adipose tissue samples were taken from the perirenal region, and stored at -20°C .

Adipose tissue triglyceride content determination

The extraction and homogenization of triglycerides from the perirenal adipose tissue respects the steps described by Gall *et al.* [19], except for triglyceride solubilisation. Instead of Tween 20, 10% w/w, Tergitol-type NP40 solution was used. The concentration of the final solution was determined using a commercially available kit for triglyceride

quantification (Diagnosticum Rt., Budapest). The measurement of absorbance was performed using a Shimadzu UV-1601 UV-Visible Spectrophotometer, at 505 nm wavelength and 37°C in a 0.5 cm light path glass cuvette against blank reagent.

Adiponectin determination

Serum levels of adiponectin were measured by a commercial sandwich ELISA assay (Adiponectin kit Rat, DRG Diagnostics GmbH, Marburg, Germany). The enzyme activity was measured spectrophotometrically at 450 nm - 590 nm. The unknown rat adiponectin level was derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of rat adiponectin.

Statistical analysis: For statistical analyses, the trial version of the software GraphPad Prism 5 was used. The comparison of groups was performed using one-way ANOVA with Dunnett's post hoc test. The results were expressed as means and standard deviation (SD), respective mean difference with 95% confidence interval (CI). Correlation between different metabolic parameters was performed with Pearson's test. For all analyses the statistical significance level was set to the p value < 0.05 .

Results and Discussion

After 6 weeks of treatment with fluoxetine, the weight of male rats increased significantly ($p < 0.001$)

(Figure 1). The mean weight gain (95% CI) was 110 g (84 - 140), 100 g (74 - 130) and 70 g (44 - 97) in the control group, 6 mg/kgbw FLX treated group and 12 mg/kgbw FLX treated group, respectively. The reached mean BW in 12 mg/kgbw FLX treated male animals was significantly lower than in 6

mg/kgbw FLX treated animals (the mean difference (95% CI) was 30 g (0.3 - 59.7), $p < 0.05$). Although the BW changes in female rats were not significant after fluoxetine treatment, a weight loss tendency was observed (Figure 1).

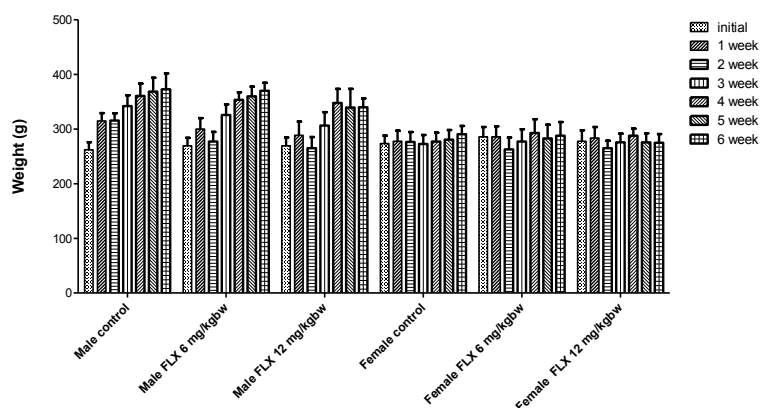


Figure 1.
Body weight changes

The perirenal adipose tissue triglyceride content, described as the percentage of adipose tissue mass (g%), showed a significant difference only in 12 mg/kgbw FLX treated male rats (Figure 2). In this group, a mean (95% CI) reduction of 5.14 (0.55 - 9.73) g% of the perirenal adipose tissue triglyceride content was observed ($p < 0.05$). Although female rats had lower perirenal adipose tissue triglyceride content, the difference between male and female control groups was not significant. After fluoxetine treatment no significant changes of perirenal adipose tissue triglyceride content was found in female animals.

The adiponectin level presented no significant differences between the studied groups (Table II).

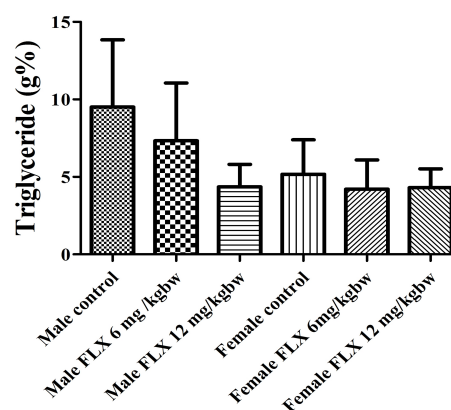
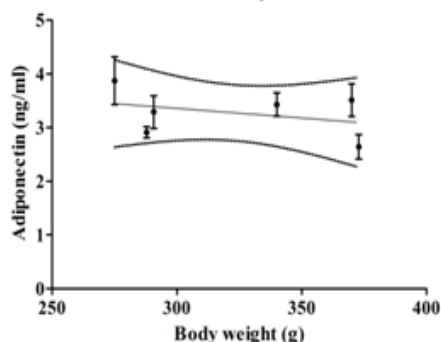


Figure 2.
The triglyceride content of the perirenal adipose tissue

Table II
Plasma adiponectin levels

	Mean adiponectin level (ng/mL) $\pm$ SD
Male control (n = 7)	2.64 $\pm$ 0.60
Male 6 mg/kgbw FLX (n = 7)	3.51 $\pm$ 0.80
Male 12 mg/kgbw FLX (n = 7)	3.43 $\pm$ 0.57
Female control (n = 7)	3.29 $\pm$ 0.80
Female 6 mg/kgbw FLX (n = 7)	2.91 $\pm$ 0.27
Female 12 mg/kgbw FLX (n=7)	3.87 $\pm$ 1.17

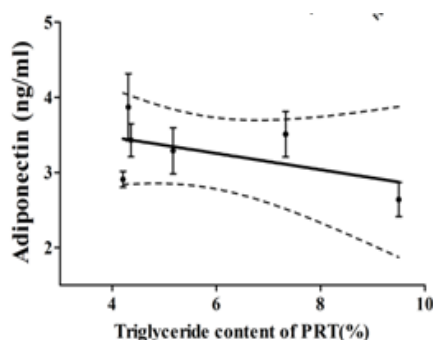
When adiponectin and body weight relationship was assessed, an inverse, but not significant (Pearson coefficient, $r = -0.35$, $p > 0.05$) correlation was found (Figure 3).

**Figure 3.**

The adiponectin level and body weight correlation

The inverse correlation was more evident when adiponectin and perirenal adipose tissue triglyceride content correlation test was performed (Pearson coefficient $R = -0.53$) but without statistical significance (Figure 4).

Inconsistent results exist in the scientific literature regarding the effect of fluoxetine on body weight changes. In a few studies weight loss was described during fluoxetine treatment [2, 5, 13, 14]. Some authors described earlier the serotonergic anorectic effect of fluoxetine in rats [33]. Conversely, other researchers discovered that fluoxetine induces weight gain [16, 26], or may not cause any changes in body weight [24]. In a prospective clinical study, the authors discovered weight loss for overweight depressed patients as a result of fluoxetine treatment, and body weight gain for normal weight patients [29]. In this experimental study sex-dependent body weight changes were observed. In male rats the weight gain observed in the control group was partially stopped by administering high doses of fluoxetine. In female rats, body weight stagnation was observed, both in the control and treated groups, with tendency of weight loss after high doses of fluoxetine.

**Figure 4.**

Adiponectin level and perirenal adipose tissue (PAT) triglyceride content correlation

In accordance with stopping weight gain, the 12 mg/kgbw doses of fluoxetine reduced the perirenal adipose tissue triglyceride content.

Triglycerides accumulated in adipose tissue can reflect the whole body's energy storage level and characterize the adipocyte's maturity [19]. The effects of fluoxetine on weight may be mediated by distinct mechanisms, which influences appetite [35] or directly metabolic effects on adipose tissue [24]. In both mechanisms serotonin is involved. As central neurotransmitter it acts on different serotonergic receptors and as peripheral modulator influences the adipokines secretion through the same receptors described in humans and rats [4, 11].

The differences in adiponectin levels between the fluoxetine treated and non-treated groups were not significant. An inverse correlation between adiponectin level and body weight, and especially perirenal adipose tissue triglyceride content was found. The primary source of adiponectin is the adipose tissue. This adipokine has insulin-sensitizing and anti-inflammatory properties [3, 10, 20, 34]. Others considered adipokines as biomarkers of metabolic syndrome (central obesity), adiponectin and omentin-1 being the only ones with lowering levels in this disease [7, 8]. Conversely, higher levels of adiponectin were found in obese individuals, who are metabolically healthy (peripheral obesity) [21]. Our findings are in lines with studies that suggest an increase of the adiponectin level during body weight reduction [25].

Conclusions

In conclusion, fluoxetine is an antidepressant with safe metabolic effects, which could prevent weight gain. Dose and gender are contributing factors on the relationship of this antidepressant with body weight changes. The perirenal adipose tissue triglyceride content reduction with a parallel elevation of plasma adiponectin level suggests that fluoxetine has important peripheral effects.

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