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Fluoxetine induces lean phenotype in rat by increasing the brown/white adipose tissue ratio and UCP1 expression

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Abstract The serotonergic system plays a crucial role in the energy balance regulation. Energy balance is mediated by food intake and caloric expenditure. Thus, the present study investigated the mechanisms that might be associated with fluoxetine treatment-induced weight reduction. Wistar male rat pups received daily injections with subcutaneous fluoxetine (Fx-group) or vehicle solution (Ct-group) from day 1 until 21 days of age. Several analyses were conducted to verify the involvement of mitochondria in weight reduction. We found that body weight in the Fx-group was lower compared to control. In association to lower fat mass in the Fx-group (25 %). Neither neonatal caloric intake nor food intake reveals significant differences. Evaluating caloric expenditure (locomotor activity and temperature after stimulus), we did not observe differences in locomotor activity. However, we observed that the Fx group had a higher capacity to maintain body temperature in a cold environment compared with the Ct-group. Since brown adipose tissue-(BAT) is specialized for heat production and the rate of heat production is related to mitochondrial function, we found that Fx-treatment increases respiration by 36 %, although after addition of GDP respiration returned to Ct-levels. Examining ROS production we observe that Fx-group produced less ROS than control group. Evaluating uncoupling protein (UCP) expression we found that Fx-treatment increases the expression by 23 %. Taken together, our results suggest that modulation of serotonin system results in positive modulation of UCP and mitochondrial bioenergetics in brown fat tissue.

Keywords Brown fat mitochondria · Uncoupling protein · Fluoxetine · Energy balance

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Introduction

The serotonin (5-HT) produced in brain's area contributes substantially to food intake and energy expenditure regulation (Simansky 1996; Heisler et al. 2006; Vickers et al. 2008). A study from Silva et al. (2010) using a selective serotonin reuptake inhibitor (i.e., fluoxetine) during lactation demonstrated morphological changes in serotonergic neurons in addition to a gradual reduction in body weight during lactation and post-weaning (Silva et al. 2010). Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants such as fluoxetine, citalopram, sertraline among others, that are often prescribed to pregnant and lactating women with varying degrees of depression, thus exposing fetuses and infants to the drug during brain development (Fleschler and Peskin 2008). It has been suggested that early exposure to SSRIs can impact the pattern of brain development (Liao and Lee 2011).

Maintenance of energy balance requires several physiological and behavioral adjustments to regulates energy, especially controlling metabolism and storage (Wynne et al. 2005). Energy imbalance is the main cause of the global epidemics such as obesity and diabetes mellitus (Zafrir 2013). Pathways that control body nutritional status and energy levels are integrated into the Central Nervous System (CNS) to produce

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appropriate responses in peripheral tissues (i.e., brown adipose tissue or skeletal muscle) and maintain energy homeostasis (Guyenet and Schwartz 2012). Studies show that electrical stimulation of hypothalamic nucleus leads to activation of brown adipose tissue and increase body temperature (De Fanti et al. 2000). These authors suggested that this signal is likely mediated by serotonin (De Fanti et al. 2000). Bross and Hoffer (1995) demonstrated that fluoxetine administration increased energy expenditure, which was associated with increased basal body temperature in humans (Bross and Hoffer 1995). A study using sibutramine (serotonin-norepinephrine reuptake inhibitor) showed activation of brown adipose tissue indirectly through activation of sympathetic system (Heal et al. 1998; Connoley et al. 1999); studies using fenfluramine (stimulates serotonin release) showed increase in fat oxidation rates mediates to decrease in body weight (Boschmann et al. 1996) and also activation of brown adipose tissue with a decrease in metabolic efficiency (uncouple electron transport from ATP synthesis and with generate heat in brown adipose tissue mitochondria) (Lupien and Bray 1985; Arase et al. 1989; Ma and Preston 1992; Rothwell and Le Feuvre 1992).

Since mechanistic control of energy balance needs the participation of the central nervous system, which is vulnerable to environmental stimuli during the critical developmental period (Bellinger et al. 2004), it is very likely that serotonin system manipulation by fluoxetine treatment during development is crucial to developing specific and permanent adaptation, which could reflect in brown adipose tissue activation. The mechanisms involved in the control of body weight induced by chronic exposure to fluoxetine during the nursing phase of early rat development have not been fully elucidated. No experimental studies have been conducted to investigate the role of serotonin modulation on energy balance and the participation of brown adipose tissue mitochondria. Thus, to investigate whether serotonergic system manipulation by fluoxetine treatment, during development would affect energy balance, we conducted several experiments to investigate what was responsible for the gain and loss of weight.

Material and methods

Animals

The animal use protocols for this study have been approved by the Ethics Committee for Animal Research at the Federal University of Pernambuco in accordance with the guidelines published in "Principles of Laboratory Animal Care" (NIH, Bethesda, USA) (Ethical Protocol 23076.015276/2012-56). Wistar rats (*Rattus norvegicus*) were maintained at a room temperature of 23±1 °C in a 12-h alternating light–dark cycle (light 6:00 a.m.–6:00 p.m.). At ninety-days old, rats were mated (1 females for 1 male). 6-Pregnant rats were then transferred to individual cages and at least four male offspring of each litter were used in the present study. The pharmacological treatment stated 24 h after birth. The dams received commercial chow *ad libitum*. After weaning, the rat pups receive the same diet as their mothers, also *ad libidum*.

Pharmacological treatment and experimental groups

All male neonates received a subcutaneous fluoxetine (10 mg/kg, dissolved in saline solution, 10 ml/kg, bw; Fx group) or vehicle (NaCl 0.9 %, 10 ml/kg, bw; control-Ct group) injection once daily from the 1st to the 21st postnatal day (i.e., during the suckling period) as our previous studies (da Silva et al. 2014; da Silva et al. 2015). To avoid a possible influence of the circadian rhythm in these studies, injections were always administered between 7:00 and 8:00 a.m. (Sanchez et al. 2008; da Silva et al. 2014).

Body weight measurement

Body weights (in grams) were measured from 1st to the 21st postnatal day (weaning) and again at 40 and 60 days after birth using a digital balance (Marte, model S-100 with a 0.001 g sensitivity) (Mendes-da-Silva et al. 2002; da Silva et al. 2014).

Food intake measurement

Neonatal food intake

Individual pup food consumption in milligrams (mg) was obtained on lactation day 7, 14 and 21. To stimulate food intake, animals were separated from their mother for 3 h. During this period, pups aged 7 and 14 days stayed together in a plastic box in a 33 °C incubator. Consumption was measured by weight gain one hour after return to the mother box (Houpt and Houpt 1975). To ensure greater food intake quantitation reliability, the stimulation was performed with soft object genital cups to promote urine and feces excretion (Hall and Rosenblatt 1978).

24-hour food intake evaluation

Food intake (in grams) was evaluated by weighing commercial chow (Labina®) consumption for 24 h at 40 days of age.

Brown and white adipose tissue quantification

Intrascapular brown adipose tissue, mesenteric and epididymal white adipose tissue were excised, rinsed and weighed (in grams) using a digital balance (Marte, model S-100 with a 0.001 g sensitivity) at 60 days of age.

Free locomotor activity measurement

At 40 days of age, the pups were individually recorded for 24 h using an infrared camera (1/3480 chip sony line), and the captured images were stored in a computer system for voluntary activity analysis. Free locomotor activity including combined exploratory behaviors (i.e., holding the cage, nest building and grooming behavior) was recorded (Grieb et al. 2013).

Rectal body temperature measurement after thermal stimulation

At 40 days of age, the animal rectal body temperature (in °C) was assessed. The record was initially performed at room temperature, and the animals were then placed in a container with air vents and subjected to freezing ambient at −15 °C, and rectal temperature was measured every 30 min for 90 min (Li et al. 2008).

Mitochondria isolation

Brown Adipose Tissue (BAT) mitochondria were prepared by homogenization followed by differential centrifugation. Male Wistar rats (at 60 days of age) were killed by decapitation, and BAT was immediately removed from interscapular deposits and homogenized in a mixture containing 225 mmol/L mannitol, 75 mmol/L sucrose, 1 mmol/L EGTA, 4 mmol/L HEPES (pH 7.2). The homogenate was centrifuged at 4000 rpm for 10 min in 4 °C. The supernatant was carefully removed and centrifuged at 15000 rpm for 10 min in 4 °C. The pellet was re-suspended in a buffer containing 250 mmol/L sucrose and 5 mmol/L HEPES (pH7.2). Mitochondrial protein concentration was determined spectrophotometrically according to Bradford with BSA as the standard.

Mitochondrial oxygen consumption

Measurement of mitochondrial respiration from BAT was performed at 28 °C in a 600 SL chamber connected to a Clark-type oxygen electrode (Hansatech Instruments, Pentney King's Lynn, UK). The mitochondria were incubated in respiration buffer containing 120 mmol/L Potassium chloride (KCl), 5 mmol/L 3-(N-morpholino) propanesulfonic acid (MOPS), 1 mmol/L Ethylene glycol tetracetic acid (EGTA), 5 mmol/L, Monopotassium phosphate (KH2PO4), and 0.2 % BSA. Mitochondria were used at 0.5 mg protein/mL buffer. Mitochondrial respiration was measured with Complex II (succinate 5 mM + rotenone 4 μM) substrates, GDP (UCP inhibitor—1 mM) and CCCP (1 μM) (Lagranha et al. 2010; Nascimento et al. 2014).

Mitochondrial reactive oxygen species (ROS) production

Mitochondrial ROS production in isolated mitochondria was performed at 28 °C using a fluorescent probe DCFDA (5- (and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester). This dye is non-fluorescent when chemically reduced, but after cellular oxidation and removal of acetate groups by cellular esterases it becomes fluorescent (Garcia-Ruiz et al. 1995). Briefly, mitochondrial suspensions (0.5 mg protein) were incubated in the presence of 1 μM DCFDA and fluorescence was monitored over 5 min of gentle shaking using a temperature-controlled spectrofluorimeter (OMEGA, USA) with excitation and emission wavelengths of 503 and 529 nm, respectively. Under these conditions, the linear increment in fluorescence for each reaction indicated the rate of ROS formation, for the calculation was used the slope from the linear portion of the are under the curve.

Western blot for UCPs expression

After mitochondrial isolation, aliquots of BAT mitochondria were used for the measurement of total protein content as described by Bradford. Equal amounts of proteins were separated using 14 % SDS-gel polyacrylamide electrophoresis. Western blotting was carried out following the method described by Towbin et al. The proteins of the gel were transferred to a nitrocellulose membrane at 100 V for 1 h. Non-specific binding was blocked by incubating the membranes with 5 % defatted milk in basal solution (10 mM Trizma, pH 7.5; 150 mM NaCl; 0.05 % Tween 20) overnight at 4 °C. Membranes were washed in basal solution three times for 5 min each and then incubated with anti-UCP1 antibodies (SCBT: SC-6528) in basal solution containing 3 % defatted milk, at 4 °C, overnight. Membranes were washed again (three times for 5 min each) and incubated with anti-IgG antibody linked to horseradish peroxidase in basal solution containing 1 % defatted milk, at 25 °C, for 4 h. Following another washing, membranes were incubated with substrate for peroxidase and chemiluminescence enhancer (ECL Western Blotting System) for 5 min and immediately exposed to X-ray film. Films were then processed in a conventional manner. Gel transfer efficiency and equal load was verified using reversible Ponceau staining (Lagranha et al. 2010). Band intensities of the Western blotting experiments were analyzed and quantified by optical densitometry using the Image J software (NIH, Maryland, USA) (Towbin et al. 1979; Lagranha et al. 2007).

Statistical analysis

All of the results are expressed as the means ± SEM. A student's *t*-test was performed to assess significant differences between the two groups. Two-way ANOVA with multiple comparisons was used for repeated analyses. The data were

considered to be statistically significant when $p \leq 0.05$. All of the data were plotted, and the statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA).

Results

Effect of modulation of serotonin system by fluoxetine in body weight, adipose tissue weight and food consumption

We measured body weight daily, and we observed that body weight was significantly lower in the treated group than the control group beginning in second week of life (Fig. 1a; 14 days after birth, Ct: 32.30±0.5673 g, n: 35, Fx: 25.10±0.2900 g, n: 35; 21st days Ct: 50.70±0.9768 g, n: 35, Fx: 41.41±0.7332 g, n: 35). Furthermore, body weights of the treated group remained lower than the control rats at 40th and 60th days (19 and 39 days after last drug application, respectively) (40th days: Ct: 131.8± 3.5 g, n: 13, Fx: 115.0±2.5 g, n:11; 60th days: Ct: 239.10±10.83 g, n: 13, Fx: 206.1±9.94 g, n: 11). Because the difference observed in in body weight, we quantified the adipose tissue weight. We observed that rats from the Fx group had significantly reduced white adipose tissue (Fx: 0.97±0.093 g, n: 11; $p<0.0001$) compared with the control group (Ct: 1.72±0.129 g, n: 11) and also significant increase in the weight of brown adipose tissue (Fx: 0.089±0.004 g, n: 11; Ct: 0.074±0.003 g, n: 11; $p<0.01$) (Figs. 2a and b). Evaluating the adipose tissue weight-to-body weight ratio we observe that fluoxetine group present less white adipose tissue/body weight ratio (WAT/BW ratio Ct: 0.0073±0.000768, n: 11; Fx: 0.0048±0.000535, n:11; $p<0.05$) and more brown adipose/body weight ratio tissue than control group (BAT/BW ratio Ct: 0.000314± 0.000016, Fx: 0.000434±0.000028 g, n: 11;$p<0.01$) (Figs. 2c and d). In addition to this observation when we compare the ratio of brown/white adipose tissue we also verify a significant increase in fluoxetine group (BAT/WAT ratio Ct: 0.0469±0.0046, Fx: 0.09277± 0.0098 g, n: 11;$p<0.001$) (Fig. 2e).

After the observation in the body weight lost, we conduct the analysis in food consumption, with the intent to verify whether the effect of serotonin modulation in brain's developmental period interferer in body weight by induce satiety. No differences were observed in food consumption between groups on the 7th days (Ct: 2.04± 0.19 g, n: 16; Fx=1.86±0.17 g, n:21); 14th days (Ct: 2.47±0.31 g, n:16; Fx: 2.24±0.22 g, n:21) and 21st days (Ct: 3.7±0.18 g, n:16; Fx: 3.05±0.29 g, n:21) (Fig. 1b). To understand the possible mechanisms behind the decrease in body weight, we evaluated food intake during a 24-hour period in rats at 40 days. Interestingly, 24 h food intake was not altered by the drug, suggesting that the weight differences between the groups were not related to food intake or possible satiety (Ct: 19.5±1.53 g, n:16; Fx: 16.84±1.139 g, n:21) (Fig. 1b).

Effect of modulation of serotonin system by fluoxetine in energy expenditure

Because energy balance is an equation between energy intake and energy expenditure, we evaluated voluntary locomotor activity to verify whether the decrease in body weight and white adipose tissue was because of increased energy expenditure from increased activity. Upon analyzing voluntary locomotor activity for 24 h, we observed that the Fx group (Fx: 25292±1377 s, n: 6) had no significant difference than the C group (C: 27666± 1170 s, n:6) (Fig. 3a).

After evaluating body weight, food intake, adipose tissue and locomotor activity, we were still unable to determine how fluoxetine modulates energy balance. One mechanism that controls energy balance is decreasing metabolic efficiency, in other words, uncoupling substrate oxidation from the production of ATP; this can occur with proton leak that is not coupled to ATP production. In addition, it is well known that cold exposure stimulates lipolysis in BAT; thereby activating uncouple protein (UCP1) and increasing heat production

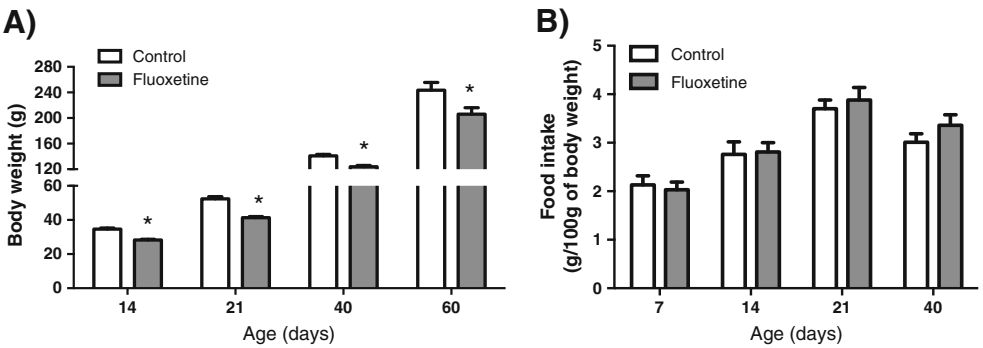

Fig. 1 Effect of neonatal fluoxetine treatment on body weight and food intake in male rat pups that were treated with fluoxetine (fluoxetine=10 mg / kg bw, sc) or saline (control=0.9 % NaCl 1 ml/kg, pc, sc) during the suckling period. Data are presented as the mean ± SEM (* $p<0.01$)

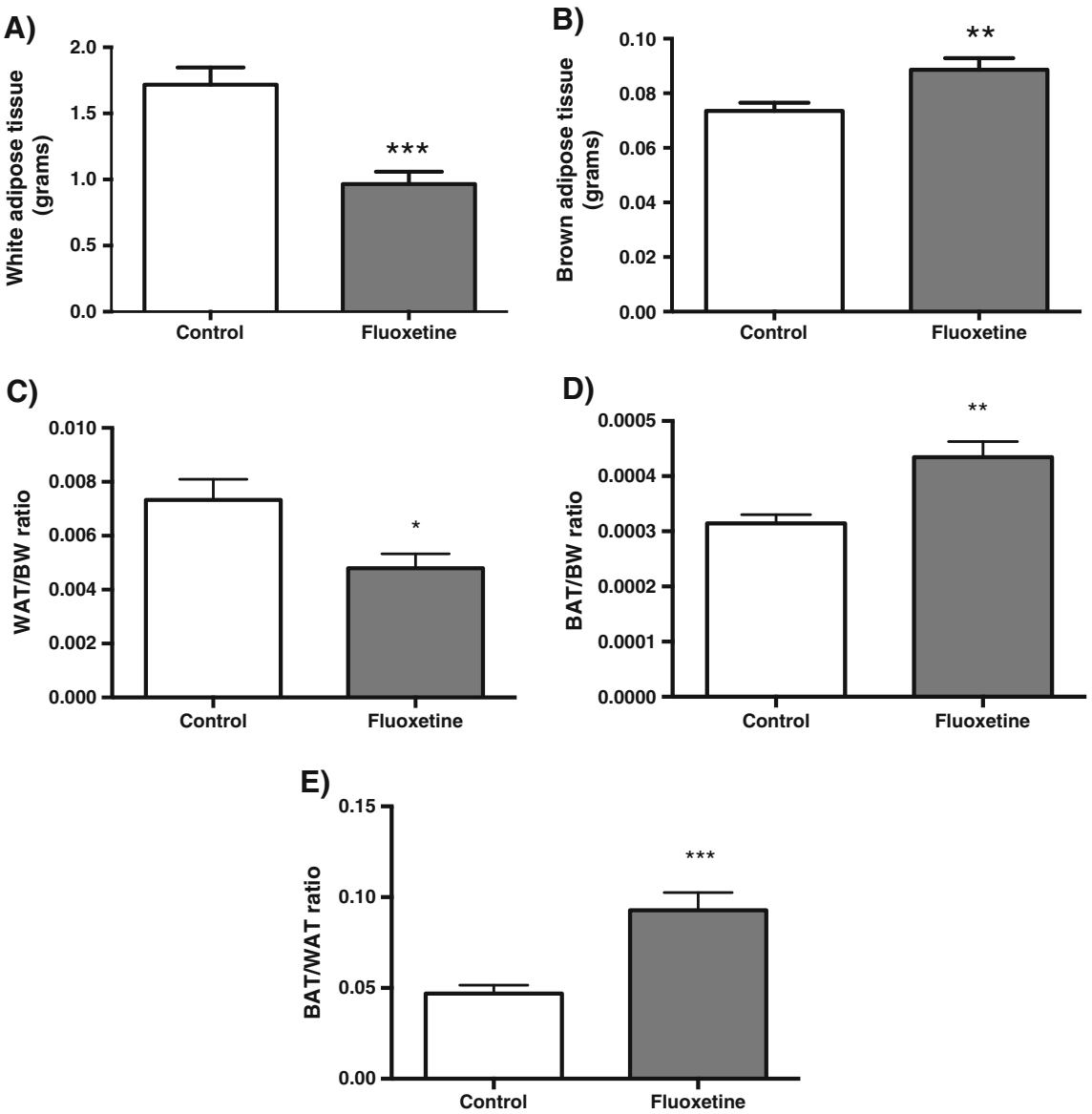

Fig. 2 Effect of neonatal treatment with fluoxetine on *White* (**a**) and *Brown* (**b**) adipose tissue weight (grams) in male rat pups that were treated with fluoxetine (fluoxetine=10 mg / kg bw, sc) or saline (control=0.9 % NaCl 1 ml/kg, pc, sc) during the suckling period. The retroperitoneal white adipose tissue and brown dorsal tissue was measured in animal with 60 days of life. Data are presented as the mean ± SEM. (* $p<0.01$)

(proton transporter). After exposure to −15 °C, body temperature was measured using rectal temperature every 30 min for a total period of 90 min. The Fx-group demonstrated a reduced decrease in body temperature (basal temperature=Ct: 33.08±0.44 °C, n:5; Fx:32.33±0.65 °C, n:6; 30 min after thermal stimulus=Ct: 25.72±0.29 °C, n:5; Fx:27.50± 0.52 °C, n:6, $p<0.05$; 60 min after thermal stimulus=Ct: 21.66±0.07 °C, n:5; Fx:24.58±0.65 °C, n:6, $p<0.01$; 90 min after thermal stimulus=Ct: 18.38±0.17 °C, n:5; Fx:23.38±0.12 °C, n:6, $p<0.001$), and the Δ temperature after 90 min demonstrated that the Fx-group lost less heat than the Ct group (Fx: 4.56±0.31, n: 5; Ct: 7.42±0.45, n: 6;$p<0.001$) (Fig. 3b).

Effect of modulation of serotonin system by fluoxetine in mitochondrial function

Because proton leak (heat production) is mainly a mitochondrial event, mitochondrial function is a good indicator of brown fat activity. To assess the role of BAT mitochondria in the regulation of body weight we examined the mitochondrial oxygen consumption and we observed that mitochondria from Fx group has a higher basal respiration than Ct group (Fx: 56.6±2.13 nmol O2/min/mg prot, $N=11$; Ct: 38.0±1.98 nmol/min/mg prot, $N=11$; $p<0.0001$); we also observed that after add an uncoupling protonophore (CCCP) the Fx group still

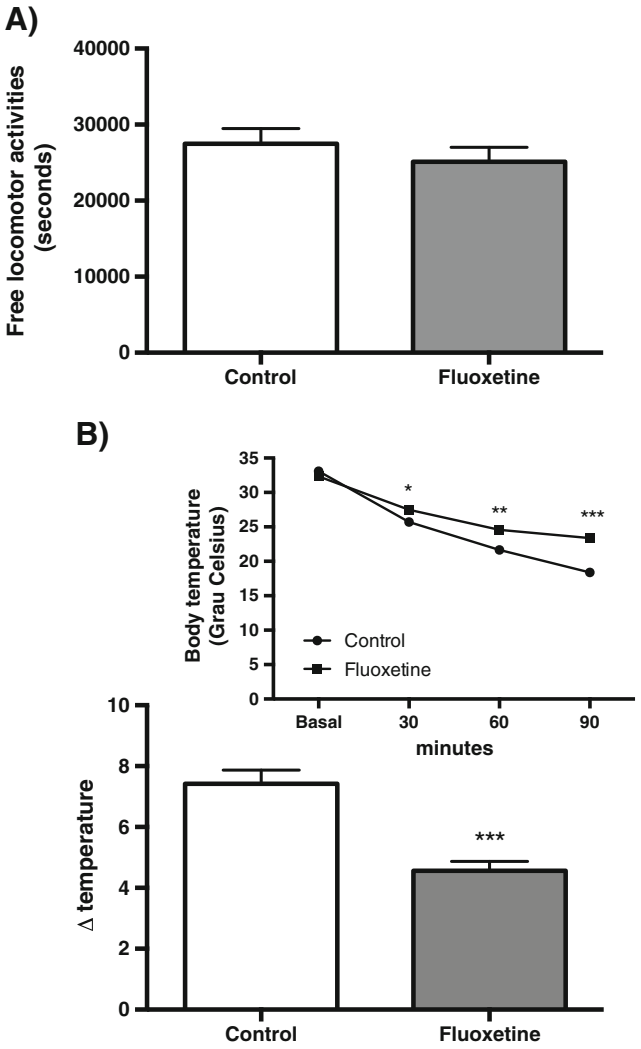

Fig. 3 Effect of neonatal fluoxetine on energy expenditure. **a** Free locomotor activity (seconds), **b** Body temperature in the male pups of rats that were fluoxetine (fluoxetine=10 mg / kg bw, sc, n=6) or saline-treated (control=0.9 % NaCl 1 ml/kg, pc, sc, n=6) during suckling period. The volunteer activities recorded in seconds are presented as the mean ± SEM. Body temperature variation was measured progressively every 30 min for 90 min total. The delta of variation (Δ) in temperature is presented as the mean ± SEM (*p<0.05; **p<0.01;***p<0.001)

showed a higher respiration rate than Ct group (Fx: 71.2 ±2.27 nmol O2/min/mg prot, N=11; Ct: 48.0± 2.35 nmol/min/mg prot, N=11; p<0.0001) (Fig. 4a). These results, together with the temperature led us to the hypothesis that uncoupling proteins could participate in the regulation of the body weight. With this in mind we evaluate the oxygen consumption in BAT mitochondria in presence and absence of GDP, a potent inhibitor for uncouple proteins (UCP), and we found a significant decrease of oxygen consumption in Fx group after added GDP (Fx—GDP: 56.6±2.13 nmol O2/min/mg prot, N= 11; Fx+GDP: 46.0±1.7 nmol/min/mg prot, N=6; p<0.01) (Fig. 4b), adding another piece that suggests an involvement of UCP in Fx-modulating body weight.

Effect of modulation of serotonin system by fluoxetine in uncouple protein-1 in brown adipose tissue

Together, our results suggest the participation of UCP1 in Fx-modulating body weight. We next measure the ROS production from BAT mitochondrial since several studies had shown that mild uncoupling from UCPs could attenuates the production of ROS (Dlaskova et al. 2006; Dlaskova et al. 2010; Oelkrug et al. 2010). As shown in Fig. 5, BAT mitochondria from the Fx group produced significantly less ROS than Ct group. As final test to understand the possible participation of UCP1 in fluoxetine-treated lean rats, we measured the UCP1 protein expression in BAT mitochondria. As shown in Fig. 6 neonatal fluoxetine treatment induces an increase in UCP1 expression (Fx: 2.45±0.07, N=3; Ct 1.98±0.11, N=4; p<0.05), suggesting that the decrease observed in body weight may be due to the increase in mitochondrial function especially UCP1 activation. Taking together, our results shown that serotonin modulation by fluoxetine treatment modulates UCP and improves mitochondrial function.

Discussion

In the present study, we demonstrated that chronic treatment (21 consecutive days) with selective serotonin reuptake inhibitors during the period of brain development reduced body weight with participation of mitochondrial UCP and mitochondrial bioenergetics. In our study, fluoxetine treatment did not induce differences in food consumption compared with controls during the neonatal (7, 14 and 21 days) period or at 40 days for 24 h of analysis. Previous studies have shown that fluoxetine decreased body weight by anticipate satiety (McGuirk et al. 1992; Leibowitz and Alexander 1998). However, these previous studies were conducted in adult animals using SSRIs such as citalopram, sertraline, fluoxetine or 5-HT or its precursor, the amino acid tryptophan (Blundell and Latham 1979; McGuirk et al. 1992). It is important to highlight that our treatment was conducted during brain development (neonates), and the effects of treatment can be very different between ages and/or developmental stages. Using same experimental model, previous study conducted in females from our laboratory also did not observe a difference in food intake, corroborating with our previous study conducted (da Silva et al. 2014). The window for nervous system maturation is until the 40th postnatal day, thus these stages are vulnerable to neurobiological changes (Hansson et al. 1998; Zhou et al. 2000). It is likely that fluoxetine handling is crucial to the development of specific adaptive behaviors, as our results demonstrate no difference in food intake; drug concentration and treatment duration are variables that affect the response of fluoxetine on food intake.

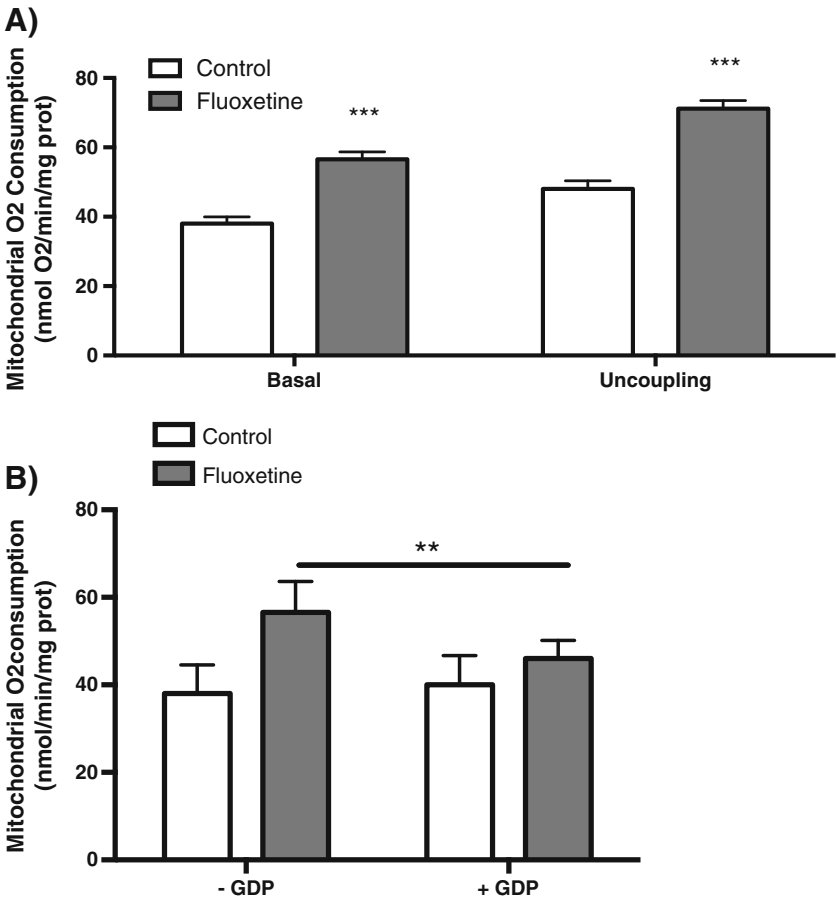

Fig. 4 Effect of neonatal fluoxetine treatment on brown adipose mitochondrial respiration in male pups of rats that were treated with fluoxetine (fluoxetine=10 mg / kg bw, sc, n=11) or saline (control=0.9 % NaCl 1 ml/kg, pc, sc, n=11) during suckling period. **a** Mitochondrial respiration was measured in State 2 respiration and Maximal respiration capacity (Basal and Uncoupled, respectively); **b** Mitochondrial respiration was measured in absence or presence of GDP during State 2 respiration (n=6 in each group). The data is presented as the mean ± SEM (**p<0.01;***p<0.001)

Our results showed a decrease in body weight associated to decrease in white adipose tissue, an increase in brown adipose tissue in association to increase in the ratio of brown/white adipose tissue. In agreement with our study, Silva et al. (Silva et al. 2010) and da Silva et al. (da Silva et al. 2014), using the same experimental model that we apply in our study, demonstrates that chronic fluoxetine treatment induced less weight gain and less body weight since lactation and maintain in adult life. Another study using the antidepressant citalopram (10 mg / kg bw, sc) also demonstrated reduced body weight (Magalhaes et al. 2006). The antidepressant fenfluramine (reuptake inhibitor and 5—HT release stimulator) increased proopiomelanocortin (POMC) expression (Heisler et al. 2002). The neuropeptide POMC is synthesized in hypothalamic nuclei and emits preganglionic neuron projections in the mediolateral spinal cord; they communicate with skeletal muscle by sympathetic postganglionic fibers (Cechetto and Saper 1988; Broberger 2005), which may activate also UCP in skeletal muscle. When this hypothalamic pathway is stimulated, changes in skeletal muscle energy metabolism can occur resulting in increased energy expenditure and decreased body weight (Angiolini et al. 2006).

The dissipated energy comprises the basal metabolism, mechanical work (locomotor activity) and adaptive temperature

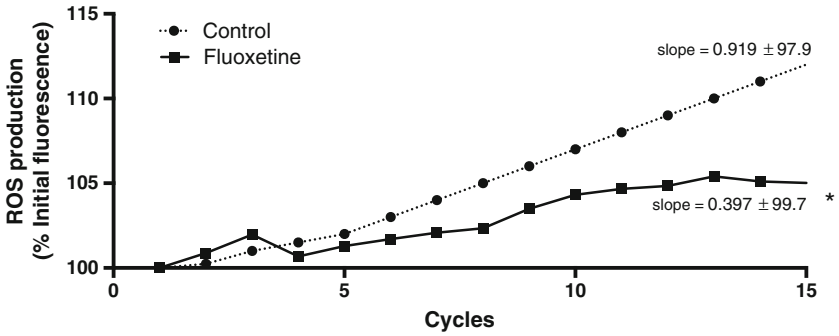

Fig. 5 Effect of neonatal fluoxetine treatment on ROS production in brown adipose mitochondria from male pups of rats that were treated with fluoxetine (fluoxetine=10 mg / kg bw, sc, n=6) or saline (control=0.9 % NaCl 1 ml/kg, pc, sc, n=6) during suckling period. The data is presented as the mean ± SEM (*p<0.05)

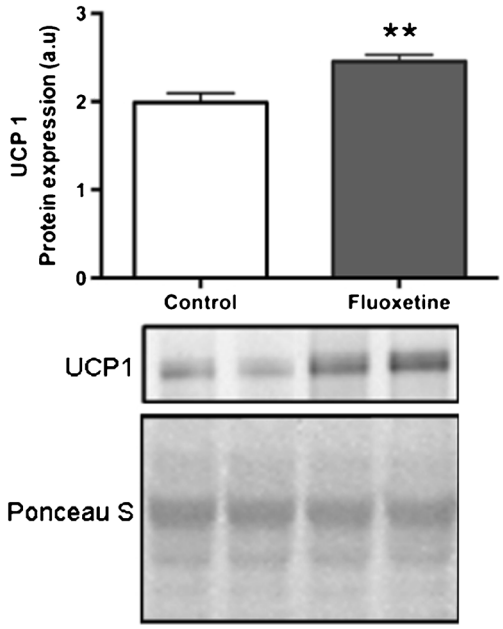

Fig. 6 Effect of neonatal fluoxetine treatment on BAT UCP1 protein expression in male pups of rats that were treated with fluoxetine (fluoxetine=10 mg / kg bw, sc, n=3) or saline (control=0.9 % NaCl 1 ml/kg, pc, sc, n=4) during suckling period. The data is presented as the mean ± SEM (*p<0.05)

control (Spiegelman and Flier 2001). Thus, high locomotor activity likely increases dissipated energy resulting in body weight loss. Our results showed that fluoxetine treatment has no effect on locomotor activity. However we found alterations in temperature control parameters after cold exposure. It is well known that in rodents, cold exposure can sufficiently activate brown adipose tissue (BAT), leading to increased levels of nonshivering thermogenesis via activation of the sympathetic system, and we observe that in the first 30 min in cold stimulus the temperature was significant difference between groups. In agreement with our data, studies with rats using another SSRIs, sibutramine, reported increased dissipated energy and reduced body weight (Missale et al. 1998; Izenwasser et al. 1999; Liu et al. 2002; Golozoubova et al. 2006; Mitchell et al. 2006).

Brown adipose tissue (BAT) is the main organ for the control of temperature in small mammals (Lowell and Spiegelman 2000; Avram et al. 2005). Drugs that increase temperature dissipation (by activating in BAT) may be possible drug targets for obesity treatment (Spiegelman and Flier 2001; Subramanian and Vollmer 2002; Major et al. 2007). Activation of β3-adrenergic receptors in brown adipocytes causes lipolysis, increased UCP-1 activity, and thermogenesis (Avram et al. 2005; Fan et al. 2005). Because thermogenesis in BAT is a process of metabolic regulation that is controlled by the hypothalamus via descending sympathetic fibers (Lowell and Spiegelman 2000), serotonin reuptake inhibitors may act both centrally and peripherally to influence body temperature control (Liu et al. 2002; Subramanian and Vollmer 2004). Increasing extracellular 5-HT using reuptake inhibitors may indirectly increase β-adrenergic receptor activation and affect neuronal activity in the melanocortin system (Myung et al. 2005). Studies in obese mice treated with leptin demonstrated an increase in hypothalamic and brainstem serotonin, decreases body weight, and increase in rectal temperature and UCP expression in BAT, suggesting the involvement serotonin in BAT and UCP (Harris et al. 1998).

Taking our current data together with the literature, this study provides additional information related to the mechanism by which selective serotonin reuptake inhibitors cause weight loss and provides additional insight into the role of serotonin in energy balance regulation. We hypothesize that modulation of serotonin system results in a reduction in body weight by direct augmentation of brown/white adipose tissue ratio and uncoupling protein activity that increases proton leak and heat production.

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