Muscular and mitochondrial effects of long-term fluoxetine treatment in mice, combined with physical endurance exercise on treadmill

Abdulkarim Tutakhai⁠¹,², Qand Agha Nazari³, Sarah Khabil⁶, Alain Gardiera, François Coudorea

¹ CESP/National Institute of Health and Medical Research INSERM UMR-S 1178, Paris-Sud University, Faculty of Pharmacy, Paris-Saclay University, France
² Pharmacology Department, Faculty of Pharmacy, Kabul University, Afghanistan
³ Received 10 April 2019; Received in revised form 22 May 2019; Accepted 23 May 2019
Available online 03 July 2019

ABSTRACT

Aim: Fluoxetine, one of the first newer SSRI antidepressant, is an extremely popular treatment for depression that could improve mental health problems. Many recent studies have suggested that SSRI have potential beneficial effects on skeletal muscle tissue.

Main method: We evaluated the potential beneficial effects of oral fluoxetine (18 mg/kg/day for 6 weeks) on muscle performance, after 6 weeks of physical exercise on treadmill. Male mice were randomly assigned to four groups (n = 12 per group) for treatment. Each group received treatment with following specifications: 1) no exercise with vehicle treatment (SED-S); 2) no exercise with fluoxetine treatment (SED-F); 3) exercise with vehicle treatment (EX-S); and 4) exercise with fluoxetine treatment (EX-F). Exercise performances were assessed based on the exhaustive running time and forelimb grip strength, anxious behavior by elevated plus-maze and open-field tests. Mitochondrial enzymes activity and ROS production were measured in the gastrocnemius and soleus muscles.

Key finding: Fluoxetine treatment had a significant effect on maximal aerobic capacity in mice without exercise, but more significant effects on gripping strength and anxiety when combined with exercise training, e.g. increased strength and decreased anxiety.

Significance: Fluoxetine treatment and exercise stimulation also had synergistic effects on strength and increased mitochondrial activity, cellular oxidative and antioxidant capacity in two different muscles.

1. Introduction

Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) are widely used to treat various mental health disorders, such as moderate-to-severe depression and anxiety [1–3]. Both symptoms contribute to insomnia, loss of appetite, lack of motivation and increased physical fatigue [4–6]. These symptoms can impair physical performances for athletes, more specifically for those who develop sport-specific skills and techniques, receive higher training volumes at various intensities, and participate in more frequent competitions [7]. Therefore athletes may use drugs that enhance motivation and/or improve overall fitness by reducing depressive symptoms. The use of antidepressants is not yet forbidden in elite sports [2]. Recent reports on doping associated with SSRIs show an increasing trend of its usage among healthy athletes [2]. The antidepressants intake among athletes has increased in different sports over the last decade, especially endurance sports [2,8]. The antidepressants Bupropion and Amineptine were removed from the list of banned substances [2]. Machnik et al. concluded that the increase in the prevalence of SSRIs in elite sports was not correlated with that of SSRI in the general population [2,9]. The mostly used class of antidepressants (AD) in sports is that of SSRIs, particularly fluoxetine. A large body of evidence shows the plasticity of the brain against various physical and chemical stimuli. In the field of SSRI therapy, fluoxetine is considered to improve motor activity and muscle strength [10,11]. However, there is increasing literature, such as the antidepressant mono-amine theory, trying to highlight that some serotoninergic regions of the brain are involved in performance through improving metabolism. According to some old and recent studies, fluoxetine has various effects on different mechanisms such as hippocampal dependent and independent neurogenesis, and hypothalamic-pituitary-adrenal (HPA) axis regulation [3,12,13]. However, no clear research can explicitly indicate their potential impact on performance. Individually or in conjunction with long-term aerobic exercise, fluoxetine has a number of common biological targets, such as brain-derived neurotrophic factor (BDNF) [9,14,15], alleviation of anxiety- and depression-like behaviors [16,17], muscle strength [11], and scavenging
inflammation [15]. Some recent studies have confirmed that fluoxetine modifies mitochondrial redox parameters in different animal models [18]. According to recent studies, three types of fibers reflect a significant increase in the level of PGC-1α expression after six weeks of endurance exercise [19,20]. The isoforms of myosin have been identified as the main determinant of functional variability among skeletal muscle fibers. Endurance exercise increases lactate tolerance, glycolytic function, mitochondrial volume, mitochondrial protein synthesis, capillary density, oxidative function and endurance capacity. While resistance exercise enhances muscle hypertrophy, muscle strength, muscle fiber size and myofibrillar protein synthesis [21]. Endurance exercise lead to changes in muscle fiber type composition which are mainly limited to type-2 fibers and involve a transformation of 2b into 2a fibers, resulting in a more oxidative muscle [22].

We therefore present a study on the mutual effects of fluoxetine, exercise, and combination of both on physical and neuro-behavioral performance, using a model of anxiety/depression phenotype in mice.

2. Method and materials

2.1. Animals

Six weeks old adult Balbc-j male mice purchased from Janvier Labs (Le Genest Sur l’Isle, France) weighing 21–25 g at the beginning of the study, were randomly assigned to five mice per cage in a temperature (21 ± 1 °C) controlled room with a 12 h light:12 h dark cycle (lights on at 06:00 h). Food and water were provided ad libitum. The protocols involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive #87-848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92-373 to FC) and in compliance with protocols approved by the Institutional Animal Care and Use Committee (CEE26 authorization #6195).

2.2. Drugs

Fluoxetine hydrochloride (18 mg/kg per day in the drinking water for six weeks) was purchased from Anawa Trading (Zurich, Switzerland).

2.3. Treadmill-running protocol

Exercise training consisted of running on a 6-lane rodent motor driven treadmill (Ugo Basile®, Italy) equipped with UB X-Pad software version 1.0.01 that automatically recorded the distance, velocity and time of animal running. To reduce their stress, mice were faced to treadmill for one-week adaptation (20 min/day, 15 m/min). To determine individual maximal aerobic running speed, treadmill band speed was increased from 6 m/min by 0.03 m/s every 2 min until their exhaustion. Subsequently, mice underwent incremental exercise training for six weeks. Mice were divided in four groups, no exercise-saline = 12, no exercise-fluoxetine = 12, exercise-Saline = 12 and exercise-fluoxetine = 12. Body weight was assessed at the beginning of each week, during the six weeks. After the last physical performance determination, animals were sacrificed by cervical dislocation before tissues harvest. Gastrocnemius muscles from both legs were dissected and their mass was measured just after sampling and before freezing in liquid nitrogen and storing in −80°C for analysis. The brain was quickly removed, hippocampus and cortex were dissected and stored at −80°C.

2.4. Physical performances

2.4.1. Gastrocnemius muscle mass

Gastrocnemius muscle mass was measured rapidly after dissection and before fixation in liquid nitrogen. Before sacrifice, the ratio of gastrocnemius mass to last body mass was calculated in mg and g for each mice.

2.4.2. The treadmill was used to determine the physical performances for each mouse: maximal aerobic velocity (VO2max in m/min), duration to exhaustion in VO2max75% and running distance in VO2max75% before and after training

The VO2max75% was measured by (VO2max75% = VO2max × 0.75). The time until exhaustion was determined after repeated failed tries toward treadmill pad and parallel shock number increase.

2.4.3. A Grip strength meter (Ugo Basile®, Italy) equipped with DCA software version 2.2 was used to measure the gripping strength of forelimb in gram force unit, by placing each mouse over a base plate in front of a grasping tool, either T-shaped, trapeze shaped or grid. The bar is fitted to a force sensor connected to the control unit, connected to a computer. This Procedure is repeated five times for each mouse alternatively.

2.5. Neurobehavioral parameters

2.5.1. Open field test

Anxiety and locomotor activity of mice were measured using the open field test [23]. Motor activity was quantified in Plexiglas open field boxes 43 cm × 43 cm (MED Associates, Georgia, VT, United States) during a 30-min session [23,]. Two sets of 16 pulse-modulated infrared photo beams were placed on opposite walls 2.5 cm apart to record x-y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100-millisecond resolutions. The center was defined as a 32 × 32-cm central area. Dependent measures were: total time spent in the center, the numbers of entries into the center and distance traveled in the center divided by total distance traveled over a 30-min period of the test. Overall motor activity was quantified as the total distance traveled (cm). The test was performed in the dark over a 30 min session; first of all, mice were acclimatized to dark for 15 min, and then placed in a corner of the boxes[24].

2.5.2. Elevated plus maze test

Behavior in the elevated plus maze was measured using a cross maze with two open and two closed arms (30 × 5 cm arms). Numbers of entries and time spent in the open arms during 5 min test measured anxiety-related behaviors. The different parameters were recorded by a digital camera and scored by video-tracking (EPM3C software, Bioseb, Vitrolles, France). The distance traveled in the maze was used as an index of locomotor activity.

2.6. Biochemical parameters

2.6.1. Reactive oxygen species (ROS) production

DCF-DA (Dichloro-dihydro-fluorescein diacetate) test. Gastrocnemius and soleus muscle were mechanically dissociated and homogenized in ten volumes of ice-cold PBS buffer using a Bertin Precellys 24 homogenizer (Bertin, Montigny-le-Bretonneux, France). Protein concentration was assessed using the bicinchoninic acid assay. After vortexing, 50 µg proteins were incubated with adequate probes for assessment of the oxidative response. Free radical generation and ROS, mainly hydrogen peroxide (H2O2) production, were detected with the 2,7-dichlorofluorescein diacetate (DCFH2-DA) probe, (Interchim, Montluçon, France) 10 µM solution in PBS, incubated with the homogenates for 20 min. The non-fluorescent polar derivative (H2DCF), in the presence of intracellular reactive oxygen species, mainly H2O2. The fluorescent intensity signal was measured with a microplate reader (λexc = 485 nm; λem = 535 nm).
2.6.2. Mitochondrial enzymes cytochrome C oxidase (COX) and citrates synthase (CS) activity measurement

Complete enzyme extractions from small pieces of frozen Gastrocnemius muscle tissues were done in an ice-cold homogenization buffer (50 mg ml\(^{-1}\); containing (in mM): Hepes 5 (pH8.7), EGTA 1, DTT 1, and 0.1% Triton X-100) using a Bertin Precellys 24 homogenizer (Bertin, Montigny-le-Bretonneux, France). Protein concentration was assessed using the bicinchoninic acid assay.

2.6.2.1. For COX activity measurement, Cytochrome C (C7752, Sigma Aldrich) 1 mM/K2HPO4 50 mM pH 7.4 was prepared and 90% reduced by sodium dithionite (157953 Sigma Aldrich). The absorbance of reduced Cytochrome C was measured, using standard spectrophotometric assays by UviKon XS (SECOMAN France) in 550 nm. 5 μl of 1/5 (3 μl sample + 12 μl homogenization buffer) diluted sample was added to the cuvette. The absorbance was re-measured after manual agitation. The difference between two absorbances was calculated as COX activity.

2.6.2.2. For CS activity measurement, 5 μl of 1/5 (3 μl sample + 12 μl homogenization buffer) diluted sample was added to the mix of Acetyl Co-A lithium salt (A2181 Sigma Aldrich) and dinitrobenzoic acid (D8130 Sigma Aldrich). First measurement was carried out in 420 nm, using standard spectrophotometric assays by UviKon XS (SECOMAN France). 50 μl, 10 mM Oxaloacetic acid (O4126 Sigma Aldrich) was added, manually agitated and the absorbance was re-measured. The difference between two absorbances was calculated as CS activity.

2.7. Statistical analysis

Prism v.6 computer software was used to conduct statistical analysis. Two-way analysis of variance (ANOVA) followed by post-hoc Tukey’s multiple comparison test was used to compare test groups to control groups: no exercise-saline (SED-S), no exercise-fluoxetine (SED-F), exercise-saline (Ex-S) and exercise-fluoxetine (Ex-F). Data were presented as mean ± SEM and the statistical significance level was set at \( p < 0.05 \).

3. Results (Table 1)

3.1. Physical performances

3.1.1. Gastrocnemius muscle mass (Fig. 1)

Fluoxetine significantly increased the gastrocnemius muscle mass in exercise group, compared to other groups: EX-F vs Ex-S (\( p < 0.05 \)).

3.1.2. Maximal aerobic velocity (Fig. 2)

Physical exercise significantly increased the maximal velocities after 6 weeks training (ratio T6/T0) as compared to no exercise animals: +32.7 ± 4.7% vs −3.2 ± 2.7% for EX-S vs SED-S, respectively (\( p = 0.001 \)) and +30.6 ± 5.0% vs +7.6 ± 2.4% for EX-F vs SED-F, respectively (\( p < 0.001 \)). Fluoxetine increased the maximal velocity only in no exercise mice: +7.6 ± 2.4% vs −3.2 ± 2.7% for SED-S FXD-S, respectively (\( p = 0.001 \)), but not after 6 weeks exercise training: +30.6 ± 5.0% vs +32.7 ± 4.6% for EX-F vs Ex-S, respectively (\( p > 0.05 \)).

3.1.3. Time to exhaustion during training at VO\(_{2}\)max\(_{75\%}\) (Fig. 3)

Durations until exhaustion were significantly increased by the exercise, whether in fluoxetine and in saline groups (+1.1 ± 0.2% vs +0.8 ± 0.2% for EX-S vs SED-S, respectively \( p = 0.01 \)) and +1.2 ± 0.1% vs +1.1 ± 0.1% for EX-F vs Sed-F, respectively (\( p > 0.05 \)). Interestingly fluoxetine administration induced only a slightly significant increase of time to exhaustion in the no exercise group: +1.0 ± 0.1% vs +0.8 ± 0.2% for SED-S vs SED-S, respectively (\( p = 0.05 \)). No significant effect of fluoxetine was observed between both exercise groups.

3.2. Neurobehavioral performances

3.2.1. Open field test (Fig. 5A, B)

Open field test was done to measure the locomotor activity between groups. Time in center (A) and ambulatory ratio (B) was calculated.

- Exercise increased the time in center significantly in saline group but this increase was not significant in fluoxetine-treated group: +168.9 ± 33.9 s vs +111.06 ± 49.0 s for Ex-S vs SED-S (\( p = 0.01 \)), for Ex-F vs SED-F, respectively. Fluoxetine significantly increased the time in center only in no exercise animals: +167.2 ± 50.5 s vs +111.06 ± 49.0 s for SED-F vs SED-S (\( p = 0.01 \)).

- Exercise increased the ambulatory ratio significantly in saline group but this increase was not significant in fluoxetine-treated group: 0.201 ± 0.03 s vs 0.141 ± 0.06 s for Ex-S vs SED-S (\( p = 0.01 \)), for Ex-F vs SED-F, respectively. Fluoxetine also increased the ambulatory ratios, but effects were not significant either in no exercise or in trained animals.

3.2.2. Elevated plus maze (EPM) (Fig. 6)

EPM test was used to measure the time in open arm and close arm. Only exercise-fluoxetine combination significantly increased time in open arm: +166.02 ± 44.9 s vs +82 ± 40.2 s for open vs close arm (\( p < 0.05 \)). Exercise and fluoxetine separately, slightly increased the time in open arm (\( p > 0.05 \)).

3.3. Biochemical results

To investigate the potential cellular mechanisms underlying the effects on performances and behaviors of exercise and fluoxetine, we...
of gastrocnemius muscle of mice.

3.3.1. Mitochondrial enzymes: cytochrome C oxidase (COX) and Citrate synthase (CS) (Figs. 7 and 8)

Kreb’s cycle enzymes of mitochondria were studied in homogenates of gastrocnemius muscle of mice.

Concerning COX, exercise significantly increased COX activity versus no exercise group only in the fluoxetine-treated group (140.7 ± 25.88 vs 107.9 ± 24.1 for Ex-F vs SED-F (p < 0.05)) but not in saline-treated groups.

CS activity was increased after exercise in saline and fluoxetine-treated groups: 157.1 ± 13.2 vs 119.5 ± 25.8 for EX-S vs SED-S (p = 0.01), 174.0 ± 25.8 vs 120.8 ± 28.2 for Ex-F vs SED-F (p = 0.01), respectively. Interestingly, fluoxetine significantly increased CS activity only in exercise group: 174.0 ± 25.8 vs 157.1 ± 13.2 for EX-F vs EX-S.

3.3.2. ROS production study (gastrocnemius: Fig. 9) (soleus: Fig. 10A and B)

DCF-DA test was carried out to measure reactive oxygen species and superoxide anion as fluorescence intensity in gastrocnemius and soleus muscles.

Exercise significantly decreased the ROS level in gastrocnemius and soleus muscles, in saline and fluoxetine-treated groups. ROS levels decreased were always higher in fluoxetine groups than in saline groups, even more important when fluoxetine was associated with physical exercise.

4. Discussion

The present study analyses the effects of physical training and fluoxetine treatment, each one individually or combined, on physical performance, neurobehavioral activity, markers of oxidative stress in muscles, cerebral cortex, and hippocampus in mice. Intrinsic endurance abilities were evaluated by a treadmill exercise. As expected, six weeks of treadmill training at an increasing speed, from 50% to 70% of the maximum running speed, has significantly improved physical performance, by increasing endurance parameters (33%) or strength (33%). The physically active lifestyle remains an important factor in muscle plasticity [25,26]. Continuous endurance training could decrease anxiety-depressive-like behaviors, as previously demonstrated by several studies. In humans, exercise improves neuroplasticity and alleviates anxiety-depressive behaviors, leading to improved mood [27]. Fluoxetine and

### Table 1
Data of various behavioral and biochemical tests in mice treated by fluoxetine (18 mg/kg/day, p.o., 6 weeks) or saline and either submitted to six weeks treadmill training exercise (exercise group) or untrained (no exercise group). Results are expressed as means ± S.D., n = 11–12 mice per group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No-exercise saline</th>
<th>No-exercise fluoxetine</th>
<th>Exercise-saline</th>
<th>Exercise-fluoxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastronemius (mg/g body)</td>
<td>4.82 ± 0.33</td>
<td>5.01 ± 0.2</td>
<td>4.61 ± 0.329</td>
<td>5.24 ± 0.361</td>
</tr>
<tr>
<td>VO2 max (m/min)</td>
<td>27.25 ± 3.1</td>
<td>27.5 ± 2.19</td>
<td>33 ± 3.20</td>
<td>35.6 ± 3.70</td>
</tr>
<tr>
<td>Endurance (min)</td>
<td>60.7 ± 10.06</td>
<td>70.5 ± 9.33</td>
<td>68.67 ± 16.24</td>
<td>84.25 ± 2.75</td>
</tr>
<tr>
<td>Grip strength (g)</td>
<td>88.56 ± 16.50</td>
<td>99.67 ± 11.77</td>
<td>113.89 ± 17.12</td>
<td>126.23 ± 12.48</td>
</tr>
<tr>
<td>Ambulatory ratio</td>
<td>0.141 ± 0.066</td>
<td>0.174 ± 0.061</td>
<td>0.203 ± 0.032</td>
<td>0.213 ± 0.037</td>
</tr>
<tr>
<td>EPM time in open arm (sec)</td>
<td>92.26 ± 61.12</td>
<td>131.23 ± 57.37</td>
<td>147.95 ± 70.04</td>
<td>166.02 ± 44.90</td>
</tr>
<tr>
<td>COX activity (IU/g protein)</td>
<td>86.39 ± 40.45</td>
<td>104.04 ± 36.02</td>
<td>122.91 ± 39.24</td>
<td>125.35 ± 37.79</td>
</tr>
<tr>
<td>CS activity (IU/g protein)</td>
<td>119 ± 25.8</td>
<td>120.8 ± 28.24</td>
<td>157.09 ± 13.21</td>
<td>174.95 ± 25.88</td>
</tr>
<tr>
<td>ROS (fluorescence intensity)</td>
<td>1,962,199 ± 630,819</td>
<td>1,553,016 ± 185,025</td>
<td>1,382,588 ± 180,395</td>
<td>897,055 ± 116,205</td>
</tr>
</tbody>
</table>

Fig. 2. Evolution of maximal aerobic velocity ratio (%) after 6 weeks treadmill exercise (T6/T0) and fluoxetine treatment: mice were treated by fluoxetine (18 mg/kg/day, p.o., 6 weeks) or saline and submitted either to six weeks treadmill training exercise (exercise group) or untrained (no exercise group). Results are expressed as means ± S.D., n = 11–12 mice per group.

Fig. 3. Evolution of running time until exhaustion at 75% of MAV ratio after 6 weeks treadmill exercise (T6/T0) and fluoxetine treatment: mice were treated by fluoxetine (18 mg/kg/day, p.o., 6 weeks) or saline and submitted either to six weeks treadmill training exercise (exercise group) or untrained (no exercise group). Results are expressed as means ± S.D., n = 11–12 mice per group.

Fig. 4. Evolution of grip strength force ratio after 6 weeks treadmill exercise (T6/T0) and fluoxetine treatment: mice were treated by fluoxetine (18 mg/kg/day, p.o., 6 weeks) or saline and submitted either to six weeks treadmill training exercise (exercise group) or untrained (no exercise group). Results are expressed as means ± S.D., n = 11–12 mice per group.
exercise, taken individually or in combination, can reduce anxiodepressive disorders by increasing hippocampal neurogenesis [3]. We also found in open field and elevated plus maze tests that exercise and fluoxetine increase ambulatory ratio, time spent in the center, and time in open arm—representatives of anxiolytic activity.

We also observed that fluoxetine treatment increased muscle strength with or without exercise. This is the first time that such a synergistic effect of an antidepressant is demonstrated on muscle strength of a resistant physical training. Fluoxetine resulted in a 36% increase of maximal forelimb resistance with grip strength in mice without exercise post experimental intervention, however this increase was even more
significant (+57%) in mice subjected simultaneously to a physical exercise. The increase in strength observed with this non-invasive and easy-to-perform test is a marker of muscle function improvement.

A considerable amount of evidence confirms the existence of very large and varied body-serotonin interactions – out of which a single close link between the serotonergic system and skeletal muscle morphology is worth-mentioning [28]. The serotonergic system, as a target of fluoxetine, stimulates glucose uptake by skeletal muscle via activation of phospho-fructo-kinase enzyme [29]. Fluoxetine has also been shown to increase motor activity via the serotoninergic system [11]. Our research on fluoxetine and skeletal muscle interactions has been very well confirmed [1,30]. Huang et al., found that grip strength was significantly correlated with intrinsic aerobic capacity [31]. Interestingly, we observed that fluoxetine alone or in combination with exercise increased muscle strength.

Despite the associated mechanism being unknown, our study proves that only treatment with fluoxetine also significantly increased muscle strength. Rodent studies investigate the interaction between SSRIs and muscle weight, myocyte count, and skeletal muscle fiber cross-sectional area. Mice treated with fluoxetine appear to have better neuromuscular adaptation to recruit more motor units for higher force production than control groups. Some studies have demonstrated that activation of the 5-HT2A serotonin receptor in skeletal muscle induces the expression of genes involved in myogenesis and confirms its participation in excitation-contraction coupling [32]. The studies show the role of fluoxetine in prevention of degeneration of skeletal muscle [33] as well as improvement of motor activity [34]. Peripheral cellular effects of SSRIs in muscle tissues are largely unexplored. In a recent study, an intramuscular application of fluoxetine results in functional changes in the electrical properties of myotonic dystrophy of type-1 muscle [34,35].

Endurance exercises are characterized by repeated and sustained low-intensity contractions for a prolonged period without fatigue. This term generally refers to training of the aerobic system (Krebs cycle, oxidative phosphorylation) versus the anaerobic system [35,36]. Endurance exercises mainly increase the rate of mitochondrial biogenesis, as well as improve functional parameters of mitochondria. Exercise-induced mitochondrial function adjustments appear to be primarily explained by increased expression of mitochondrial enzymes that facilitate aerobic metabolism. We confirmed these changes after 6 weeks of endurance exercise in mice. We found that long-term administration of fluoxetine combined with physical exercise significantly increased citrate synthase and cytochrome oxidase activity in adult mice. With respect to the combined effect of fluoxetine on mitochondria, Visco et al., have previously shown that selective serotonin reuptake inhibitors would alter skeletal muscle electrical activity, muscle weight, myocyte count, and muscle thickness as well as changes in energy metabolism by various mitochondrial enzymes in rodents [1].

It has also been reported that fluoxetine increases blood flow via vasodilation in skeletal muscle by interfering with Ca^{2+} signal transduction in vascular smooth muscle cells [37]. Preferably, it was due to mitochondrial biogenesis, prevention of oxidative stress, myogenesis and plasticity of muscle fibers [1], and blockage of neuromuscular transmission [38]. As a part of literature review, we found ample of evidence confirming the predominant role of fluoxetine in mitochondrial biogenesis [1,18,22,30,37,39]. While physical endurance exercises increase muscle volume, strength and mitochondrial biogenesis, their combination will potentially have synergistic effects [18,40-43] and will have greater significance for improving endurance.

We also observed that lean body mass was increased by exercise and fluoxetine. Total body mass decreased while gastrocnemius muscle mass increased. As previously shown, the relative contribution of fat to muscle-producing machinery increases with a parallel reduction in the rate of glycogen depletion [44,45]. Thirty-day voluntary exercise prevented hyperglycemia in streptozotocin-induced diabetic mice by increasing the mitochondrial oxygen consumption capacity in muscles and improving the state of oxidation-reduction [46]. Fujimakie et al., also found that exercise training was very helpful in preventing neuronal and skeletal muscle diabetes-induced impairment by enhancing the neurogenic capacity of neural stem cells and the proliferation and differentiation capabilities of satellite cells [47]. In response to muscle injury or exercise, the satellite cells are activated and could proliferate, renew automatically, and differentiate into new mature fibers [47]. Considerable number of literature suggests that the type of oxidative fiber and the number of satellite cells increase after chronic resistance exercise, mainly in type-2 fibers [48,49].

In the DCF-DA test, we also found out a decrease in the level of reactive oxygen species in gastrocnemius muscle cells in mice that were either trained or treated with fluoxetine, or received a combination of the two. Balanced regular physical training can alter mitochondrial content by reducing oxidative stress and enhancing antioxidant capacity, resulting in upregulation of the expression of certain important proteins that induce muscle growth and hypertrophy in mechanically stimulated muscle cells, such as Human Insulin Growth Factor I Protein (IGF1), Mechano Growth Factor (MFG), and Neuregulin 1 (NRG1) [50]. Reduced levels of reactive oxygen species, which may also decrease myostatin expression in skeletal muscle via NF-kB signaling of TNF-α, are likely to strengthen the role of myostatin in the adaptation of muscle forces [51]. However, chronic treatment with fluoxetine had a synergistic effect on mitochondrial bioenergetics with higher oxygen uptake and lower production of reactive oxygen species in rat skeletal muscle [30]. According to recent studies, physical exercise also attenuates the intensity of oxidative stress by inducing the Nrf2-HO-1 cascade [52].

5. Conclusion

In this study, we provided basic protocols for assessing physiological strength. Skeletal muscle is made of different types of fibers that form the basis of muscle plasticity in response to various functional requirements. This study confirms that long-term treatment with fluoxetine combined with prolonged exercise improves physical performance and muscle strength. Adaptive changes are strongly influenced by various structural, metabolic, and functional characteristics of individual fiber types. We observed that the exercise-fluoxetine protocol improved mitochondrial function in skeletal muscle, increased muscle strength and induced anxiolytic-like effects.

Acknowledgment

This project was supported by funding from the France anti-doping...
agency. The authors would like to thank Mrs Alexandra Malgyre for her aid in determination of physical performances, Melanie Gressette for measuring enzyme activity and Valerie Domergue for her assistance in management of animal care.

Compliance with ethical standards

The authors had no competing financial interest relevant to this article to disclose. This article does not contain any studies with human participants performed by any of the authors. All animals were carried out in accordance with “the guide for the care and use of laboratory animals”, Right Edition (2011).

References