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"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

Marcelo Rafanelli Rosatti

The effects of anabolic steroids abuse on breathing and body temperature in mice: a chronic and progressive study

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Dissertation presented to the São Paulo State University (UNESP), Faculty of Odontology, to obtain the title of Master by the Graduate Program of Physiological Sciences (PIPGCF), in physiology.

Supervisor: Profª. Drª. Mirela Barros Dias

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The effects of anabolic steroids abuse on breathing and body temperature in mice: a chronic and progressive study

Examining board

For obtaining a master's degree in physiological sciences

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Dedicated to my friends at the Dragon’s Cave, one of the best things that ever happened in my life was living with you.

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“But I am constant as the Northern Star,
Of whose true fixed and resting quality
There is no fellow in the firmament.
The skies are painted with unnumbered sparks;
They are all fire, and every one doth shine.
But there’s but one in all doth hold his place.
So in the world: ’tis furnished well with men,
And men are flesh and blood, and apprehensive.
Yet in the number I do know but one
That unassailable holds on his rank,
Unshaked of motion; and that I am he.”
William Shakespeare*

* Shakespeare W. Julius Caesar. Act III, Scene I, lines 66-76; 1599.

Rosatti MR. The effects of anabolic steroids abuse on breathing and body temperature in mice: a chronic and progressive study [master's dissertation]. Araraquara: São Paulo State University (UNESP), School of Dentistry; 2025.

ABSTRACT

Sexual hormones alter basal breathing, the hypercapnia and hypoxic responses, and thermoregulation. However, the effects and its progression of chronic high-dose testosterone administration were previously unexplored. We performed a chronic and progressive study using supra-physiological administration of testosterone cypionate (25mg/kg/week), in C57Bl6 adult male mice for 5, 10 and 24 weeks. Unanesthetized mice were submitted to whole-body plethysmography during exposition to room air, hypercapnia and hypoxia. In baseline conditions, testosterone treatment increased the respiratory frequency (fR) in all treated groups, [fR 5 weeks = 194.4±16.3 breaths.min^{-1} (n = 6), versus the vehicle 152.2±11.5 breaths.min^{-1} (n = 5); P = 0.0003; one-way ANOVA, Tukey's multiple comparisons], but this did not lead to an increase in pulmonary ventilation ($\dot{V}_E$). After 5 weeks, the hypercapnic ventilatory response was severely reduced [% of $\dot{V}_E$ min 20 = 94.2% ± 53.2 (n = 6), versus the vehicle 296.6% ± 77.7 (n = 5); P = 0.0005; Two-way ANOVA, Bonferroni's multiple comparisons], but it did not persist after 10 weeks, although it returned mildly after 24 weeks. The hypoxic ventilatory response changed its biphasic pattern after 10 weeks, which was restored after 24 weeks. Body temperature (Tbº) was unaltered during exposition to room air, hypercapnia and hypoxia, but the circadian cycling of Tbº was altered, treated animals having higher Tbº during the night, in a manner dependent of treatment length. In conclusion, supraphysiological TC alters baseline breathing, the respiratory chemoreflexes and Tbº in a manner that depends on the duration of administration and the light-dark cycle.

Keywords: Respiration. Hypercapnia. Hypoxia. Testosterone. Body temperature.

Rosatti MR. Efeitos do abuso de anabolizantes esteroides na respiração e temperatura corporal: um estudo crônico e progressivo [dissrtação de mestrado]. Araraquara: Universidade Estadual Paulista (UNESP), Faculdade de Odontologia; 2025.

RESUMO

Hormônios sexuais alteram a respiração basal, os quimioreflexos e a termorregulação. Porém, o efeito e sua progressão durante administração de testosterona crônica em alta dose não havia sido investigado. Executamos um estudo crônico e progressivo com doses supra-fisiológicas de cipionato de testosterona (25mg/kg/semana), em camundongos adultos C56Bl6, por 5, 10 e 24 semanas. Camundongos não anestesiados foram submetidos a registros de variáveis respiratórias por meio de pletismografia de corpo-inteiro durante exposição ao ar ambiente, hipercapnia e hipóxia. Testosterona aumentou a frequência respiratória (fR) basal em todos os grupos [fR 5 semanas = 194.4±16.3 breaths.min^{-1} (n = 6), versus o veículo 152.2±11.5 breaths.min^{-1} (n = 5); P = 0.0003; one-way ANOVA, pós teste de Tukey], mas isso não levou a um aumento na ventilação pulmonar ($\dot{V}_E$). Após 5 semanas, a resposta ventilatória hipercápnica foi severamente reduzida, [% da $\dot{V}_E$ min 20 = 94.2% ± 53.2 (n = 6), versus grupo controle 296.6% ± 77.7 (n = 5); P = 0.0005; Two-way ANOVA, Bonferroni’s multiple comparisons], mas não persistiu após 10 semanas, e retornou de modo leve após 24. A resposta ventilatória hipóxica perdeu sua natureza bifásica após 10 semanas, mas foi restaurada após 24. Temperatura corporal (Tbº) não foi alterada durante a pletismografia, mas o ciclo circadiano da Tbº foi alterado, animais tratados apresentando Tbº mais elevada durante a noite, dependendo do tempo de tratamento. Em conclusão, Administração de TC suprafisiológica altera a respiração basal, os quimioreflexos respiratórios e Tbº de maneira dependente da duração do tratamento e do ciclo claro-escuro.

Palavras-chave: Respiração. Hipercapnia. Hipóxia. Testosterona. Temperatura corporal.

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1 INTRODUCTION

Breathing is different between males and females. This results from central neuromorphological differences[1] from hormonal differences[2-4] and others[5]. These differences can be observed not only under basal conditions, but also during exercise, and in response to challenges such as hypercapnic and hypoxic conditions. The effects of normal levels of sexual hormones on breathing and the other differences mentioned have been thoroughly studied for decades, from the 1980's[6], through the 1990's[7] and today[8]; although the literature on this subject can be rather conflicting due to different experimental designs. Two influential literature reviews on the subject are those by Behan and Kinkead (2011)[9], and more recently Gargaglioni et al. (2019)[5]. Research has primarily focused on studying normal levels of sex hormones in the context of hormone replacement therapy and fertility treatment, as these represent the most common medical uses of exogenous sex hormones, aside from female contraception.

However, there is another context in which exogenous sex hormones are used extensively, though not for medical purposes: the non-therapeutic illegal use of performance enhancing drugs in sports, especially androgenic anabolic steroids (AAS) such as Testosterone Cypionate (TC)[10]. And, differently from hormonal replacement therapy, in this context the doses used are always supra-physiological, and athletes who use AAS are known to show respiratory symptoms, such as dyspnea, reduced endurance exercise capacity, mouth breathing and trenbolone cough[11,12].

The deleterious effects of chronic abuse of supra-physiological testosterone on the cardiovascular system are well known, and its pathological progression also well stablished. In fact, cardiopathy is the most common cause of death in athletes using AAS, since it leads to left ventricular concentrical hypertrophy, endothelial dysfunction, hypertension and blood hyper viscosity[13-15]. Although originally restricted to elite Olympic athletes, the deleterious abuse of AAS has now reached the general population and is used by average gym-goers and individuals seeking weight loss or improved aesthetics for non-professional reasons, and thus AAS is today considered an important worldwide public health problem[16].

The acute effects testosterone replacement therapy and antiandrogens on the chemoreflexes have been recently studied in rats[4], and studies on the acute effects of hormonal replacement therapy in humans on the chemoreflexes and metabolism exist for decades[6]. In these cases, testosterone replacement increased ventilation. Nevertheless, although the non-therapeutic supra-physiological effect of testosterone is well characterized in the context of the cardiovascular system[17], its effect on breathing and chemoreflexes, their chronic profile and symptom progression remained uninvestigated. The same applies to the effects on thermoregulation mechanisms such as hypoxia-induced anapyrexia, and the circadian cycling of Tbº.

Thus, we designed a chronic and progressive protocol of AAS abuse, to study how this would affect basal breathing, the hypercapnic and hypoxic chemoreflexes and Tbº during exposure to these respiratory challenges, and now AAS might affect the daily cycle of Tbº and understand if the effects of sexual hormones in these aspects depend on the doses, length of administration and light-dark cycle.

We hypothesized that the HVR would be affected the most, and that the change would be a progressive increase while the HcVR would be only mildly affected. Concerning the Tbº we hypothesized that the treatment would reduce anapyrexia and that there would be no effect during exposition to hypercapnia.

7 CONCLUSIONS

AAS abuse alters basal breathing and both chemoreflexes, each in a different way: it increases basal fR, reduces the HcVR and affects the biphasic nature of the HVR. These chronic effects not only are different from acute effects but also change according to the duration of the chronic treatment, and when it comes to Tbº it is furthermore dependent of the light-dark cycle. This opens the doors for future studies to understand how exactly sex hormones change the functioning of neurons and astrocytes that participate in the control of breathing, especially on how testosterone/DHT/estradiol might affect the molecular mechanisms chemosensitivity. Our experimental design leads to a testosterone/estradiol ambiguity, a common challenge in studies on sex hormones and breathing. Future research should focus on distinguishing the effects of these hormones, either by using AR antagonists or by employing alternative drugs, such as nandrolone, which is also commonly used in sports performance but is not converted into estradiol.

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