

## Project Review

### Beta2-agonists: modes of action and new tools for their detection

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This project, which constitutes a follow-up from a previous work funded by WADA, aims at widening our understanding of the effects of  $\beta$ 2-agonists on muscle function, cognitive function and exercise performance. The programme is composed of 10 complementary studies and involves 6 Universities of 4 different countries. Three main issues will be addressed:

- 1- Results of two bronchial provocation tests widely used for asthma detection in athletes (mannitol and eucapnic voluntary hyperpnea) will be confronted;
- 2- ergogenic and stimulant effects associated with the administration of  $\beta$ 2-agonists will be specified;
- 3- the precise signalling pathways involved in physiological adaptations induced by  $\beta$ 2-agonists will be clarified.

The expected outcomes are as follow:

- 1-Eucapnic Voluntary Hyperpnea should remain the "Gold Standard" for asthma detection in athletes;
- 2- since  $\beta$ 2-receptors are ubiquitous, the psychotropic effects of Terbutaline (i.e., improved cognitive function and increased level of arousal) contribute to its ergogenic effects. At a muscular level,  $\beta$ -agonists facilitate excitation-contraction coupling and protect against fatigue during intermittent high intensity exercise. A synergic effect, characterized by a marked hypertrophy and a proportional increase in maximal tension, is noticed when clenbuterol is associated to strength training;
- 3- myostatin and interleukin-6 are controlling factors in muscle growth. As such, both those molecules will be proposed as new detection tools for  $\beta$ 2-agonists abuse in athletes

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### **Results and Conclusion**

We showed that 3 week treatment of high doses of a beta-2 agonist with anabolic and lipolytic properties such as clenbuterol depressed specific isometric tension, particularly in slow twitch muscle. Another potential negative effect for slow twitch muscle lies in the increased ATP cost of shortening, suggesting an altered efficiency in mechano-chemico transduction. However, probably the most marked negative effect is the lesser resistance to fatigue observed with such high doses. These negative effects are of particular importance in sports events where performance depends on endurance and muscle oxidative capacity. In fast-twitch muscles, the slowing of the time course of skeletal muscle contraction and relaxation during force generation induced by clenbuterol treatment could be considered as a negative effect, contrasting the anabolic effect. These last negative impacts had a functional relevance in sport performance since the rate constants of force development and relaxation both control maximal shortening velocity and thus sprint performance. These negative impacts of anabolic beta-2 agonist must be highlighted for prevention strategies against doping.

We also reported that clenbuterol regulates negatively the expression of myostatin a master regulatory factor of muscle mass. Interestingly, we found that recombinant myostatin was sufficient to antagonize the hypertrophy activities of clenbuterol. Reciprocally, we also found that the genetic deletion of myostatin renders satellite cells refractory to the hypertrophic effect of clenbuterol. These results suggest that the clenbuterol-induced decrease of myostatin plays a functional role during hypertrophy. Although the beneficial health effects of regular moderate exercise are well established, there is substantial evidence that the heavy training carried out by endurance athletes can cause skeletal muscle damage. This damage is repaired by satellite cells that can undergo a finite number of cell divisions. Our results indicate that clenbuterol-induced hypertrophy can involve the recruitment of reserve cells in human skeletal muscle. From a sport viewpoint, it predicts that any benefits of clenbuterol treatment are likely to impose

any extra stress on the satellite cells and this method could risk the regenerative capacity in the long term.

In another study, we established the molecular mechanisms of the anabolic action of formoterol. In addition to protein kinase A, cAMP produced by the  $\beta$ 2-adrenergic signaling can also activate Epac. This protein is an exchange factor (guanine nucleotide) for the small G protein Rap. Rap juggles between a GDP inactive form and a GTP active form. Epac protein, activated by cAMP, catalyzes the exchange of GTP in the GDP form. Rap results in the activation of the PI3K/Akt/mTOR pathway and induces protein synthesis.

In a separate study conducted in trained athletes, we showed that oral administration of terbutaline at supra-therapeutic dose seems to alter the balance that exists between endogenous beta-adrenergic stimulation and exercise-induced stress. Exogenous stimulation of the beta-adrenergic pathway with terbutaline produced the disadvantage of an artificially stimulus and results in negative psychological effects in athletes. In prevention against doping, it is important to highlight the deleterious psychological effects induced by a supra-therapeutic terbutaline administration before a competitive sports event.

Finally in our last study in athletes we showed that inhalation of terbutaline prevents bronchoconstriction induced by hyperventilation with dry air, without limiting bronchial epithelial damages. Since epithelial damages may be involved in the development of respiratory asthma in many elite athletes, it seems important to identify new pharmacological strategies or nonpharmacological to minimize such damages.