“Single vs. combinatory effects of non-prohibited Beta-2 agonists at threshold doses on skeletal muscle metabolism and endurance performance”

Juergen Steinacker, Martina Zuegel, Daniel Bizdjak, Leoni Fink, Helmut Geiwiz (University of Ulm, Germany), **Maria Parr** (University of Berlin, Germany), **Patrick Diel** (German Sports University, Germany)

Project Overview

Background: High prevalence rates of β2 agonists used particularly amongst athletes of endurance disciplines makes it tempting to speculate that illegitimate use of β2 agonists beyond medical reason might be a common practice to boost performance in competitive sports. However, there is a lack of data regarding the underlying molecular basis of potential performance enhancing effects of β2 agonists.

Methods: In this study, 24 highly endurance trained participants (12 female, 12 male) participated in a double-blinded balanced 4-way complete block cross-over human pharmacological trial to investigate dosing and combinatory β2 agonist effects in order to evaluate their performance-enhancing potential. Single vs. combinatory threshold doses of non-prohibited, short-acting (salbutamol) and long-acting (formoterol) β2 agonists were administered by inhalation and potential effects were investigated by measuring skeletal muscle gene and protein expression of nuclear NR4A receptors, endocrine regulation, urinary β2 agonist concentrations, cardiac biomarkers, cardiopulmonary and lung function and the 10-min time trial performance (TT) with Cardiac Output (CO) measurement on a bicycle ergometer. Blood and urine samples were collected at four distinct time points: Pre TT (Pre), Post TT (Post), 3h Post TT (3h Post) and 24h Post TT (24 Post).

The possible stimulating effect of the β2 agonists formoterol and salbutamol on C2C12 myotubes was determined in vitro. Hypertrophy was assessed by measuring diameter of the fibers.

Results and Conclusions

All medication combinations were reliably detected in the urine samples by LC-MS/MS meeting WADA standards. None of the samples collected after application of verum medication resulted in concentrations exceeding the threshold concentrations set for doping control analysis. Mean Power Output during TT was not different between the different study arms.

There was a treatment effect regarding lung function observable without any influence on performance or health. There was a treatment effect on myocardial contractility measured by Echocardiographic Longitudinal Strain which increased for both strains and there was a marked effect of combined treatment.

Microarray subsample analysis revealed no significant treatment effect on gene expression of NR4A1 or NR4A3, but an effect was observable for NR4A2 with the most significant difference between Placebo and salbutamol + formoterol. The β2-combination influenced up- and downregulation of differently expressed genes most compared to the other study arms. Muscle analysis did not show any treatment effect on NR4A protein and NR4A1/NR4A3 gene expression, whereas a whole group treatment effect was observable for NR4A2. Further pathway analysis with gene expression software TACx and linked WikiPathways revealed treatment effects in energy metabolism related genes ATF3 (e.g. Hypertrophy model; TGF-beta signaling pathway), PDK4 (e.g. Estrogen receptor pathway; nuclear receptors meta-pathway), LPL (e.g., Metabolic pathway of LDL, HDL, and TG; PPAR signaling pathway), CREM (e.g. mBDNF and proBDNF regulation of GABA neurotransmission), and ATP1B3/ATPase (e.g. Calcium regulation in cardiac cells). Noradrenaline, adrenaline and TGF-β concentrations in blood were not affected by treatment or gender, whereas IGF concentrations showed a treatment effect 24h Post compared to Pre for women. CO data determined by Clearsight® device were not reliably reproduced in all measurements due to technical artefacts.

Both β2-agonists stimulated hypertrophy in a dose dependent manner compared to negative control in C2C12 myotubes. Diameters relative to control were increased for all β2-agonists treatments, but an additive effect were clearly observed for salbutamol+formoterol compared to control or the respective β2-agonists alone.

In conclusion: there is presumably no performance enhancing effect in this study design with the used doses of β2-agonists either alone (salbutamol or formoterol) or in combination (salbutamol + formoterol) compared to Placebo, whereas it was shown that in cell culture, β2-agonists may indeed have a strong hypertrophic effect and exert their effects in an additive manner that can be relevant for human in vivo pharmacologic kinetics.

An acute effect on the lung function was observable without side effects and with presumably no impact on exercise performance capacity in healthy subjects. Acute effects were observable for heart contractility but without objective impact on aerobic performance capacity or health.

The impact of chronic β2-application in healthy and asthmatic subjects on TT performance of longer duration, which simulates real life competition even closer, has still to be determined.