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**Section:** Original Investigation

**Article Title:** Improved Sprint Performance With Inhaled Long-Acting B_2-Agonists Combined With Resistance Exercise

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Improved sprint performance with inhaled long-acting β2-agonists combined with resistance exercise

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Abstract

Purpose: To investigate the impact of twice daily inhalation of 100 µg of salmeterol or 12 µg of formoterol in addition to a strength and power training programme over a 5-wk period on 30-m sprint, strength, power, mood, stress, and skinfold thickness.

Methods: In a randomized single-blind study, 23 male and 15 female nonasthmatic, recreationally active individuals were recruited (mean ± SD age 26.3 ± 5.4 y, weight 76.2 ± 11.5 kg, height 176.9 ± 8.5 cm). Participants completed 3 standardized whole-body strength and power training sessions per week for 5 wk. During the 5-wk training period they were assigned to a salmeterol (SAL), formoterol (FOR), or placebo (PLA) group. Participants used their inhaler twice per day as instructed and completed assessments of sprint, strength, and power at baseline and 1 wk after cessation of the training program. The assessments included 30-m sprint, vertical jump, 1-repetition-maximum (1RM) bench press, 1RM leg press, peak torque flexion and extension, anthropometric evaluation, and Rest-Q questionnaires.

Results: After 5 wk of strength and power training, 30-m sprint time reduced in FOR (0.29 ± 0.11 s, \(P = .049\)) and SAL (0.35 ± 0.05 s, \(P = .040\)) groups compared with PLA (+0.01 ± 0.11 s). No significant change was found in other assessments of strength, mood, or skinfold thickness.

Conclusions: When strength and power training is combined with the inhalation of FOR or SAL over a 5-wk period, moderately trained individuals experience an improvement in 30-m sprint performance.

Key words: performance, asthma, doping
Improved Sprint Performance With Inhaled Long-Acting $\beta_2$-Agonists Combined With Resistance Exercise

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**Introduction**

The 2019 World Anti-Doping Agency (WADA)\(^1\) permits athletes to use inhaled therapeutic doses of $\beta_2$-agonists salbutamol (1600 $\mu$g/d, no more than 800 $\mu$g in a 12-h period), formoterol (54 $\mu$g/d), and salmeterol (200 $\mu$g/d). However, there is some debate as to whether the current rules allow unscrupulous athletes, with and without asthma-related conditions, to use inhaled $\beta_2$-agonists for the purpose of benefitting from a potential ergogenic action.

Previous research investigating the acute and short term use (e.g. 2 weeks) of inhaled $\beta_2$-agonists suggests they do not have an ergogenic action on endurance performance.\(^2\) Furthermore, endurance performance is not improved from acute doses of inhaled formoterol\(^3\) and salmeterol.\(^4\) However, moderately and highly trained individuals may experience enhanced strength and power performance from the acute use of short acting\(^ 5\) and long acting $\beta_2$-Agonists.\(^6\)

The mechanisms behind the ergogenic action from acute doses that have been observed in skeletal muscle include: $\beta_2$-adrenergic stimulation that counteracts exercise-induced reductions in Na$^+\text{-K}^+$ ATPase maximum rate of activity, elevated glycolytic activity during high intensity exercise and enhanced rates of Ca$^{2+}$ release and uptake from the sarcoplasmic reticulum.\(^5\) Furthermore, increased anaerobic ATP utilisation has been suggested as a potential mechanism.\(^6\) However, others have failed to demonstrate changes in peak force velocity, and have shown maximal strength deteriorates following acute oral terbutaline administration.\(^7\)

Long-term use of $\beta_2$-Agonists also has the potential to produce an ergogenic action. Data from animal models suggests long-term $\beta_2$-adrenergic stimulation results in muscle hypertrophy.\(^8\) Studies investigating the long-term $\beta_2$-adrenergic stimulation in humans suggest increases in skeletal muscle mass\(^9\) and portioning of amino acids from oxidative loss toward protein synthesis \(^{10}\) may occur. Furthermore, salbutamol has been shown to counteract a negative net protein balance following resistance training in males.\(^{11}\) These changes to skeletal
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Muscle from long-term use of B<sub>2</sub>-Agonists has been shown to increase peak muscle strength<sup>12</sup> and power output,<sup>13</sup> whilst also inducing a slow-to-fast twitch muscle phenotype transition in humans.<sup>14</sup>

Long-term use of B<sub>2</sub>-Agonists may also decrease body fat due to increased fat mobilization from adipose tissue,<sup>13</sup> decreased fat synthesis in adipose tissue and liver<sup>15</sup>, or a combination of both.<sup>16</sup> Although there is clear potential for ergogenic action with oral or supra-therapeutic inhaled doses of B<sub>2</sub>-Agonists, we do not know whether long term stimulation of B<sub>2</sub>-adrenoreceptors via therapeutic doses of long acting inhaled B<sub>2</sub>-Agonists has a similar effect.

Endurance training has been shown to confound the ergogenic action of inhaled short acting B<sub>2</sub>-Agonists<sup>17</sup>. However the ergogenic action of inhaled short acting B<sub>2</sub>-Agonists is augmented with resistance training.<sup>9</sup> It is not known whether there is a similar interaction when long acting B<sub>2</sub>-Agonists are inhaled whilst engaging in strength training. This is a realistic consideration as athletes using long acting inhaled B<sub>2</sub>-Agonists (salmeterol or formoterol) are prescribed to do so on a daily basis, which may modify their response to strength and power training.

Accordingly, the purpose of this study was to investigate the impact of therapeutic doses of inhaled salmeterol or formoterol combined with a resistance exercise training programme on 30 m sprint, strength, power, mood, stress and skinfold thickness.

Methods

The study procedure was approved by the Faculty of Science Research Ethics Committee at the University of Kent and followed the ethical principles for medical research involving human subjects set by the World Medical Association Declaration of Helsinki.
To ensure none of our participants had asthma or exercise induced bronchospasm (EIB) they were required to declare they had no history of asthma diagnosis and objectively demonstrate this via a eucapnic voluntary hyperpnoea challenge (EVH).

Participants completed a series of assessments prior to and following a five week resistance exercise training programme, which included: 30 m sprint, peak concentric strength of the knee extensors and flexors, maximal one repetition of bench and leg press, vertical jump and skinfold thickness. Participants were randomly allocated to one of three treatments to be inhaled twice daily: placebo inhaler (PLA), 100 µg inhaled salmeterol (SAL) or 12 µg inhaled formoterol (FOR). Over the course of five weeks participants administered their inhaler as instructed and completed supervised strength and power training sessions three times per week. The training programme included lower body and upper body exercises that progressed appropriately over the five weeks. Sprint training focused on quickness and coordination. To assess the status of recovery from day to day training the participants completed the Recovery-Stress questionnaire (figure 1).

Participants: We initially recruited twenty-four male participants however one male participant withdrew from the study due to an injury not related to the study in week four. We therefore had twenty-three healthy recreationally active males (mean ± SD: age 27.9 ± 5.5 years; height 179.8 ± 7.3 cm; weight, 78.8 ± 10.3 kg) and fifteen recreational active females (age 24.1 ± 4.1 years; weight 65.4 ± 9.5 kg; height 168 ± 4.3 cm) who volunteered for the study, provided informed consent and completed the study. All participants had been involved in strength and power activities over the past year during their weekly training habits. The heterogeneous nature of the male participants taking part in the study was characterised by their involvement in a variety of sports at an amateur competitive level including: football (n = 9); basketball (n = 4); track and field (n = 2); martial arts (n = 3); swimming (n = 1); running (n = 2); and cycling (n = 3). Female participants were characterised by their involvement in a variety
of sports at an amateur competitive level including: basketball (n = 8); football (n = 4); boxing (n = 2); running (n = 1). Prior to participation in the study none of the participants competed in strength and power lifting sports. Participants engaged in strength and power training sessions at least three times per week completing a diary to record their training engagement and progress.

**Broncho-provocation Challenge:** All participants were free from asthma and EIB, which was confirmed by the presentation of a negative EVH challenge. The EVH challenge consisted of six minutes breathing cold dry air from a compressed gas cylinder at a target minute ventilation of 85% of estimated maximal voluntary ventilation (30 x baseline FEV$_1$). Maximal flow-volume loops were measured at baseline and 3, 5, 7, 10 and 15 minutes post EVH. An EVH challenge was deemed positive if the individuals FEV$_1$ fell >10% from baseline FEV$_1$ at two consecutive time points following the EVH challenge.

**Treatment groups:** Participants were randomly assigned to one of three groups using a minimisation method. As part of this randomisation we factored in gender balance between groups so that they were balanced eight males to five females in each group. In a single blinded randomised design each group was allocated to use either:

- Placebo inhaler (containing water vapor) twice daily (PLA)
- Inhaled 100 µg salmeterol twice daily (Serevant, Accuhaler 50 µg/dose, GSK, UK)
- Inhaled 12 µg formoterol twice daily (Oxis Turbohaler 6 µg/dose, Astra Zeneka, UK)

These doses were chosen as they are high therapeutic doses permitted for use by athletes. Participants were instructed about their inhaler technique. At each training session researchers checked the participants were using their inhalers as instructed by reading the inhalation counter on their device to confirm they were adhering to the protocol.
Assessments

Participants completed each of the following assessments at baseline and one week after the final inhaler dose and training session (figure 1). Prior to the start of the study participants attended two familiarisation sessions for all assessments.

30 meters sprint: Participants were asked to complete a maximal 30 m sprint on a non-motorised calibrated treadmill (Force Treadmill System, Woodway, SA). The peak speed data collected from the non-motorised treadmill has been described in literature to be approximately 80% of that achieved in free-sprint track performance. Each participant completed three 30 m sprints separated by 5 minutes. The fastest 30 m sprint was recorded.

Isokinetic dynamometry: Participants performed three maximal voluntary contractions of the knee extensors at $120^\circ \cdot s^{-1}$ and three maximal voluntary contractions of the knee flexors at $120^\circ \cdot s^{-1}$ (Biodex 830-210, Biodex Medical System, Shirley, New York, USA). The highest peak torque measurement was taken as a measurement of maximal strength in the knee extensors and knee flexors.

Maximal One Repetition Bench Press and incline Leg Press: Participants progressively worked toward a maximum one repetition for both incline leg press and bench press. The incline leg press (CF800 Leg Press/Hack Squat Machine, Bodymax, UK) was performed at $45^\circ$ by first completing a six repetitions maximum. This was followed by a four repetition maximum and a two repetition maximum at increasing weights. The bench press was performed using a 20 Kg Olympic bar with weights added to it accordingly. The participants continued to complete one-repetition efforts at increasing weight until they reached failure. Each effort was separated by four minutes. The weight lifted during the last complete repetition was taken as their maximal one repetition.

Power – Counter movement vertical jump (CMJ): A counter movement jump was performed on a jump mat (Probiotics Inc., Huntsville, AL, USA). The participants were
instructed to jump as high as they could by performing a CMJ with an arm swing. Coaching of technique was only provided if participants consistently landed off the jump mat demonstrating poor technique. Participants performed three counter movement jumps and the greatest vertical jump height achieved was recorded.

**Body Composition:** Skin-fold thickness was taken at the following recognised sites on the right hand side of the body: triceps, biceps, subscapular and supraspinale. All measurements were taken by the same technician using a single set of Harpenden skinfold callipers (Baty International, Sussex, UK). Skin-fold thickness measurements were taken from each site consecutively a total of two times with the mean of the two measurements taken as the skin fold thickness for each specific site. The criterion for a valid measurement was a difference of less than 1 mm between the two totals. If this was not the case the measurements were repeated until the criterion was met. The sum of four mean skin-folds thickness measurement was calculated.

**Recovery, Sleep and Mood Questionnaires:** Participants completed questionnaires in week three and five to measure recovery and stress from training and mood. Recovery and stress from training were assessed via the Recovery-Stress Questionnaire for athletes. Mood was assessed using the Brunel Mood Scale.

**Strength and Power Training Programme:** Following the completion of the above baseline assessments participants began a strength and power training programme. The training programme focused on developing strength, power and sprinting. Participants training was individualised and supervised by a strength and conditioning specialist.

Participants training incorporated lower body exercise such as lunges, squat, leg press and leg curl; upper body exercises included chest and shoulder press, shoulder dumbbell raise and arm exercises using both barbell and dumbbells. Each training session consisted of twelve exercises. Each exercise was completed with a target of completing three sets of eight
repetitions, with each set separated by two minutes. When participants were able to complete all three sets they increased the load. Sprint training included exercises involving quickness and coordination with a set of 5 to 10 m sprint accelerations performed five times at the end of the training session. Participants recorded total work done during each session. Participants were asked not to engage in strength, power or sprint training outside of the programme. Aerobic training outside of the study was restricted to two sessions per week. It was not feasible to accurately record or control for the intensity and duration of any additional endurance based training.

**Statistical Analysis:** Changes in sprint performance, strength, power, mood, recovery, sleep and skinfold thickness from baseline to week five between PLA, SAL and FOR were analysed using a mixed model repeated measures ANOVA (3 group x 2 time). Assumptions for this analysis was checked and corrected for according to the methods described by Atkinson and Nevill. A P≤0.05 was deemed significant. Effect size was calculated according to Cohen’s statistical power analysis used to indicate the standardised difference between two means measuring small, medium and large effect sizes (d= 0.20, 0.50, 0.80.).

**Results**

There were no differences at baseline between groups for any of the sprint (p=0.670), strength and power (p=0.226), anthropometric (p=0.438) and skinfolds (p=0.762). Psychological variables were different at baseline (p=0.001) but not at week 3 and week 5 between groups (p=0.234) (table 1, 2 and 3).

**30 m Sprint**

Between baseline and week five 30 m sprint time improved in both the FOR (−0.29 ± 0.11 s; p=0.049; ES= 0.50) and SAL (−0.35 ± 0.05 s; p=0.040; ES= 0.41) groups when compared to the placebo group (+0.01 ± 0.11 s; see table 1 and figure 2).
**Strength and Power Assessments**

Over the five weeks all groups improved markers of strength and power (see table 1). There was no difference in the rate of improvement between groups.

**Anthropometric measures**

Over the five weeks of training the sum of skinfold thickness across four sites did not change significantly (p=0.762; table 2). No significant changes in body mass between groups were observed over the five weeks of training (p=0.915; table 2).

**Recovery, Sleep and Mood Questionnaires**

Recovery-Stress Questionnaire index did not change (p=0.395) across the five week training period in PLA, SAL or FOR groups (table 3).

**Discussion**

Our study suggests that 30 m sprint performance is improved when daily doses of inhaled formoterol or salmeterol are combined with strength, power and sprint training over a five week period. However we did not observe significant changes in strength, power, mood, recovery or skinfold thickness between formoterol, salmeterol and placebo over the five-week period.

The improved 30 m sprint performance following five weeks of inhaled formoterol or salmeterol administration in our study was similar to previous reports examining acute and long term use of $\beta_2$-Agonists. Likewise, administration of oral $\beta_2$-Agonists enhances muscle strength and peak power output during maximal cycling. Improvements in sprint and power performance from short-term use of $\beta_2$-Agonists has been suggested to be as a result of increased skeletal muscle mass and maximal muscle force production, leading to greater initial peak power. Furthermore, high doses of formoterol can augment resting energy expenditure and fat utilization in active males. However, in some cases where authors report changes in
peak power this does not correspond to significant improvements in the mean power produced during a Wingate test.\textsuperscript{12} Although Wingate performance is related to sprint performance, the duration of our 30 m sprint was approximately seven seconds compared to the 30 s Wingate challenge. It may be long-term use of formoterol and salmeterol have a greater potential for ergogenic action for explosive sprints lasting under 10 s, compared to longer sprinting activities. Further research is required to confirm this hypothesis.

In our study although we observed an improvement in sprint performance we did not see changes in strength, power or skinfold thickness. This may due to the smaller therapeutic doses of salmeterol and formoterol use in our study, compared to other studies using supra-therapeutic doses.\textsuperscript{30} Although, our method of using skin fold thickness to measure changes in body composition may not have been sensitive enough to detect changes in muscle mass. By using other means of measuring changes in body composition (e.g. DEXA) we may have detected changes in muscle mass. In a recent study by Jessen et al.,\textsuperscript{9} they observed a significant increase in lean body mass of 1.03 – 1.04 kg as detected by DEXA following daily inhalation of 4 mg terbutaline over a four week period whilst participants engaged in strength training and also those in an habitual life-style group, but this was not the case for those engaging in endurance training.

Gender differences in pulmonary anatomy may influence the potential ergogenic action of formoterol and salmeterol. Although previous studies that have suggested this may be the case for salbutamol, the hypothesis has not been rigorously investigated.\textsuperscript{31} Due to the relatively small number of females in our study, our data was underpowered to conduct meaningful sub analyses.

Future studies should investigate the relationship between long acting $\beta_2$-Agonists administration in male and female athletes and the bio-availability required to stimulate an increase in muscle protein turnover and synthesis between sexes. Previous studies suggest
Formoterol induces opposing effects between oxidation and synthesis but ultimately results in net anabolic gain because of a greater anti-catabolic effect (oxidation) over reduced synthesis. In females, these anti-catabolic and synthesis effects were three-fold larger when compared to men.

A limitation of this study is that we cannot assume the observations we have seen in our participants who take part in recreational sport translate to elite athletes. However, if elite athletes did take part in our study they may have been subject to a doping test, in which they may have provided a urine sample with a concentration of formoterol that is above the permitted level and therefore committed an anti-doping violation. For this reason we excluded elite athletes from participating. Future studies may incorporate highly trained individuals to investigate whether they experience a similar response to sprint performance following five weeks of inhaling either formoterol or salmeterol.

Previous studies have demonstrated that the potential ergogenic action of long-term use of β2-Agonists can be confounded by endurance training. In our study we specifically focused on strength and power training which has previously been shown not to confound increases in lean mass. We have not investigated whether endurance training would confound the improvements in 30 m sprint performance, which has been previously reported when short acting β2-Agonists have been used over a four to six week period incorporating endurance training.

Athletes use formoterol and salmeterol to protect against bronchoconstriction. Both drugs have side effects including: increased heart rate, headaches, tremors, muscle cramps and palpitations. It is not known whether athletes using either inhaled formoterol or salmeterol increase the risk of these side effects. However, in our study we did not observe athletes reporting these symptoms throughout the study. Furthermore, we did not see any significant differences between the recovery, sleep and mood between groups. We investigated these
parameters to see if salmeterol or formoterol may have influenced perception of recovery between training days. As we have not detected any differences between groups we can exclude this as a potential mechanism to explain improvements in 30 m sprint performance.

**Conclusion**

This study was the first to demonstrate five weeks of therapeutic doses of either inhaled salmeterol or formoterol in combination with strength, power and sprint training may improve 30 m sprint performance. At this stage we are not able to conclude that similar effects will occur in highly trained athletes using similar doses. Therefore anti-doping stake-holders may wish to commission investigations into whether highly trained athletes experience a similar ergogenic action from inhaled formoterol or salmeterol. These studies should be conducted before changes to the WADA Prohibited List are recommended. However, our findings suggest that consideration should be given to closer monitoring of inhaled long acting β2-Agonists use by athletes in and out of competition. Future research is required to investigate the mechanism behind the potential improvement in sprint performance in both males and females.

**Practical Application**

Our results demonstrate long-term use of long acting beta-2-agonists may lead to improvements in sprint performance. Before changes are made to the WADA anti-doping code, similar research project on highly trained athletes should be conducted.

**Acknowledgements**

This study was funded by a grant from the World Anti-Doping Agency.

**Conflict of Interest**

None to report
“Improved Sprint Performance With Inhaled Long-Acting B2-Agonists Combined With Resistance Exercise”
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References


Figure 1: Schematic representation of the study across five week training intervention
Figure 2: 30 m sprint performance at pre and post five weeks training for (a) participants in PLA, (b) participants in SAL, (c) participants in FOR and (d) mean 30 m sprint performance in PLA, SAL and FOR groups.
Table 1: 30 meters Sprint, Power and Strength Performance in SAL, FOR and PLA groups at week 0 and week 5 (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>SAL</th>
<th>FOR</th>
<th>PLA</th>
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<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 5</td>
<td>Mean Change</td>
</tr>
<tr>
<td>1RM Inc. Leg Press (Kg)</td>
<td>102 ±</td>
<td>135 ±</td>
<td>33 ± 41</td>
</tr>
<tr>
<td></td>
<td>59 ±</td>
<td>92 ±</td>
<td></td>
</tr>
<tr>
<td>1RM Hack Squat (Kg)</td>
<td>135 ±</td>
<td>201 ±</td>
<td>66 ± 40</td>
</tr>
<tr>
<td></td>
<td>57 ±</td>
<td>57 ±</td>
<td></td>
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<tr>
<td>1RM Bench Press (Kg)</td>
<td>55 ±</td>
<td>64 ±</td>
<td>9 ± 26</td>
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<tr>
<td></td>
<td>20 ±</td>
<td>27 ±</td>
<td></td>
</tr>
<tr>
<td>Leg Extension (N•m)</td>
<td>226 ±</td>
<td>228 ±</td>
<td>1 ± 41</td>
</tr>
<tr>
<td></td>
<td>48 ±</td>
<td>22 ±</td>
<td></td>
</tr>
<tr>
<td>Leg Flexion (N•m)</td>
<td>123 ±</td>
<td>137 ±</td>
<td>14 ± 25</td>
</tr>
<tr>
<td></td>
<td>23 ±</td>
<td>10 ±</td>
<td></td>
</tr>
<tr>
<td>Vertical Jump (cm)</td>
<td>49.6 ±</td>
<td>53.5 ±</td>
<td>3.9 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>10.7 ±</td>
<td>9.4 ±</td>
<td></td>
</tr>
<tr>
<td>30 m Sprint (s)</td>
<td>7.38 ±</td>
<td>7.03 ±</td>
<td>-0.35 ±</td>
</tr>
<tr>
<td></td>
<td>0.70 ±</td>
<td>0.72*</td>
<td>0.05 ±</td>
</tr>
</tbody>
</table>

Abbreviations: 1RM = one maximal repetition; SAL = salmeterol; FOR = formeterol; PLA = placebo

* = Significantly different from PLA (P < 0.05).

Incline Leg press 45°, Hack Squat and Bench Press measured is reported at 1RM; Leg Extension, Leg Flexion is reported as

Peak Torque measured at 120°/s.
Table 2: Skinfolds and Body Mass from week 0 to week 5 in SAL, FOR and PLA groups. Mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>SAL</th>
<th>FOR</th>
<th>PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 5</td>
<td>Week 0</td>
</tr>
<tr>
<td>Skinfolds</td>
<td>46 ± 18</td>
<td>41 ± 12</td>
<td>47 ± 16</td>
</tr>
<tr>
<td>Σ4 (mm)</td>
<td>18</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Body Mass</td>
<td>80.8 ± 12</td>
<td>81.6 ± 10</td>
<td>76.6 ± 7</td>
</tr>
<tr>
<td>(Kg)</td>
<td>12</td>
<td>10.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Abbreviations: Σ4 = Sum of the four skinfold sites (triceps, biceps, subscapular and supraspinale); RM = repetition maximum; SAL = salmeterol; FOR = formoterol; PLA = placebo
Table 3: Rest Q Recovery and Stress Index values in weeks 3 and 5 in SAL, FOR and PLA groups (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>SAL</th>
<th>FOR</th>
<th>PLA</th>
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<tbody>
<tr>
<td></td>
<td>Week 3</td>
<td>Week 5</td>
<td>Mean Change</td>
</tr>
<tr>
<td>Rest Q Recovery Index (A.U.)</td>
<td>2.5 ± 0.1</td>
<td>2.7 ± 0.4</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Rest Q Stress Index (A.U.)</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>- 0.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Abbreviations: A.U. = arbitral units; SAL = salmeterol; FOR = formoterol; PLA = placebo