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The effect of beta2-agonists on aerobic performance – A systematic review and meta-analysis of randomized controlled trials

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What is already known?

- Asthma is the most common chronic disease in athletes
- The gold standard for asthma therapy is inhaled glucocorticoids with inhaled beta2-agonists pre-exercise and as a reliever for symptoms
- The use of beta2-agonists in sports is regulated by WADA due to a possible performance enhancing effects.

What are the new findings?

- Beta2-agonists do not affect physical aerobic performance or maximal oxygen consumption (VO_{2max}) in healthy subjects.
- Route of administration, type of beta2-agonist, duration of treatment or dose were not related to the effect of beta2-agonists in healthy subjects.
- Study bias or performance level of the subjects had no impact on the effect of beta2-agonists on aerobic performance in healthy subjects.

ABSTRACT

Objectives. We aimed to examine the effect of beta2-agonists on aerobic performance in healthy non-asthmatic subjects.

Design. Systematic review and meta-analysis.

Eligibility criteria. We searched four databases (PubMed, Embase, SPORTDiscus and Web of Science) for randomized controlled trials, published until December 2019. Studies examining the effect of beta2-agonists on maximal physical performance lasting longer than one minute were included in the meta-analysis. Data are presented as standardized difference in mean (SDM) with 95% confidence intervals.

Results. The present meta-analysis includes 47 studies. The studies comprise 607 participants in crossover trials including 99 participants in three-way crossover trials and 27 participants in a four-way crossover trial. Seventy-three participants were included in parallel trials. Beta2-agonists did not affect aerobic performance compared to placebo (SDM 0.051 (95%CI -0.020-0.122). The SDM for the included studies was not heterogeneous (I^2= 0%, p=0.893) and the effect was not related to type of beta2-agonist, dose, administration route, duration of treatment or performance level of the participants. Beta2-agonists had no effect on time trial performance, time to exhaustion or maximal oxygen consumption (VO_{2max}) (p<0.218).

Conclusion/implication. The present study shows that beta2-agonists do not affect aerobic performance in non-asthmatic subjects regardless of type, dose, administration route, duration of treatment or the performance level of the participants. The results of the present study should be of interest to the World Anti-Doping Agency and anyone who is interested in equal opportunities in competitive sports.

Systematic review registration. PROSPERO CRD42018109223

INTRODUCTION

Asthma is the most common chronic disease in elite athletes (1) and endurance athletes regularly performing heavily increased ventilation are at increased risk of developing asthma (2). Asthma is usually associated with airway hyper responsiveness (AHR) and the recommended therapy for asthma is inhaled glucocorticoids with inhaled beta2-agonists pre-exercise and as a reliever of symptoms (3). However, ever since inhaled beta2-agonists became available just before the Olympic Games in 1972, the anti-doping authorities have regulated the use of beta2-agonists in athletes, due to a possible performance enhancing effect (1). The World Anti-Doping Agency (WADA) annually updates the prohibited list, a list of substances and methods prohibited in elite sports. The prohibited list, effective from January the 1st 2020, prohibits all use of beta2-agonists except inhaled salbutamol (maximum 1600 micrograms over 24 hours in divided doses not to exceed 800 micrograms over 12 hours starting from any dose), inhaled formoterol (maximum delivered dose of 54 micrograms over 24 hours) and inhaled salmeterol (maximum 200 micrograms over 24 hours) (4).

Asthmatic athletes have consistently outperformed non-asthmatic athletes during the Olympic Games (2) and it has been suspected that non-asthmatic athletes use beta2-agonists with intention to improve their performance (5). Thus, the possible performance enhancing effect of beta2-agonists has been examined in multiple studies. In 2007, Kindermann reviewed the effect of beta2-agonists and concluded that inhaled beta2-agonists do not enhance endurance performance, while oral beta2-agonists enhance endurance performance (5). The year after the International Olympic Committee (IOC) consensus statement claimed that inhaled beta2-agonists do not enhance endurance performance (6) and a joint Task Force of European Respiratory Society (ERS) and European Academy of Allergy and Clinical Immunology (EAACI) concluded that there were no evidence to suggest that asthma drugs can improve physical performance in healthy athletes (7). In 2011, Pluim et al. published the first systematic review and meta-analysis on the effect of beta2-agonists on physical performance in healthy athletes (8). They did not detect any effect of inhaled

beta2-agonists on endurance, strength or sprint performance, but some weak evidence indicating a performance enhancing effect of systemic beta2-agonists on anaerobic performance. Since august 2009, multiple studies have investigated the effect of beta2-agonists on aerobic performance, and continuous controversy regarding the use of beta2-agonists in sports exists, which has been highlighted in recent beta2-agonist anti-doping investigations involving world-class athletes (9, 10). In a recent meta-analysis (11), we assessed the effect of beta2-agonists on anaerobic performance in non-asthmatic subjects. Therefore, the aim of this systematic review and meta-analysis was to assess the effect of beta2-agonists on aerobic performance in healthy non-asthmatic subjects.

METHODS

Search strategy and selection criteria

The study protocol for this systematic review and meta-analysis was registered at Prospero on September 18th, 2018, with registration number CRD42018109223 and complied with the PRISMA 2009 Guidelines (12).

Literature search

We systematically searched for published randomised controlled trials (RCT) that examined the effect of beta2-agonists on physical performance in healthy humans on October 29th 2018. Peer-reviewed articles published in English were identified from four electronic databases: PubMed (All fields), Embase (All fields), SPORTDiscus (Text) and Web of Science (Topic). The search strategy consisted of four blocks of terms: (healthy OR non-asthmatic OR athletes) AND (salbutamol OR formoterol OR salmeterol OR terbutaline OR albuterol) AND (exhaustion OR power OR endurance OR strength OR aerobe OR anaerobe OR exercise OR performance) AND (rct OR randomized controlled trial* OR randomized control trial* OR controlled trial*). The search identified 398 records (PubMed 100, Embase 105, Web of Sience, 36 and SPORTDiscus 157). After elimination of duplicates, 290 records remained. The 18th of December 2019 we performed an updated search in the four databases adding the term beta2-agonist and all beta2-agonists listed in the WADA prohibited list (4) but not included in the original search (beta2-agonist OR Fenoterol OR Higenamine OR Indacaterol OR Olodaterol OR Procaterol OR Reproterol OR Tretoquinol OR Tulobuterol OR Vilanterol). The updated search identified 57 records (PubMed 11, Embase 22, Web of Sience, 5 and SPORTDiscus 19). After elimination of duplicates, 44 records remained (Figure 1). The searches were performed by the first author and a librarian.

Inclusion criteria and selection process

Two authors, (AR and LBA) independently assessed the studies for eligibility with subsequent consensus by discussion. We included RCTs involving healthy, non-asthmatic subjects examining the effect of beta2-agonists on maximal physical performance.

Studies investigating the effect of salbutamol/albuterol, salmeterol, formoterol, and terbutaline alone or in various combinations, administered by inhalation, orally or by infusion were included. There were no restrictions related to dose or duration of treatment.

We excluded studies examining physical performance with a duration of 60 seconds or less and non-performance variables like neuromuscular function, oxygen kinetics, and ventilation.

Included studies

From the first search 45 studies were selected for full text eligibility assessment after screening of titles and abstracts, while 22 other studies were included based on previous knowledge of the studies or screening of the reference lists of the studies included. From the updated search four studies were selected for full text eligibility assessment after screening of titles and abstracts. In total 71 studies met the primary inclusion criteria. As the present study includes only performance outcomes lasting longer than one minute, 21 studies only presenting data from performance outcomes with a duration of one minute or less were excluded. Two studies presented results that were published in two other studies and were therefore excluded, and one study reported no performance outcome. Thus, 47 studies were included in the present meta-analysis (figure 1).

----Insert **Figure 1.** Flow chart of included studies as proposed by PRISMA statement 2009(12) here ---

Study quality assessment

The included studies were assessed using the Cochrane Collaboration Risk of Bias Tool to evaluate seven bias domains (13). The domains were scored as low risk of bias, high risk of bias or unknown

risk of bias according to the tool criteria. For the domain "blinding of participants and personnel" the studies were scored as high risk of bias if the subject experienced side effects of the beta2-agonists even if the blinding procedure in the study was performed according to the criteria for low risk of bias. The domains "incomplete data" and "selective reporting" were set to "low risk of bias" due to the nature of the studies. The 7th domain (other bias) was defined as "Participants screened for AHR". For a study to be classified as "Low risk for bias" on the 7th domain, an objective measure of AHR was required. Studies which screened participants using lung function measurement at rest, stethoscopy or a questionnaire on medical history or bronchial complaints were considered as a "high risk of bias". An 8th domain was added to the risk of bias tool reporting the days between the treatments in crossover studies. Carryover effect and period effect were also extracted from the study as a part of the bias assessment. Two authors (AR and JS) independently assessed the included studies that were not assessed in the meta-analysis by Pluim et al. (8). Any discrepancies in the assessments were resolved by discussion.

Analysis

AR and TS conducted data extraction of study results separately and settled discrepancy by mutual agreement. The main outcome was aerobic performance defined as maximal physical performance lasting more than one minute. Other outcomes were maximal/peak oxygen consumption ($VO_{2max/peak}$) measured by analysis of expiratory gas, and physical performance, measured as time to exhaustion, distance covered in a pre-set time, time to cover a distance/amount of work or contractions to task failure. If a study reported both $VO_{2max/peak}$ and physical performance, physical performance was included in the main analysis. If only $VO_{2max/peak}$ was reported, $VO_{2max/peak}$ was included as outcome in the main analysis. Performance tests were categorised into time to exhaustion/open-end tests when the subjects performed running, walking, cycling or quadriceps contractions for as long as they could and time trials/closed end tests where the subjects performed a given amount of work as fast as

possible. The interventions were categorised in four different ways; 1) Type of beta2-agonist: short acting (salbutamol and terbutaline) and long acting (formoterol, and salmeterol). 2) Administration route: Inhaled, oral and infusion (infusion was only used in one comparison and was not included as a category in the meta-regression). 3) Duration of treatment: Acute treatment and multiple weeks of treatment 4) Dose: approved and prohibited by WADA (4). The four interventions were treated as categorical variables in the meta-regression analysis. The included studies were classified as high risk of bias if they scored "high risk of bias" in one domain or more, and low risk of bias if all domains in the risk of bias assessment tool were scored as "low risk of bias" or "unclear risk of bias". If the subject in a study performed endurance training for more than 10 hours per week or had VO_{2max} > 65 $ml \cdot kg^{-1} \cdot min^{-1}$ (females > 60 $ml \cdot kg^{-1} \cdot min^{-1}$), the subjects were classified as high performance endurance athletes. Correlations between performance with active treatment and placebo were seldom reported in the included studies, thus a correlation coefficient of 0.5 was imputed for all comparisons.

Statistics

Extracted data from individual studies were collated and prepared for meta-analysis (computing standard deviations (SD) when standard error of the mean and 95% confidence intervals were reported) in Excel (Microsoft Corp) prior to transfer into Comprehensive Meta-Analysis version 3, (CMA V3) (Biostat, Inc., Englewood, NJ, USA). Further analyses were performed in CMA. The meta-analyses were performed with random effects models and effect estimates are presented as standardized difference in means (SDM) with 95 % confidence intervals (CI). Heterogeneity is presented as I^2, and p-values. Whether or not the effect size was related to type of beta2-agonist, administration route, duration of treatment, dose, publication bias or level of fitness was analyzed by meta-regression (test of model). In addition, meta-regression was used to perform the Goodness of fit to assess the presence of unexplained variance in the model. The proportion of total between study variance explained by the covariate is expressed as R^2 analog. Potential publication bias was

assessed by funnel plot Begg and Mazumdar rank correlation test. Adequacy of sample size in each included study was assessed by calculation of the sample size required for the effect found in the respective study to obtain an alpha of 0.05 and a beta of 0.2 (14). Skewness of outcomes was assessed as baseline mean/SD. Variables with a mean/SD ratio > 2 was considered skewed (15). Significance level was set to $p<0.05$

RESULTS

Study characteristics

The present meta-analysis consists of 47 RCTs including 43 studies with a crossover design (16-58) of which eight studies comparing two (33, 35, 39, 44, 46, 53, 55, 58) and one study comparing three different interventions (40) with placebo, and four studies with parallel design (59-62) (one with both acute and multiple week intervention (62)). The meta-analysis of aerobic performance include 60 different randomized and placebo controlled comparisons with beta2-agonists comprising 607 participants in crossover trials including 99 participants in three-way crossover trials comparing two different treatments with placebo and 27 participants in a four-way crossover comparing three different beta2-agonist treatments with placebo. Seventy-three participants were included in parallel trials. The included studies are presented in table 1.

Table 1: Characteristics of the studies included in the systematic review and meta-analysis.

Study, year	Design	Subjects: n, sex, age, years ± SD or range	Fitness	Intervention	Outcomes
Molphy et al. 2019 (58)	Three-way crossover	16, m/f 8/8, 23 ± 3	Recreationally active >2 times/week	Inhaled terbutaline 2000 µg Inhaled terbutaline 4000 µg	3000 m treadmill time trial
Merlini et al., 2019 (57)	Crossover	13, m, 18 ± 1	Amateur football	Inhaled salbutamol 1600 µg	Shuttle run test
Hafedh et al., 2019 (56)	Crossover	12, 6/6, 22 ± 1	Recreationally active	Oral terbutaline 8000 µg	Shuttle run test
Laurent et al., 2018 (55)	Three-way crossover	14, m, 25 ± 5	Endurance athletes 6 ± 2 h/w	Inhaled salbutamol 800 µg Oral salbutamol 4000 µg	Quadriceps contractions to task failure
Eckerstrom et al., 2018 (54)	Crossover	36, m/f 18/18, 26 ± 5	Non-athletes	Inhaled salbutamol 900 µg	$VO_{2\text{-max}}$
Molphy et al., 2017 (53)	Three-way crossover	7, m, 22 ± 1	Recreational exercise ≥ 3 h/w	Inhaled salbutamol 400 µg	3000 m treadmill time trial
Halabchi et al., 2017 (52)	Crossover	20, m, 17 ± 1	Junior professional football players	Inhaled 200 µg salbutamol	20 m multistage shuttle run test
Hostrup et al., 2016 (62)	Parallel	20, m, 26 ± 4	National level endurance athletes *	Oral salbutamol 8000 µg acute and 8000 µg/day for two weeks	$VO_{2\text{-max}}$ Time to exhaustion
Koch et al., 2015 (51)	Crossover	35, m, 28 ± 5	Experienced cyclists/ triathletes, $VO_{2\text{-max}}$ ≥ 60 $ml \cdot kg^{-1} \cdot min^{-1}$ or 5L	Inhaled salbutamol 400 µg	10km ergometer time trial
Koch et al., 2015 (50)	Crossover	15, f, 30 ± 5	Cyclists and triathletes $VO_{2\text{-max}}$ 50 $ml \cdot kg^{-1} \cdot min^{-1}$ or 4L	Inhaled salbutamol 400 µg	10km ergometer time trial
Koch et al., 2015(49)	Crossover	12, m, 31 ± 7	Competitive cyclists $VO_{2\text{-max}}$ ≥ 60 $ml \cdot kg^{-1} \cdot min^{-1}$or 5L	Inhaled salbutamol 1600 µg	10km ergometer time trial

Hostrup et al., 2015 (61)	Parallel	18, m, 24 ± 3	Recreationally active 4-8 h/w	Oral terbutaline 5000 µg/30 kg body weight, twice daily for 28 ± 1 days	$VO_{2\text{-max}}$, Cycling to exhaustion, incremental
Kalsen et al., 2014 (48)	Crossover	9, m, 24 ± 3	Moderately trained	Inhaled terbutaline 15000 µg	300 kcal cycling time trial
Hostrup et al., 2014 (47)	Crossover	9, m, 24 ± 3	Recreational active	Inhaled terbutaline 15000 µg	100 kcal cycling time trial
Dickinson et al., 2014a (60)	Parallel	16, m, 20 ± 2	Amateur-level competition	Inhaled salbutamol 1600 µg/day for 6 weeks	$\dot{V}O_{2\text{-peak}}$ 3 km treadmill time trial
Dickinson et al., 2014b (46)	Three-way crossover	7, m, 22 ± 4	Runners, > 2 t/w	Inhaled salbutamol 800 and 1600 µg	5 km treadmill time trial in 18C and 30C
Sanchez et al., 2013 (45)	Crossover	7, m, 29 ± 6	Competitive recreational athletes, 10 h/w	Oral terbutaline 8000 µg	Cycling to exhaustion $VO_{2\text{-max}}$
Decorte et al., 2013 (44)	Three-way crossover	11, m 33 ± 6	Highly trained cyclists/triathletes/ runners, 12 ± 3 h/w *	Inhaled salbutamol 200 µg and 800 µg	Quadriceps contractions to task failure
Elers et al., 2012 (43)	Crossover	9, m, 27 ± 5	Endurance-trained 11 h/w *	Inhaled 8000 µg salbutamol	Peak power output, incremental $VO_{2\text{-max}}$
Beloka et al., 2011 (42)	Crossover	21, m, 23 ± 2	Healthy non-athletes	Infused salbutamol 350 µg or 700 µg	$VO_{2\text{-max}}$
Andersen et al., 2009 (41)	Crossover	7, m, 25, 18-30	Highly endurance-trained *	Oral salbutamol 4000 µg	Running to exhaustion $VO_{2\text{-max}}$
Sporer et al., 2008 (40)	Four-way crossover	27, m, 29 ± 6	Competitive cyclists and triathletes *	Inhaled salbutamol 200 µg, 400 µg and 800 µg	20 km cycling time trial
Descorte et al., 2008 (39)	Three-way crossover	10, m, 23 ± 3	Healthy non-athletes	Inhaled salbutamol 200 µg 800 µg	Cycling to exhaustion, incremental $VO_{2\text{-max}}$
Tjorhom et al., 2007 (38)	Crossover	23, m, 29 ± 5	Endurance athletes, $VO_{2\text{-max}}$ 60.6 $ml \cdot kg^{-1} \cdot min^{-1}$	Inhaled formoterol 18 µg	Running to exhaustion at -20C at 107% $VO_{2\text{-max}}$, $VO_{2\text{-max}}$
Riiser et al., 2006 (37)	Crossover	20, m, 29 ± 4	Endurance athletes, $VO_{2\text{-max}}$ 61.1 $ml \cdot kg^{-1} \cdot min^{-1}$	Inhaled formoterol 18 µg	Running to exhaustion in hypobaric conditions at 107% $VO_{2\text{-max}}$, $VO_{2\text{-max}}$
Van Baak et al., 2004 (36)	Crossover	16, m, 23 ± 3	Cyclists and triathletes, training 11 ± 3 h/w *	Inhaled 800 µg salbutamol	Cycling time trial
Stewart et al., 2002 (35)	Three-way crossover	10, m, 26 20-30	Highly trained athletes *	Inhaled formoterol 12 µg or Inhaled salbutamol 400 µg	$VO_{2\text{-max}}$
Collomp et al., 2002 (34)	Crossover	8, m, 26 ± 2	Moderately trained	Oral salbutamol 6000 µg	10 min cycling time trial
Collomp et al., 2000a (29)	Crossover	9, m, 25 ± 1	Moderately trained	Oral salbutamol 6000 µg	Cycling to exhaustion at 85% of $VO_{2\text{-max}}$
Collomp et al., 2000b (30)	Crossover	8, m, 23 ± 3	Recreational athletes, cycling/running 3-5 t/w	Oral salbutamol 12000 µg/day for 3 weeks	Cycling to exhaustion at 85% of $VO_{2\text{-max}}$
Goubault et al., 2001 (33)	Three-way crossover	13, m 23 ± 2	Competitive triathlete	Inhaled salbutamol 200 µg and 800 µg	Cycling to exhaustion at 85% of $VO_{2\text{-max}}$
Carlsen et al., 2001 (32)	Crossover	24, m, 25 ± 3	Competitive athletes *	Inhaled formoterol 9 µg	Running to exhaustion at 105% of $VO_{2\text{-max}}$ $VO_{2\text{-max}}$

Van Bak et al., 2000 (31)	Crossover	16, m, 23 ± 2	Healthy non-athletes	Oral salbutamol 4000 µg	Cycling to exhaustion at 70% of $VO_{2\text{-max}}$
Sue-Chu et al., 1999 (28)	Crossover	8, m, 19-28	Highly trained cross-country skiers *	Inhaled salmeterol 50 µg	Running to exhaustion at -15 C, incremental $VO_{2\text{-max}}$
Sandsund et al., 1998 (27)	Crossover	8, m, 25 ± 4	Highly trained cross-country skiers *	Aerosolised salbutamol 1200 µg	Running to exhaustion, incremental, $VO_{2\text{-max}}$
Larsson et al., 1997 (26)	Crossover	20, m, 24 18-31	Elite endurance athletes *	Inhaled terbutaline 3000 µg	Running to exhaustion, incremental, $VO_{2\text{-max}}$
Carlsen et al., 1997 (25)	Three-way crossover	18, m, 23 ± 6	Running >3 t/w	Inhaled salbutamol 800 µg Inhaled salmeterol 50 µg	Running until exhaustion, incremental, $VO_{2\text{-max}}$
Norris et al., 1996 (24)	Crossover	15, m, 25 ± 4	Highly trained cyclists	Inhaled 400 µg salbutamol	20 km cycling time trial $VO_{2\text{-max}}$
Heir et al., 1995 (23)	Crossover	17, m, 18-30	Highly conditioned endurance athletes *	Aerosolised salbutamol 50 µg/kg	Running to exhaustion at 110% $VO_{2\text{-max}}$, $VO_{2\text{-peak}}$
Unnithan et al., 1994 (22)	Crossover	10, m, 10 ± 1	Healthy non-athletes	Inhaled terbutaline 500 µg	Total running time $VO_{2\text{-peak}}$
Fleck et al., 1993 (21)	Crossover	21, m, 24 ± 5	Elite cyclists	Inhaled salbutamol 360 µg	W-max $VO_{2\text{-max}}$
Morton et al., 1992 (20)	Crossover	17, m/f 16/1, 22 ± 4	High performance runners *	Inhaled salbutamol 200 µg	Running to exhaustion, incremental $VO_{2\text{-max}}$
Meeuwisse et al. 1992 (19)	Crossover	7, m; 24 ± 4	Trained cyclists	Inhaled salbutamol 200 µ g	Endurance sprint time $VO_{2\text{-max}}$
Violante et al., 1989 (18)	Crossover	7, m, 34 ± 8	Sedentary non-athletes	Iv salbutamol 4 µg/kg followed by 3 µg/kg/h	Walking to exhaustion, incremental
Bedi et al 1988 (16)	Crossover	15, m/f 14/1, 23 ± 5	Cyclists, triathletes	Inhaled salbutamol 180 µg	Cycling to exhaustion after 60-min submaximal exercise $VO_{2\text{-max}}$
Booth et al., 1988 (17)	Crossover	10, f, 21 ± 7	Trained cyclists	Inhaled salbutamol x2, therapeutic dose	Cycling to exhaustion
McKenzie et al. 1983(59)	Parallel	4, m, 25 ± 8 5, f, 27 ± 10 5, m, 24 ± 9 5, f, 26 ± 13	Highly trained track and field Athletes *	Inhaled salbutamol 800 µg	$VO_{2\text{-max}}$

* Denotes high performance endurance athletes

m: male, f: female, s: seconds h/w: hours per week, d/w: days per week, t/w: times per week, iv: intra venous, VO_2: oxygen consumption, W-max: maximal workload during incremental cycling.

Risk of bias

Twenty-five studies (53%) had high risk of bias in one domain or more and the washout period varied from overnight to four weeks between the studies (Appendix table 1). The effect of beta2-agonists was not related to risk of bias (table 3). One study (52) reported period effect (p=0.12) and carryover effect (p=0.51) in addition to treatment effect (p= 0.54). Examination of potential publication bias by

assessing the funnel plot indicated no publication bias (figure 1 appendix). The Begg and Mazumdar rank correlation test found no publication bias with a 1-tailed p-value of 0.466.

The effect of beta2-agonists

Beta2-agonists did not affect aerobic performance as compared to placebo (SDM 0.051 (95%CI -0.020-0.122)). The SDMs for the included studies was not heterogeneous (I^2= 0%, p=0.893) (table 2). Neither type of beta2-agonist, administration route, duration of treatment, nor dose of beta2-agonist did influence the SMD (p>0.340) (table 3). In stratified analysis beta2- agonists prohibited by WADA (SDM 0.032 (95%CI -0.082-0.146)) and beta2- agonists approved by WADA (SDM 0.063 (95%CI -0.027-0.153)) had no effect on aerobic performance.

Table 2: Meta-analysis for each outcome measure

Outcome	Number of comparisons	Meta-analysis of each outcome			Test of heterogeneity	
		SDM	CI	p-value	I^2	p-value
Aerobic performance	60	0.051	-0.020-0.122	0.156	0%	0.893
Physical performance	53	0.047	-0.028-0.121	0.218	0%	0.778
VO_{2max}	28	-0.013	-0118-0.092	0.809	0%	0.999
Time trial	17	0.059	-0.064-0.182	0.345	0%	0.999
To exhaustion	36	0.043	-0.059-0.144	0.411	26%	0.260

SDM: Standardized difference in mean. CI: Confidence interval. Aerobic performance: maximal physical performance lasting more than one minute. $VO_{2max/peak}$: maximal oxygen consumption. Physical performance: time to exhaustion, distance covered in a pre-set time, time to cover a distance/amount of work or contractions to task failure. If a study reported both $VO_{2max/peak}$ and physical performance, physical performance was included aerobic performance. If only $VO_{2max/peak}$ was reported, $VO_{2max/peak}$ was included in aerobic performance; Time trial: closed-ended tests. To exhaustion: open-ended tests. I^2 : the proportion of variance that is due to real differences in effect size

Table 3. Regression of standardized difference in means against type of beta2-agonist, administration route, duration of treatment, dose and risk of bias treated as categorical variables.

	Aerobic performance	VO_{2max}	Physical performance	Time trial	To exhaustion
Type of beta2-agonist; Reference Long acting				*	
Test of model, p-value	0.552	0.687	0.693		0.724
Goodness of fit, p-value	0.826	0.998	0.723		0.196
R^2 analog	0	0	0		0
Administration route; Reference inhaled					
Test of model, p-value	0.340	0.464	0.325	0.99	0.300
Goodness of fit, p-value	0.790	0.998	0.752	0.999	0.236
R^2 analog	0	0	0	0	0
Duration of treatment; Reference acute					
Test of model, p-value	0.953	0.249	0.944	0.861	0.888
Goodness of fit, p-value	0.816	0.999	0.718	0.998	0.193
R^2 analog	0	0	0	0	0
Dose; Reference prohibited					
Test of model, p-value	0.659	0.913	0.467	0.338	0.869
Goodness of fit, p-value	0.821	0.997	0.737	0.999	0.193
R^2 analog	0	0	0	0	0
Type, route, duration, dose				*	
Test of model, p-value	0.644	0.820	0.496		0.693
Goodness of fit, p-value	0.809	0.997	0.738		0.180
R^2 analog	0	0	0		0
Risk of bias; Reference high risk					
Test of model, p-value	0.928	0.845	0.744	0.130	0.485
Goodness of fit, p-value	0.816	0.997	0.721	0.999	0.210
R^2 analog	0	0	0	0	0.00
Performance level; Referance high performance					
Test of model, p-value	0.808	0.538	0.639	0.557	0.865
Goodness of fit, p-value	0.717	0.998	0.725	0.999	0.193
R^2 analog	0	0	0	0	0

R^2 analog: Proportion of total between-study variance explained by the covariate. *: Could not be assessed because of a problem with collinearity. Aerobic performance: maximal physical performance lasting more than one minute. $VO_{2max/peak}$: maximal oxygen consumption. Physical performance: time to exhaustion, distance covered in a pre-set time, time to cover a distance/amount of work or contractions to task failure. If a study reported both $VO_{2max/peak}$ and physical performance, physical performance was included aerobic performance. If only $VO_{2max/peak}$ was reported, $VO_{2max/peak}$ was included in aerobic performance. Time trial: closed-ended tests. To exhaustion: open-ended tests.

The effect of beta2-agonists on physical performance was assessed in 53 comparisons. Beta2-agonists did not improve aerobic physical performance (SDM 0.047 (95%CI -0.028-0.121). The SDM for the included studies was not heterogeneous (I^2= 0%, p=0.778) (table 2, figure 2). Neither type of beta2-agonist, administration route, duration of treatment, nor dose of beta2-agonist did influence the SMD (p>0.325) (table 3).

Insert Figure 2

The SDM from 28 comparisons showed that beta2-agonists did not affect VO_{2max} (p=0.809) (table 2, figure 3) and type of beta2-agonist, administration route, duration of treatment, or dose of beta2-agonist did not influence the SMD (p>0.249) (table 3).

Insert Figure 3

The effect on time trial performance was assessed in 17 comparisons and the effect on performance to exhaustion was assessed in 36 comparisons. Beta2-agonists did not improve time to exhaustion (p=0.411) or time trial performance (p=0.345) (table 2).

Sample size and skewness

Two of the 47 included studies included adequate numbers of participants to obtain an alpha < 0.05 and a beta < 0.2 (supplementary table 2). Baseline performance values were skewed in four comparisons and sensitivity analysis excluding these four comparisons found no effect of beta2-agonists on aerobic performance (SMD 0.046, p=0.213).

Sensitivity analysis

Eleven studies included high performance endurance athletes (table 2) and there was no difference in response to beta2-agonists in the high performance endurance athletes compared to the less fit subjects included in the other studies (table 3, p= 0.808).

In a sensitivity analysis excluding the 16 comparisons with ten or less pairwise comparisons between beta2-agonists and placebo we did not find any effect of beta2-agonists on aerobic performance (SMD 0.059, p=0.171).

Discussion

This meta-analysis of RCTs that examined the effect of beta2-agonists on aerobic performance provides the most comprehensive quantitative summary of the evidence to date, including 47 RCTs with 60 placebo-controlled comparisons comprising 680 participants. Twenty-five studies had high risk of bias due to side effects, single blinding or inadequate screening for AHR. Our study extends previous reviews by including 21 studies not previously meta-analysed and with an in-depth analysis of aerobic performance. The results from our analysis demonstrated that beta2-agonists had no effect on aerobic performance. The result was consistent and not heterogeneous.

To our knowledge, no other studies have pooled data and meta-analysed the effects of beta2-agonists on aerobic performance to this extent. Pluim et al. (8) presented a meta-analysis stratified by administration route (oral or inhaled) and analysed test specific outcomes separately and did not find any effect of inhaled or oral beta2-agonists on any aerobic performance outcome. In our study, we included comparisons with inhaled and oral beta2-agonists in the same analysis, as we hypothesized that inhalation and oral ingestion may provide the same physiological stimuli, which depends on the dose and systemic bioavailability, because the two administration routes may induce similar serum concentrations of beta2-agonists (63). This assumption was supported by the findings in the present study, as route of administration was not related to the effect of beta2-agonists on aerobic performance. We also investigated whether beta2-agonists prohibited by WADA had different effect than beta2-agonists (type and dose) approved by WADA and found no difference. In addition, we meta-analysed different types of aerobic performance and stratified by $VO_{2\text{-max}}$, performance, time trials (closed-end tests) and performance until exhaustion (open-end tests). We combined 59 comparisons as compared to 2-18 comparisons (depending on the number of studies measuring aerobic performance in the same way) in the study by Pluim et al (8). Thus, our results strengthen the findings from Pluim et. al (8). Type of beta2-agonist, administration route, duration of treatment, dose, fitness level of the participants or study quality did not affect the result.

Analysis of performance categories

Maximal oxygen consumption was unaffected by the use of beta2-agonists. The effect size from the 27 comparisons included was not heterogeneous and type of beta2-agonist, administration route, duration of treatment or dose did not affect the result. The finding builds on the evidence from Pluim et al. (8) who meta-analysed 18 studies with inhaled beta2-agonists.

Beta2-agonists did not improve aerobic physical performance measured by closed-end or open-ended tests. Closed-end tests are usually recommended over open-end tests due to a better reliability (64) and possibly a better chance of detecting minor differences in performance. However, in the present study neither type of protocol indicated an effect of beta2-agonists on aerobic performance.

Bias

The funnel plot and the Begg and Mazumdar rank correlation test were negative for publication bias indicating that results do not influence whether the studies are published or not.

Tachycardia and tremor are characteristic adverse side effects of beta2-agonists (65). These side effects may break the blinding if the participants are aware of whether they receive beta2-agonists or placebo, and possibly motivate them to perform differently when receiving beta2- agonists. Fifteen studies were classified as high risk of bias due to lack of blinding (single-blind design or reported side effects of the beta2-agonists) and 10 additional studies did not screen the participants sufficiently for AHR. However, high risk of bias did not influence the SDM in any analysis.

Our study included 16 comparisons between placebo and beta2-agonists that comprised less than 10 pairs (less than 20 subjects in parallel studies and 10 subjects in crossover studies) and only two of the included studies had a sample size providing an alpha < 0.05 and a beta < 0.2 for the measured effect. This low sample size in the individual studies may have introduced sparse data bias in the SMD

(66, 67). However, when we performed a sensitivity analysis excluding these 16 comparisons the effect of beta2-agonists on aerobic performance was still not statistically significant. Normal distribution of data is an important assumption in meta-analysis of continuous data and five comparisons had skewed baseline data, however execution of these comparisons from the meta-analysis did not influence the result.

Strength and limitations

The present study is strengthened by the systematic search of the literature in multiple databases. It is therefore likely that all relevant studies were identified, and we included RCTs only. We consider all maximal performance tests lasting more than one minute to be a measure of aerobic performance thus we used SMD as outcome in the meta-analysis (68). This resulted in a large sample size and a high statistical power. We also investigated the effect of beta2-agonists separately on $VO_{2\text{-max}}$ and physical performance that was further divided into open- and closed-ended tests to investigate any physiological (oxygen consumption) or performance test specific effects. Further, we performed subgroup analyses on outcome categories and meta-regression to investigate the effect of the different types of intervention. For example, two recent studies (69, 70) reported a potential negative effect of multiple weeks of beta2- agonist treatment while one study (44) reported a positive effect of acute administration. If this was representative for the included studies the opposite effects could even each other out in a meta-analysis but show difference in effects in a meta regression categorised by duration of treatment.

Splitting up the interventions into sub-categories can also be a limitation as the statistical power is reduced. Categories with few studies/participants (multiple weeks of treatment) usually have larger uncertainties in the effect estimates and it is difficult to interpret if the lack of effect is due to no effect or to low statistical power to reveal the real effect. Another weakness in the present study is that all outcomes are assessed by laboratory tests, not identical to actual athletic competitions. Therefore, reliable, sensitive and valid test protocols are of importance. Closed-end tests are

recommended over open-end tests due to a better reliability and the coefficients of variation for open-end tests are reported to decrease with increased intensity or decreased duration (64). Thus, we performed separate meta-analyses on open and closed end tests. However, both types of tests failed to demonstrate any effect of beta2-agonists. The fitness level of the participants included in our study varied from untrained to elite athletes and fitness level has been suggested to confound the effect of beta2- agonists on physical performance (71). However, in the present study beta2-agonist did not affect aerobic performance differently in high performance endurance athletes compared to less endurance trained participants. The meta-analysis assumes independence between the subjects included. In the present study, the same subjects are included twice in the analysis if they participated in a three-way crossover study with two different interventions with beta2-agonists, or if the same subjects are assessed after acute treatment and subsequently after multiple weeks of beta2-agonist treatment or placebo. To investigate the effect of this potential bias we performed a meta-analysis including comparisons with different people only, and the effect size was practically the same as when all relevant comparisons were included. There is also a possibility that the same subjects are included in different studies. Few studies reported correlation between trial results, thus the correlation coefficient for pre and post-test has been set to 0.5 for all studies. This is lower than data made available from Dickinson et al. (60) by request and similar to what Pluim et al. (8) reported. Randomised controlled trials are regarded as high quality studies, but there is a possibility for bias especially related to the side effects from beta2-agonists breaking the blinding for the participants. In addition, the meta-analysis includes several single blinded studies where the investigators knew when the subjects received beta2- agonists. This lack of blinding may allow the investigator to treat the subjects systematically different when knowing what the subjects have received. The possible difference in the way the investigator interacts with the subjects may lead to a systematic difference in performance. Many studies did not include objective tests for AHR and thus may have failed to exclude participants with AHR. However, risk of bias did not influence the effect size in the present meta-analysis and Koch et al. (51) found no difference in time trial performance

between cyclists with and without AHR. In addition, a systematic review from 2014 (72) concluded the current evidence is insufficient to prove a negative effect of AHR on physical performance.

Based on the previously mentioned limitations the findings should be interpreted with caution, especially the results from sub-groups with few studies/participants, but there is consistency in the results demonstrating that beta2-agonists do not affect aerobic performance in non-asthmatic subjects.

Conclusion

The present study, which summarize the best scientific evidence, shows that beta2-agonists do not affect aerobic performance in non-asthmatics. Beta2- agonists had no effect on performance tests or VO_{2max}. The results from the present study should be of interest to the World Anti-Doping Agency, when revising the anti-doping regulations and planning anti-doping sample analysis, and anyone who is interested in equal opportunities in competitive sports.

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DISCLOSURE

Riiser, Stensrud, Stang and Andersen have nothing to disclose.

Author contributions: All authors reviewed the report. AR generated the hypotheses did the literature search analyzed the data and wrote the first draft of the manuscript. AR, TS, JS and LBA revised the manuscript critically for important intellectual content. All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. AR and TS extracted the data. JS and AR assessed bias. AR and LBA evaluated studies for inclusion.

LEGENDS

Figure 1: Flow chart of included studies as proposed by Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement 2009 [12]

Figure 2: Forest plot for the effect of beta2-agonists on aerobic physical performance

Figure 3: Forest plot for the effect of beta2-agonists on maximal oxygen consumption (VO_{2max})

Supplementary figure 1: Funnel plot for the effect of beta2-agonists on aerobic performance. The plot does not indicate publication bias.

1.

1. McKenzie, D.C. and K.D. Fitch, The asthmatic athlete: inhaled Beta-2 agonists, sport performance, and doping. *Clin J Sport* Med2011;21:46-50.
2. Fitch, K.D., An overview of asthma and airway hyper-responsiveness in Olympic athletes. Br J Sports Med2012;46:413-416. doi:10.1136/bjsports-2011-090814
3. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2019. Available from: www.ginasthma.org [Accessed 26.09.2019].
4. World Anti-Doping Agency. Prohibited list, January 2020. https://www.wada-ama.org/sites/default/files/wada_2020_english_prohibited_list_0.pdf. [Accessed 17.02.2020]
5. Kindermann W. Do inhaled beta(2)-agonists have an ergogenic potential in non-asthmatic competitive athletes? *Sports med*2007;37:95-102. doi:10.2165/00007256-200737020-00001.
6. Fitch KD, Sue-Chu M, Anderson SD, et al. Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. *J Allergy Clin Immunol*2008;122:254-260 doi:10.1016/j.jaci.2008.07.003.
7. Carlsen KH, Anderson SD, Bjermer L, et al. Treatment of exercise-induced asthma, respiratory and allergic disorders in sports and the relationship to doping: Part II of the report from the Joint Task Force of European Respiratory Society (ERS) and European Academy of Allergy and Clinical Immunology (EAACI) in cooperation with GA(2)LEN. *Allergy*2008;63:492-505. doi:10.1111/j.1398-9995.2008.01663.x.
8. Pluim BM, de Hon O, Staal JB, et al. B2-Agonists and Physical Performance. *Sports Med*. 2011;41:39-57. doi:http://dx.doi.org/10.2165/11537540-000000000-00000.
9. Norway's cross-country Olympic success clouded by asthma drug ethics https://www.reuters.com/article/us-olympics-2018-nors-norway/norways-cross-country-olympic-success-clouded-by-asthma-drug-ethics-[Accessed 14.05.2019]
10. More details of Chris Froome's successful salbutamol defence http://www.cyclingnews.com/news/more-details-of-chris-froomes-successful-salbutamol-defence/[Accessed 13.05.2019].
11. Riiser A, Stensrud T, Stang J, et al. The effect of beta2-agonists on anerobic performance – A systematic review and meta-analysis of randomized controlled trials. *BJSM*2020;In review.
12. Moher D, Liberati A, Tetzlaff J, et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS med*2009;6. doi:ARTN e1000097
13. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*2011;343:d5928. doi:10.1136/bmj.d5928
14. SP K. Sample Size Calculator https://clincalc.com/stats/SampleSize.aspx. Updated November 10, 2018. Accessed May 16, 2019.
15. Altman DG, Bland JM. Detecting skewness from summary information. *BMJ*1996;313(7066):1200.
16. Bedi JF, Gong H, Jr., Horvath SM. Enhancement of exercise performance with inhaled albuterol. *Can J Sport Sci*1988;13:144-148.
17. Booth ML. The effect of ventolin on a maximal work capacity test in trained female cyclists. *NZ J Physiotherapy*1988: 17.
18. Violante B, Pellegrino R, Vinay C, et al. Failure of aminophylline and salbutamol to improve respiratory muscle function and exercise tolerance in healthy humans. *Respiration*1989;55:227-236.
19. Meeuwisse WH, McKenzie DC, Hopkins SR, et al. The effect of salbutamol on performance in elite nonasthmatic athletes. *Med Sci Sports Exerc*1992;24:1161-1166.
20. Morton AR, Papalia SM, Fitch KD. Is salbutamol ergogenic?: The effects of salbutamol on phisical performance in high-performance nonasthmatic. *Clin J Sport Med*1992;2:93-97.
21. Fleck SJ, Lucia A, Storms WW, et al. Effects of Acute Inhalation of Albuterol on Submaximal and Maximal Vo2 and Blood Lactate. *Int J Sports Med*1993;14:239-243.

22. Unnithan VB, Thomson KJ, Aitchison TC, et al. Beta-2-Agonists and Running Economy in Prepubertal Boys. *Pediatr Pulm*1994.17:378-382.
23. Heir T, Stemshaug H. Salbutamol and high-intensity treadmill running in nonasthmatic highly conditioned athletes. *Scand J Med Sci Sports;*1995;5:231-236.
24. Norris SR, Petersen SR, Jones RL. The effect of salbutamol on performance in endurance cyclists. *Eur J Appl Physiol Occup Physiol*1996;73:364-368.
25. Carlsen KH, Ingjer F, Kirkegaard H, Thyness B. The effect of inhaled salbutamol and salmeterol on lung function and endurance performance in healthy well-trained athletes. *Scand J Med Sci*1997;7:160-165.
26. Larsson K, Gavhed D, Larsson L, et al. Influence of a beta2-agonist on physical performance at low temperature in elite athletes. *Med Sci Sports Exerc*1997;29:1631-1636.
27. Sandsund M, Sue-Chu M, Helgerud J, et al., Effect of cold exposure (-15 degrees C) and salbutamol treatment on physical performance in elite nonasthmatic cross-country skiers. Eur J Appl Physiol Occup Physiol1998;77:297-304.
28. Sue-Chu M, Sandsund M, Helgerud J, et al. Salmeterol and physical performance at -15 degrees C in highly trained nonasthmatic cross-country skiers. *Scan J Med Sci Sports* 1999;9:48-52.
29. Collomp K, Candau R, Collomp R, et al. Effects of acute ingestion of salbutamol during submaximal exercise. *Int J Sports Med*2000;21:480-484.
30. Collomp K, Candau R, Lasne F, et al. Effects of short-term oral salbutamol administration on exercise endurance and metabolism. *J Appl Physiol*2000;89**:**430-436.
31. van Baak MA, Mayer LH, Kempinski RE, et al. Effect of salbutamol on muscle strength and endurance performance in nonasthmatic men. *Med Sci Sports Exerc*2000;32:1300-1306.
32. Carlsen KH, Hem E, Stensrud T, et al. Can asthma treatment in sports be doping? The effect of the rapid onset, long-acting inhaled beta2-agonist formoterol upon endurance performance in healthy well-trained athletes. *Respir Med*2001:95:571-576.
33. Goubault C, Perault MC, Leleu E, et al. Effects of inhaled salbutamol in exercising non-asthmatic athletes*. Thorax*2001;56:675-679.
34. Collomp K, Candau R, Millet G, et al. Effects of salbutamol and caffeine ingestion on exercise metabolism and performance*. Int J Sports Med*2002;23:549-554.
35. Stewart IB, Labreche JM, McKenzie DC. Acute formoterol administration has no ergogenic effect in nonasthmatic athletes. *Med Sci Sports Exerc*2002;34;213-217.
36. van Baak MA, de Hon OM, Hartgens F, et al. Inhaled salbutamol and endurance cycling performance in non-asthmatic-athletes. *Int J Sports Med*2004;25:533-538.
37. Riiser A, Tjorhom A, Carlsen KH. The effect of formoterol inhalation on endurance performance in hypobaric conditions. *Med Sci Sports Exerc*2006;38:2132-2137.
38. Tjorhom A, Riiser A, Carlsen KH. Effects of formoterol on endurance performance in athletes at an ambient temperature of -20 degrees C. *Scand J Med Sci Sports*2007;17:628-635.
39. Decorte N, Verges S, Flore P, al. Effects of acute salbutamol inhalation on quadriceps force and fatigability. *Med Sci Sports Exerc*2008;40:1220-7. doi:10.1249/MSS.0b013e31816b87aa.
40. Sporer BC, Sheel AW, McKenzie DC. Dose response of inhaled salbutamol on exercise performance and urine concentrations. *Med Sci Sports Exerc*2008;40:149-157.
41. Andersen K, Kanstrup I. Effects of acute oral administration of 4 mg salbutamol on exercise performance in non-asthmatic elite athletes. *J Exerc Physiol online*2009;12:36-47.
42. Beloka SP, Janssen C, Woff E, et al. Effects of beta2-adrenergic stimulation on exercise capacity in normal subjects. *Eur J Appl Physiol*2011;111:2239-2247. doi:10.1007/s00421-011-1856-9.
43. Elers J, Morkeberg J, Jansen T, et al. High-dose inhaled salbutamol has no acute effects on aerobic capacity or oxygen uptake kinetics in healthy trained men. *Scand J Med Sci Sports*2012;22**:**232-239.

44. Decorte N, Bachasson D, Guinot M, et al. Effect of salbutamol on neuromuscular function in endurance athletes. *Med Sci Sports Exerc*2013;45:1925-1932. doi:10.1249/MSS.0b013e3182951d2d.
45. Sanchez AM, Borrani F, Le Fur MA, , et al. Acute supra-therapeutic oral terbutaline administration has no ergogenic effect in non-asthmatic athletes. *Eur J Appl Physiol*2013;113:411-8.
46. Dickinson J, Hu J, Chester N, et al. Acute Impact of Inhaled Short Acting B-2-Agonists on 5 Km Running Performance. *J Sport Sci Med2014*;13:271-279.
47. Hostrup M, Kalsen A, Bangsbo J, et al. High-dose inhaled terbutaline increases muscle strength and enhances maximal sprint performance in trained men. *Eur J Appl Physiol*2014;114:2499-2508. doi:10.1007/s00421-014-2970-2.
48. Kalsen A, Hostrup M, Karlsson S, , et al. Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance. *J Appl Physiol*2014;117:1180-1187.
49. Koch S, Ahn JR, Koehle MS. High-Dose Inhaled Salbutamol Does Not Improve 10-km Cycling Time Trial Performance. *Med Sci Sports Exerc*2015;47:2373-2379.
50. Koch S, Karacabeyli D, Galts C, et al. Effects of inhaled bronchodilators on lung function and cycling performance in female athletes with and without exercise-induced bronchoconstriction. *J Sci Med Sport*2015.18:607-612.
51. Koch S, MacInnis MJ, Sporer BC, et al. Inhaled salbutamol does not affect athletic performance in asthmatic and non-asthmatic cyclists. *Br J Sports Med*2015;49:51-55.
52. Halabchi F, Abarashi M, Mansournia MA, et al. Effects of Inhaled Salbutamol on Sport-Specific Fitness of Non-Asthmatic Football Players. *Acta Med Iran*2017;55:324-332.
53. Molphy J, Dickinson JW, Chester NJ et al. The Effect of 400 mu g Inhaled Salbutamol on 3 km Time Trial Performance in a Low Humidity Environment. *J Sport Sci Med*2017;16: 581-588.
54. Eckerstrom F, Rex CE, Maagaard M, et al. Exercise performance after salbutamol inhalation in non-asthmatic, non-athlete individuals: a randomised, controlled, cross-over trial. *BMJ Open Sport Exerc Med*2018;4:e000397.
55. Laurent J, Guinot M, Mallouf TL, et al. Effects of Acute Salbutamol Intake on Peripheral and Central Fatigue in Trained Men. *Med Sci Sports Exerc*2018;50:1267-1276.
56. Hafedh H, Slimani M, Miarka B, et al. Effects of Terbutaline Sulfate on Physiological and Biomechanical as Well as Perceived Exertion in Healthy Active Athletes: A Pilot Study. *J Hum Kinet*2019;69:169-78.
57. Merlini M, Beato M, Marcora S, Dickinson J. The Effect of 1600 mu g Inhaled Salbutamol Administration on 30 m Sprint Performance Pre and Post a Yo-Yo Intermittent Running Test in Football Players. *J Sport Sci Med*2019;18:716-21.
58. Molphy J, Dickinson JW, Chester et al. The Effects of Inhaled Terbutaline on 3-km Running Time-Trial Performance. *Int J Sports Physiol Perform*2019;14:822-8.
59. Mckenzie DC, Rhodes EC, Stirling DR, et al. Salbutamol and Treadmill Performance in Non-Atopic Athletes. Med Sci Sports Exerc. 1983;15:520-2.
60. Dickinson J, Molphy J, Chester N, et al. The Ergogenic Effect of Long-term Use of High Dose Salbutamol. *Clin J Sport Med*2014;24:474-81.
61. Hostrup M, Kalsen A, Onslev J, et al. Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic beta(2)-adrenergic stimulation in men. *J Appl Physiol*2015;119:475-86.
62. Hostrup M, Kalsen A, Auchenberg M, et al. Effects of acute and 2-week administration of oral salbutamol on exercise performance and muscle strength in athletes. *Scand J Med Sci Sports*. 2016;26:8-16.
63. Hostrup M, Kalsen A, Ortenblad N, et al. beta2-adrenergic stimulation enhances Ca2+ release and contractile properties of skeletal muscles, and counteracts exercise-induced reductions in Na+-K+-ATPase Vmax in trained men. *J Physiol*. 2014;592:5445-59.
64. Currell K, Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. Sports Med2008;38(4):297-316.

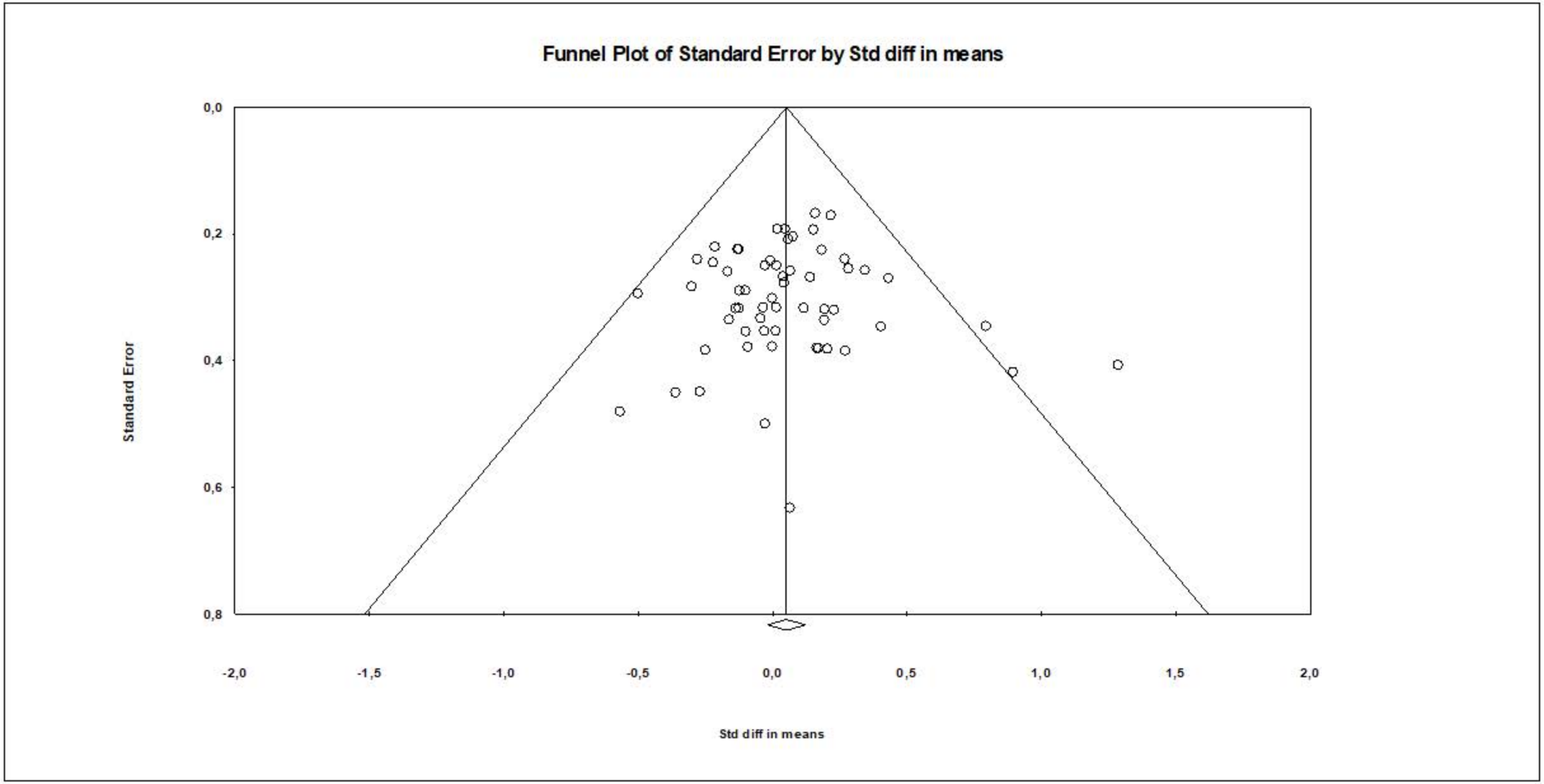
65. Cazzola M, Matera MG. Tremor and β2-adrenergic agents: Is it a real clinical problem? *Pulm Pharmacol Ther*2012;1:4-10.
66. Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in plain sight. *BMJ*2016;352:i1981.
67. Lin L. Bias caused by sampling error in meta-analysis with small sample sizes. *PLoS One*2018;13:e0204056.
68. Gastin PB. Energy system interaction and relative contribution during maximal exercise. *Sports Med*2001;31:725-41.
69. Hostrup M, Onslev J, Jacobson GA, et al. Chronic β2-adrenoceptor agonist treatment alters muscle proteome and functional adaptations induced by high intensity training in young men. J Physiol. 2018;:231-52.
70. Lemminger AK, Jessen S, Habib S, et al. Effect of beta2 -adrenergic agonist and resistance training on maximal oxygen uptake and muscle oxidative enzymes in men. *Scand J Med Sci Sports*. 2019.
71. Hostrup M, Reitelseder S, Jessen S, Kalsen A, Nyberg M, Egelund J, et al. Beta2 -adrenoceptor agonist salbutamol increases protein turnover rates and alters signalling in skeletal muscle after resistance exercise in young men. *J Physiol*. 2018;596(17):4121-39.
72. Price OJ, Hull JH, Backer V, et al. The impact of exercise-induced bronchoconstriction on athletic performance: a systematic review. *Sports Med2014*;44(12):1749-61.

Appendix table 1: The quality of the included studies assessed using the Cochrane Collaboration Risk of Bias Tool.[13]

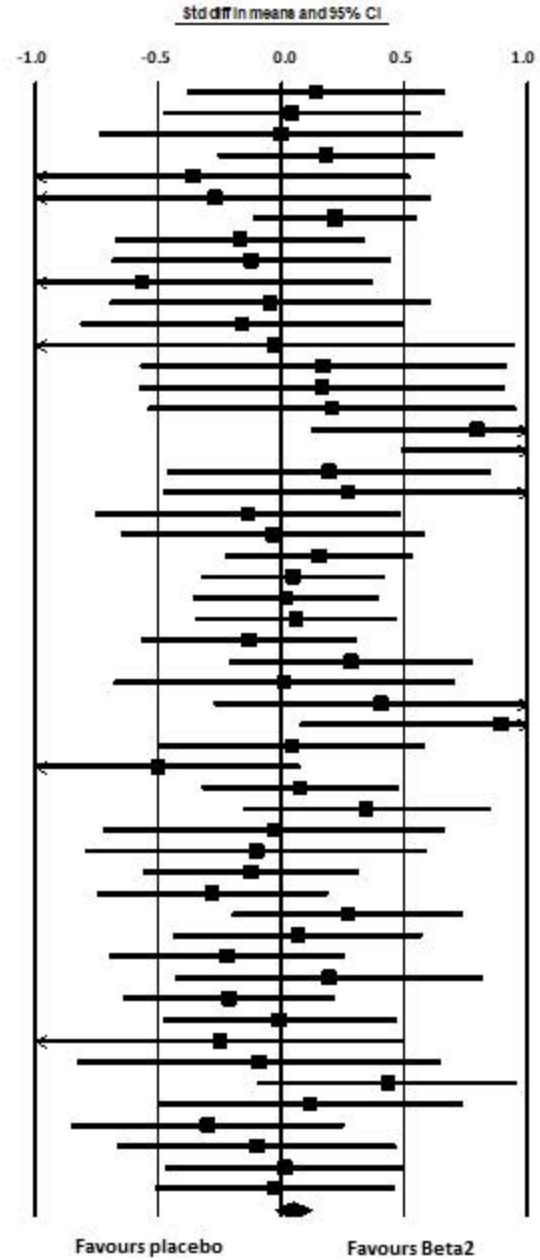
Study, year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Participants screened for AHR	Washout period between treatments	Adequate sample size*
Molphy et al. 2019 [58]	Low risk	Low risk	**High risk**	Low risk	Low risk	Low risk	Low risk	At least 7 days	4360
Merlini et al., 2019 [57]	Low risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	Unclear risk	48 hours	14
Hafedh et al., 2019 [56]	Unclear risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	**High risk**	Not reported	131
Laurent et al., 2018 [55]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	At least 4 days	663
Ekstrom et al., 2018 [54]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	2 to 14 days of interval	866
Molphy et al., 2017 [53]	Low risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	At least 7 days	15447600
Halabchi et al., 2017 [52]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**High risk**	1 week	489
Hostrup et al., 2016 [61]	Unclear risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	na	3532
Koch et al., 2015 [51]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	3 to 14 days	392
Koch et al., 2015 [50]	Unclear risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	3 to 14 days	698
Koch et al., 2015 [49]	Unclear risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	3 to 14 days	1108
Hostrup et al., 2015 [60]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	na	16
Karlsen et al., 2014 [48]	Low risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	**High risk**	3 days	9168
Hostrup et al., 2014 [47]	Low risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	**High risk**	3 days	649
Dickinson et al., 2014a [59]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	na	194
Dickinson et al., 2014b [46]	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk	Separate days	514
Sanchez et al., 2013 [45]	Unclear risk	Unclear risk	**High risk**	Low risk	Low risk	Low risk	Low risk	1 week	496
Decorte et al., 2013 [44]	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	**High risk**	4 days to3 weeks	28
Elers et al., 2012 [43]	Low risk	Low risk	**High risk**	Low risk	Low risk	Low risk	**High risk**	1 week	392
Beloka et al., 2011 [42]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	Overnight	No difference
Andersen et al.2009, [41]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Separate days	282
Sporer et al., 2008 [40]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 3 days	651
Descorte et al., 2008 [39]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	3 days to 3 weeks	883
Tjorhom et al., 2007 [38]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	2 to 8 days	4876

Riiser et al., 2006 [37]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	2 to 7 days	1081
Van Baak et al., 2004 [36]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	4 to 14 days.	123
Stewart et al., 2002 [35]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 1 wk.	76919
Collomp et al., 2002 [34]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	3 days	124
Collomp et al., 2000a [29]	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	**High risk**	2 days to 3 weeks	7883
Collomp et al., 2000b [30]	Low Risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	On two different days	2430
Goubault et al., 2001 [33]	Unclear risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	**High risk**	Approximately 1 week	9
Carlsen et al., 2001 [32]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	4 weeks	124
Van Bak et al., 2000 [31]	Unclear risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	3-4 weeks	26
Sue-Chu et al., 1999 [28]	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 2 days	15517
Sandsund et al., 1998 [27]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 1 day	1949
Larsson et al., 1997 [26]	Unclear risk	Unclear risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	24 hours.	1005
Carlsen et al., 1997 [25]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	On three different days	209
Norris et al., 1996 [24]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 24 hours	3210
Heir et al., 1995 [22]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	2 to 7 days	323
Unnithan et al., 1994 [22]	Unclear risk	Low risk	**High risk**	Unclear risk	Low risk	Low risk	Low risk	At least 2 days	429
Fleck et al., 1993 [21]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	1 day	796
Morton et al., 1992 [20]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	4 to 10 days	284822
Meeuwisse et al. 1992 [19]	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Approximately 1 week	316
Violante et al., 1989 [18]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	**High risk**	Not reported	1823
Bedi et al 1988 [16]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	At least 1 week	75
Booth et al., 1988 [17]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	**High risk**	7 days	1303
McKenzie et al. 1983[58]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	na	411

* Adequate sample size assessed by calculation of the sample size (pairwise comparasons between placebo and beta2-agonist) required for the effect to obtain an alpha of 0.05 and a beta of 0.2 [43]. na: not applicable, as it is a parallel trial. AHR: airway hyperresponsiveness. No difference: no difference in effect between groups.


Funnel Plot of Standard Error by Std diff in means
Standard Error
0,0
0,2
0,4
0,6
0,8
-2,0
-1,5
-1,0
-0,5
0,0
0,5
1,0
1,5
2,0
Std diff in means

Study name	Outcome	Statistics for each study						
		Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Laurent et al., 2018, Inhaled	To exhaustion, contractions	0,141	0,269	0,072	-0,386	0,667	0,525	0,600
Laurent et al., 2018, Oral	To exhaustion, contractions	0,041	0,267	0,071	-0,483	0,565	0,152	0,879
Mulphy et al., 2017	Time trial, running	-0,001	0,378	0,143	-0,742	0,740	-0,003	0,998
Halabchi et al., 2017	To exhaustion, running	0,184	0,225	0,051	-0,258	0,626	0,815	0,415
Hostrup et al., 2016, Acute	To exhaustion, cycling	-0,360	0,451	0,203	-1,243	0,524	-0,796	0,425
Hostrup et al., 2016, Multiple weeks	To exhaustion, cycling	-0,289	0,449	0,202	-1,150	0,611	-0,599	0,549
Koch et al., 2015a	Time trial, cycling	0,218	0,171	0,029	-0,117	0,553	1,276	0,202
Koch et al., 2015b	Time trial, cycling	-0,166	0,260	0,068	-0,676	0,343	-0,640	0,522
Koch et al., 2015c	Time trial, cycling	-0,122	0,290	0,084	-0,690	0,446	-0,421	0,674
Hostrup et al., 2015	To exhaustion, cycling	-0,567	0,481	0,231	-1,509	0,376	-1,178	0,239
Karlsen et al. 2014	Time trial, cycling	-0,044	0,333	0,111	-0,698	0,610	-0,132	0,895
Hostrup et al., 2014	Time trial, cycling	-0,161	0,335	0,113	-0,818	0,497	-0,479	0,632
Dickinson et al., 2014a	Time trial, running	-0,027	0,500	0,250	-1,007	0,953	-0,055	0,956
Dickinson et al. 2014b 800	Time trial, running	0,172	0,381	0,145	-0,575	0,918	0,451	0,652
Dickinson et al. 2014b 1600	Time trial, running	0,165	0,381	0,145	-0,581	0,911	0,434	0,665
Sanchez et al., 2013	To exhaustion, cycling	0,205	0,382	0,146	-0,543	0,954	0,538	0,591
Decorte et al. 2013, 200	To exhaustion, contractions	0,795	0,346	0,120	0,117	1,473	2,298	0,022
Decorte et al. 2013, 800	To exhaustion, contractions	1,288	0,407	0,166	0,487	2,084	3,155	0,002
Elers et al. 2012	To exhaustion, cycling	0,193	0,336	0,113	-0,466	0,853	0,574	0,566
Andersen et al. 2009	To exhaustion, running	0,271	0,385	0,148	-0,483	1,026	0,705	0,481
Descorte et al 2008, 200	To exhaustion, cycling	-0,136	0,318	0,101	-0,759	0,487	-0,428	0,668
Descorte et al 2008, 800	To exhaustion, cycling	-0,034	0,316	0,100	-0,654	0,586	-0,108	0,914
Sporer et al, 2008, 200	Time trial, cycling	0,153	0,194	0,037	-0,226	0,532	0,791	0,429
Sporer et al, 2008, 400	Time trial, cycling	0,047	0,193	0,037	-0,330	0,425	0,245	0,806
Sporer et al, 2008, 800	Time trial, cycling	0,019	0,192	0,037	-0,358	0,396	0,099	0,921
Tjarhom et al. 2007	To exhaustion, running	0,059	0,209	0,044	-0,350	0,468	0,283	0,778
Riiser et al. 2006	To exhaustion, running	-0,130	0,225	0,050	-0,570	0,310	-0,578	0,563
Van Baak et al 2004	Time trial, cycling	0,283	0,255	0,065	-0,217	0,783	1,111	0,267
Collomp et al. 2002	Time trial, cycling	0,012	0,354	0,125	-0,681	0,705	0,035	0,972
Collomp et al 2000a	To exhaustion, cycling	0,404	0,347	0,120	-0,275	1,084	1,166	0,244
Collomp et al 2000b	To exhaustion, cycling	0,895	0,418	0,175	0,075	1,715	2,139	0,032
Goubault et al. 2001, 200	To exhaustion, cycling	0,044	0,277	0,077	-0,500	0,588	0,158	0,875
Goubault et al. 2001, 800	To exhaustion, cycling	-0,500	0,294	0,087	-1,077	0,076	-1,700	0,089
Carlsen et al. 2001	To exhaustion, running	0,077	0,204	0,042	-0,324	0,478	0,377	0,706
Van Baak et al 2000	To exhaustion, cycling	0,345	0,257	0,066	-0,160	0,849	1,340	0,180
Sue-Chu et al. 1999	To exhaustion, running	-0,030	0,354	0,125	-0,723	0,663	-0,084	0,933
Sandsund et al. 1998	To exhaustion, running	-0,099	0,354	0,126	-0,794	0,596	-0,279	0,780
Larsson et al. 1997	To exhaustion, running	-0,125	0,224	0,050	-0,565	0,315	-0,557	0,578
Carlsen et al. 1997, salb	To exhaustion, running	-0,280	0,240	0,058	-0,750	0,191	-1,163	0,245
Carlsen et al. 1997, salm	To exhaustion, running	0,289	0,240	0,058	-0,201	0,740	1,123	0,261
Norris et al., 1996	Time trial, cycling	0,067	0,258	0,067	-0,440	0,573	0,259	0,796
Heir og Stemshaug., 1995	To exhaustion, running	-0,220	0,245	0,060	-0,701	0,261	-0,898	0,369
Unnithan et al. 1994	To exhaustion, running	0,194	0,319	0,102	-0,431	0,820	0,609	0,543
Reick et al. 1993	To exhaustion, cycling	-0,213	0,221	0,049	-0,646	0,220	-0,965	0,334
Morton et al. 1992	To exhaustion, running	-0,007	0,243	0,059	-0,482	0,468	-0,029	0,977
Meeuwisse et al. 1992	To exhaustion, cycling	-0,249	0,384	0,147	-1,001	0,503	-0,649	0,516
Violante et al. 1989	To exhaustion, walking	-0,091	0,379	0,143	-0,833	0,652	-0,240	0,811
Bedi et al 1988	To exhaustion, cycling	0,432	0,270	0,073	-0,097	0,961	1,600	0,109
Booth et al. 1988	To exhaustion, cycling	0,117	0,317	0,101	-0,505	0,739	0,369	0,712
Merlini et.al. 2019	To exhaustion, running	-0,300	0,284	0,080	-0,856	0,256	-1,058	0,290
Hafedh et al. 2019	To exhaustion, running	-0,100	0,289	0,084	-0,667	0,467	-0,346	0,730
Mulphy et al. 2019, 2000	Time trial, running	0,015	0,250	0,063	-0,475	0,505	0,061	0,952
Mulphy et al. 2019, 4000	Time trial, running	-0,027	0,250	0,063	-0,517	0,463	-0,108	0,914
		0,047	0,038	0,001	-0,028	0,121	1,231	0,218



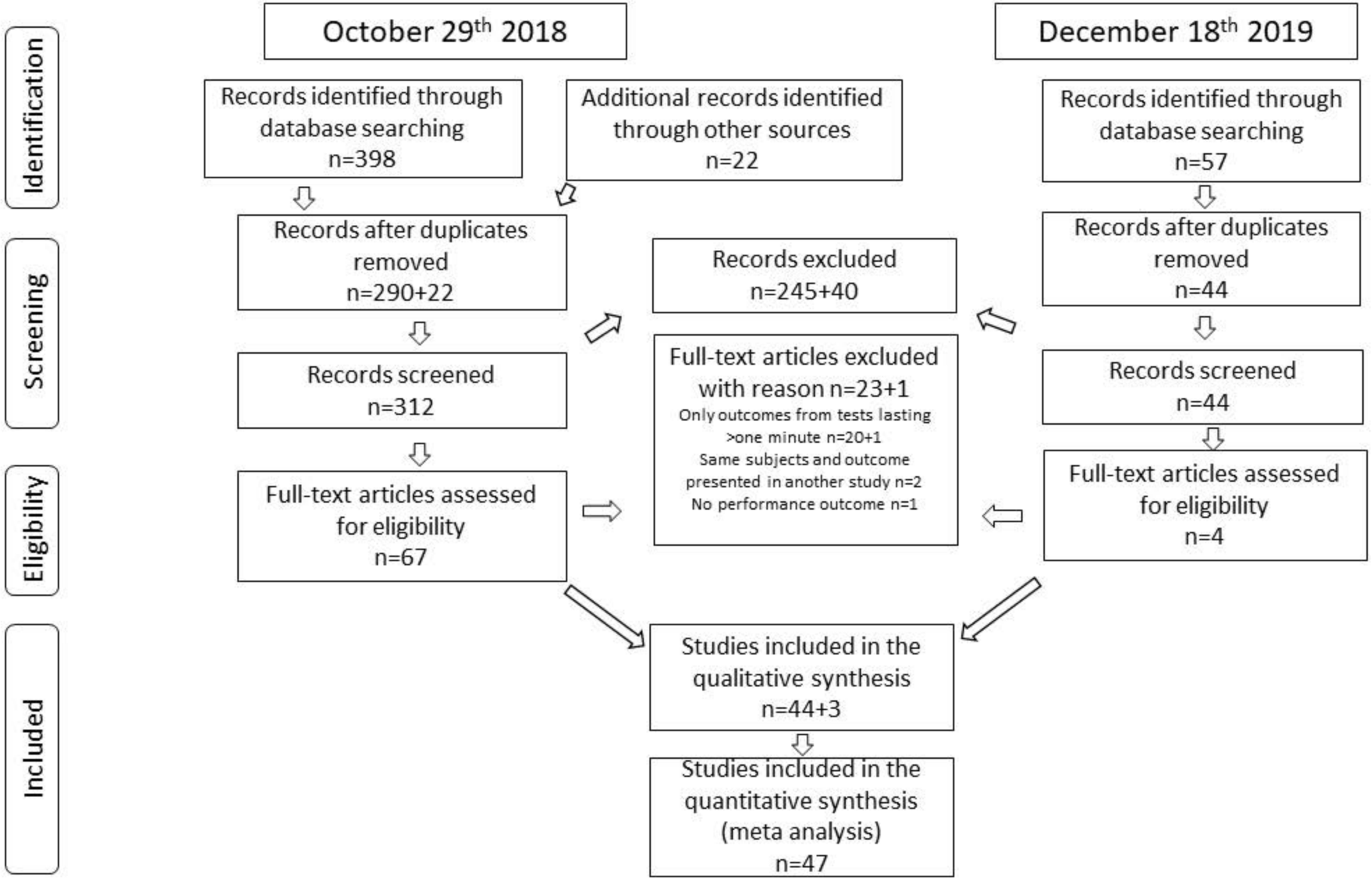


Figure 1. Flow chart of included studies as proposed by Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement 2009. [12]

Study name	Outcome	Statistics for each study						
		Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Ekstrom et al., 2018	Vo2-max	0,160	0,168	0,028	-0,169	0,488	0,952	0,341
Hostrup et al., 2016, Multiple weeks	Vo2-max	0,055	0,447	0,200	-0,821	0,932	0,124	0,901
Hostrup et al., 2015	Vo2-max	-0,667	0,484	0,235	-1,616	0,283	-1,376	0,169
Dickinson et al., 2014a	Vo2-max	-0,484	0,507	0,257	-1,457	0,529	-0,915	0,360
Elers et al. 2012	Vo2-max	0,000	0,333	0,111	-0,653	0,653	0,000	1,000
Beloka et al. 2011, 350	Vo2-max	0,000	0,302	0,091	-0,591	0,591	0,000	1,000
Beloka et al. 2011, 700	Vo2-max	0,229	0,320	0,103	-0,398	0,857	0,716	0,474
Andersen et al. 2009	Vo2-max	-0,094	0,379	0,143	-0,836	0,649	-0,247	0,805
Desconte et al 2008, 200	Vo2-max	-0,335	0,325	0,106	-0,972	0,302	-1,031	0,302
Desconte et al 2008, 800	Vo2-max	-0,083	0,317	0,100	-0,683	0,557	-0,199	0,842
Tjorhom et al. 2007	Vo2-max	-0,040	0,209	0,044	-0,448	0,369	-0,189	0,850
Riiser et al. 2006	Vo2-max	0,190	0,231	0,054	-0,263	0,644	0,822	0,411
Stewart et al. 2002, for	Vo2-max	-0,124	0,317	0,101	-0,746	0,498	-0,390	0,697
Stewart et al. 2002, sal	Vo2-max	0,015	0,316	0,100	-0,605	0,635	0,047	0,962
Carlsen et al. 2001	Vo2-max	0,000	0,204	0,042	-0,400	0,400	0,000	1,000
Sue-Chu et al. 1999	Vo2-max	0,000	0,354	0,125	-0,693	0,693	0,000	1,000
Sandsund et al. 1998	Vo2-max	0,345	0,364	0,132	-0,368	1,059	0,949	0,343
Larsson et al. 1997	Vo2-max	-0,134	0,225	0,050	-0,574	0,306	-0,597	0,550
Carlsen et al. 1997, salb	Vo2-max	-0,019	0,236	0,056	-0,481	0,443	-0,082	0,935
Carlsen et al. 1997, salm	Vo2-max	-0,019	0,236	0,056	-0,481	0,443	-0,080	0,936
Norris et al., 1996	Vo2-max	-0,077	0,259	0,067	-0,584	0,429	-0,299	0,765
Heir og Stemshaug., 1995	Vo2-max	-0,035	0,243	0,059	-0,510	0,441	-0,143	0,887
Unnithan et al. 1994	Vo2-max	-0,068	0,317	0,100	-0,689	0,552	-0,216	0,829
Morton et al. 1992	Vo2-max	-0,184	0,245	0,060	-0,664	0,295	-0,753	0,451
Meeuwisse et al. 1992	Vo2-max	-0,278	0,385	0,148	-1,034	0,477	-0,723	0,470
Bedi et al 1988	Vo2-max	0,186	0,260	0,068	-0,324	0,697	0,716	0,474
McKenzie et al. 1983, female	Vo2-max	0,066	0,633	0,400	-1,174	1,305	0,104	0,918
McKenzie et al. 1983, male	Vo2-max	0,028	0,671	0,450	-1,287	1,343	0,042	0,966
		-0,013	0,054	0,003	-0,118	0,092	-0,242	0,809