Effects Of Acute Metformin Intake On Physiological Parameters And Performance Before, During And After High-intensity Interval Training Of Swimmers

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Abstract

BACKGROUND: Metformin has shown potential to improve metabolic efficiency in short-intense efforts, prolonged-continuous efforts and recovery after supramaximal effort (i.e., phosphocreatine resynthesis). Metformin administration may be beneficial for high-intensity interval training session. The aim of the present study was to investigate the acute metformin administration effects on performance, rating of perceived exertion (RPE), blood lactate, blood glucose and neuromuscular parameters related to a swimming series of high-intensity.

METHODS: A double-blind, cross-over, randomized and placebo-controlled study was carried out. Seven healthy swimmers ingested MET (500mg) or placebo capsule on different days and performed a typical glycolytic session of high-intensity (i.e., lactate production objective). Bout time, RPE, neuromuscular parameters, blood lactate and glucose were analyzed. Cohen's *d* analysis with inference based on magnitude was adopted (confidence interval set at 90%).

RESULTS: There was an improvement in the performance of the second effort (72/28/0). Blood glucose during the series presented possible effects for decrease (96/3/1, 78/21/0, 93/6/1 after the 4th, 6th and 8th effort respectively) followed by a possible increase effect at the end of the series (1/6/93).Blood lactate showed a similar behavior of a possible decrease during the series (94/5/1, 60/40/0, 90/9/1 after the 4th, 6th and 8th effort respectively), followed by a possible increase effect at the end of the series (1/5/94). It was evidenced a possible increase effect on voluntary activation for lower limbs (91/8/1) without characterization of central and peripheral fatigue.

CONCLUSION: Metformin alters physiological parameters during and after maximal intermittent efforts in swimming without enhancement on performance.

Introduction

Metformin (MET) has been studied for more than 50 years as a treatment for type II diabetes (1). It has been well reported that metformin is an antihyperglycemic drug since it increases peripheral glucose uptake mediated by AMP-activated protein kinase (AMPK), increases the translocation of glucose transporters 4 (GLUT-4) (2, 3), induces glycolysis (4) and suppresses gluconeogenesis in the liver (5, 6). Furthermore, the metformin mildly inhibits ATP production in mitochondria, changes the AMP/ATP ratio, and as consequence glycolysis and phosphocreatine metabolism (7). Despite these interferences on the energy system in pathological or cell culture conditions, the ergogenic metformin effects on healthy subjects have been poorly studied (8-11), and the lack is even greater when related to the athletes (8, 9).

In healthy volunteers, a meta-analysis concluded that metformin ingestion increases the aerobic capacity (i.e., ventilatory anaerobic threshold), but not maximum oxygen uptake (12). The acute effect of metformin in healthy subjects not change the maximum oxygen uptake during a graded exercise test, but induces a lower level of blood lactate concentration on low-intensity exercise (10). Regarding performance, chronic metformin treatment (3 days) increased the muscle glycogen content, but was not

enough to improve the endurance performance (13). On the other hand, the evidence of acute metformin effect on high-intensity exercise still not clear. The acute administration of metformin enhanced the time to exhaustion at 110% of maximal oxygen uptake and the fast component of the Excess Post-Exercise Oxygen Consumption (EPOC fast), increasing the anaerobic alactic contribution (8). Thus, three positives shreds of evidence on acute metformin and exercise can be postulated in healthy individuals to date: i) increases the aerobic capacity; ii) induces a lower level of blood lactate concentration on low-intensity exercise iii) increases time to exhaustion at 110% of maximal oxygen uptake; iv) increases the phosphocreatine resynthesis. In this context, it is expected in athletes that acute metformin may be beneficial for maintaining efforts during training series in order to improve EPOC fast, phosphocreatine resynthesis and recovery.

Swimming sport is characterized by different efforts magnitudes either performed continuously (competition context) or at different distances intermittently (training context). In some specific training series, the swimmer can be submitted to high-intensity intermittent efforts, in which anaerobic metabolism is predominant during bouts and the aerobic pathway is required for recovery during the recovery intervals (14). In addition to the possible benefits inherent in the aforementioned metabolic pathways of metformin, a possible benefit for swimming training would be related to drug's capacity to potentiate blood glucose uptake and maintenance through the muscle (6, 15), a response that may attenuate peripheral fatigue development (16) (i.e., reduction of energy substrates) and consequently increases tolerance in high-intensity interval exercises.

Thus, the aim of this study was to investigate if the acute effects of metformin administration improve the swimmers' performance during high-intensity training. In order to explain the expected enhancement in performance, this study also proposed to verify the metformin effects on neuromuscular fatigue and physiological parameters (blood glucose and lactate).

Materials & Methods

Participants

Seven male swimmers with age of 21 ± 3 height of 179.9 ± 7.9 cm, weight of 72.7 ± 7.7 kg who were regularly training in the last two months prior to the study and had at least 2 years of experience (university and regional level) participated in this study. Their 50m front crawl performance represents 66.1% of the 25-meters pool World Record.

Experimental design

A randomized double-blind cross-over model was used and evaluations performed in two different days. Participants ingested a capsule containing 500 mg of metformin or 500 mg of the placebo 90 minutes prior to the exercise (the final 15 minutes of the absorption time counted on the heating for the series) (8). The experimental and placebo situations were separated by 72 hours, regarding the MET wash-out (17, 18).

Participants were instructed to ingest the capsule with a light feed (e.g., fruits, cereals and foods less greasy) and not to use coffee, energy drinks, supplements or alcoholics 24 hours prior to the evaluations. Before each evaluation, swimmers performed a 15 minutes standardized warm-up (coordinated by the head coach) which was repeated identically for the two evaluation days.

The chosen typical series had 10 maximal bouts of 50 m with 3-minute interval (Vel-2). Time and Rating of Perceived Exertion (RPE) were monitored each bout during the series. Blood lactate ([Lac^-]) and glucose ([Gli]) were measured before, during the series and in the recovery. In addition, the neuromuscular fatigue of the elbow extensor and knee extensor muscles was evaluated with electrical stimulation technique, being the procedure applied previously and immediately after the efforts. One week before the experiment all participants were familiarized with the neuromuscular techniques. The familiarization consisted of perform maximal voluntary contraction (MVC) with electrical stimuli on the portion analyzed (elbow extensor and knee extensor muscles) in an isometric force analyzing chair.

Metformin intake

A 99.6% pure metformin was administered, while placebo was composed of aerosil (1%), microcrystalline cellulose (10%) and maize starch (89%). Both contents were encapsulated in a laboratory specialized, which used rapid release capsules (size: 23.3 mm, diameter: 8.18 mm, volume: 95 ml, capacity 950 mg). Capsules intake were administered approximately 90 minutes prior to each typical swimming session of high-intensity intermittent training (i.e.Vel-2)(14). There were no reports of adverse reactions or sensations after capsules intake.

Collection and analysis of blood samples

All the procedure involving blood samples was carried out by the responsible researchers, which preceded 70% alcohol asepsis for posterior manual lobe puncture with a stainless-steel sterilizing lancet (Wiltex), sterilized with single use Gamma Ray. Twenty-five microliters (25 µL) of blood were punctured from the earlobe into previously calibrated and heparinized capillary tubes. Samples were immediately deposited in 1.5 ml Eppendorf tubes containing 50 µl Sodium Fluoride (NaF-1%) for further analysis on an electrochemical lactimeter (Yellow Springs Instruments Model 2300, Ohio, USA).

Samples of [Lac^-] e [Gli] were collected at rest, each 2 bouts (during the series), immediately after the end of exercise and during the recovery (3, 5, 7 e 10 minutes after the last bout).

Typical swimming training

Consisted of 10 maximum bouts of 50 m, separated by 3 min of passive interval, this series is in accordance with the models proposed by Maglischo et al. (14) who characterized this training series with a anaerobic training or velocity training model due to the metabolic predominance of the efforts and the short interval. This model was chosen due to the characteristic loss of performance between efforts (19).

Neuromuscular fatigue measurement

Neuromuscular fatigue was assessed by the measurement of muscle strength and stimulation in the analyzed muscle. The electrical stimulation was assessed by a prototype of an electric stimulator developed for this purpose with a capacity of 200 V peak-to-peak (Bioestimulador, Insight®, RibeirãoPreto – Brazil). All efforts were performed against load cells positioned towards the ankle and the wrist of the participant (CSR-1T, MK Control®, São Paulo, Brazil) and the data acquisition was done in Labview 2015 environment (National Instruments®) with 1000Hz frequency.

Neuromuscular parameters of the extensor muscles of the knee and elbow were measured by double electric pulses (duration of 1 ms and intervals of 10 ms) applied to the muscular belly. Two round electrodes (3 cm diameter) were used on triceps brachii (EletrodosAutoadesivos CF3200 ValuTrode®) (20) and two 5 x 9 cm electrodes were used on quadriceps femoris (EletrodosAutoadesivos CF5090 ValuTrode®).

After the trichotomy and skin cleaning by abrasion and alcohol 70% of the anatomical points of each musculature, the electrodes were bound in the regions suitable to muscle stimulation. For elbow extensor muscles neuromuscular fatigue evaluation, the cathode electrode was positioned at the end of the long head of the triceps brachii muscle, near the lowest border of the deltoid muscle, and the anode electrode was positioned in the distal tendon of the triceps brachii, near to the olecranon (21). For knee extensor muscles neuromuscular fatigue evaluation, the cathode was positioned in the upper limit of the vastuslateralis muscle and the anode positioned at the lowest limit of the same musculature (22).

Muscular strength was measured in a chair built to measure the elbow and knee extension of isometric strength. The participant remained with the trunk secured to the chair so that the dominant arm (intended for evaluation) remained relaxed and parallel to the trunk with an elbow flexed in 90º. For the power assessment of the lower limbs, the participant sat with his dominant leg at 90º of knee flexion. The load cells, used to measure the force by deformation during the accomplishment of muscular contraction, were fastened the respective bracelet/ankle and fixed in inextensible adjustable handles that allowed the measurement of the force exerted by the extension of the knee and elbow.

In the knee extensor neuromuscular fatigue evaluation two MVC and a progressive electrostimulation test (PET) was performed as first and second step. The PET has the purpose of finding the best intensity for stimulation and was composed of a progressive electrical pulse with the analyzed muscles relaxed (10 mA increase at each stimulus). The third step consisted of 5-seconds duration MVC (moment which the peak force - PF_K was verified) with an electrical stimualtion during the MVC and other stimulation 3-second at the end of the MVC(with the relaxed muscles)(23). The difference between PF_K and the force evocated was Twitch Superimposed (TS_K) and the difference between relaxed force and force evocated was Twitch Potentiated (TP_K). The voluntary activation of the knee extensor muscles was calculated with a formula proposed by Allen (24) (Equation 1).

$$\text{Equation 1. } VA_K\ (\%) = [1 - (TS_K / TP_K)] * 100$$

VA – voluntary activation; TS_K – Twitch Superimposed (represents the difference between maximum force rate and stimulation force evocate); TP_K – Twitch Potentiated (force evocated with relaxed muscles after maximum voluntary contraction test).

The neuromuscular fatigue evaluation for elbow extensor muscles was performed in two stages, the accomplishment of a PET, to establish the best stimulation intensity, and in a second moment a single pulse was applied with the relaxed muscles. The difference between relaxed force and force evocated was Twitch Control (TC_{TRI}).

Statistical analysis

The data were processed through version 20.0 SPSS (IBM corp©, Armonk, New York, USA). Magnitude-based inference analysis was used as a mathematical procedure between subjects (MET vs Placebo) to establish effect size, confidence interval (set at 90%) and qualitative inference in order to identify the effect of metformin for “decrease”, “increase” or “trivial” effects (25).

Effect probabilities were identified according to their propensity to be substantially positive, negative (> 5%) or trivial. Thus, the changes were qualitatively assessed as follows: <1% = Highly unlikely; 1% -5% = Very unlikely; 5% -25% = Unlikely; 25% -75% = Slight possibility; 75% -95% = Possible; 95% -99% = High possibility (26). Due to data density and aiming a better result compiled, blood lactate, blood glucose, RPE and performance had their statistics ordered for moment effect (fixed values) while the neuromuscular parameters were ordered for time effect (Δ%).

Results

The statistical procedure indicated that at rest the intake of metformin might have a possible effect of reduced blood glucose (86/13/1). After the 4º, 6º and 8º bouts, there were possible effect of reduce blood glucose (95/3/1, 78/21/0, 93/6/1 respectively), whereas at the end of the swimming training was found a possible increasing effect (1/6/93). Metformin shows a possible effect of reduced blood glucose on the 5-minutes recovery (92/7/1) (Figure 1).

Metformin shows no effects on resting blood lactate. However, it was possible to find possible decrease effects at the end of the 4º, 6º and 8º bout (94/5/1, 60/40/0, 90/9/1 respectively). Metformin intake evidenced a possible increase effect after the last bout (1/5/94). A possible reduce effect occurred on the 5-minutes recovery (91/8/1) (Figure 2).

Statistical analysis applied to neuromuscular parameters indicates a possible increase effect on VA_K (91/8/1) and a possible decrease effect on TP_K (1/8/91) (Table 1).

Par.	Placebo		Δ%	Metformin		Δ%	Effect size ± confidence interval (90%)	Increase/ Trivial/ Reduce	Inference
	Pre	Post		Pre	Post				
TC_{TRI} (N)	26,4 ± 6,8	22,5 ± 9,8	-15,5	23,5 ± 6,8	18,6 ± 6,8	-20,2	-0,08 ± 0,06	0/99/1	Very likely trivial
TS_K (N)	42,1 ± 34,3	38,2 ± 32,3	-11,1	49,1 ± 46,1	35,3 ± 36,2	-28,4	-0,52 ± 0,42	1/8/91	Likely -ive
TP_K (N)	261,8 ± 20,5	220,6 ± 62,7	-15,6	272,6 ± 37,2	230,5 ± 47,1	-15,3	0,02 ± 0,02	0/100/0	Most likely trivial
PF_K (N)	645,3 ± 95,1	602,1 ± 105,9	-6,7	659,1 ± 48,0	668,8 ± 104,9	4,1	0,06 ± 0,05	0/100/0	Most likely trivial
VA_K (%)	86,4 ± 11,7	86,1 ± 10,8	-0,3	84,7 ± 11,4	88,2 ± 10,7	3,5	0,53 ± 0,42	91/8/1	Likely +ive

Table 1. Neuromuscular parameters in mean ± standard deviation before and after the typical series in two different conditions (placebo and metformin intake). Difference between conditions, effect size, confidence interval and qualitative inference of the magnitude-based inference analysis.

Par. – Parameter; Δ% - variation percentile; TC_E – Twitch Control of elbow extensor muscles; VA_K – Voluntary activation of knee extensor muscles; TS_K – Twitch Superimposed applied on quadriceps femoris; TP_K –Twitch Potentiated of knee extensor muscles; PF_K – Peak force of knee extensor muscles. Despite the similar behavior in each condition, metformin intake showed a possible reduce effect on RPE response after the first bout (70/29/1) and possible increase effect after the seventh effort (0/40/60) (Figure 3).

A general overview indicates that MET does not improve performance in a large scale. Only the second bout shows a possible decrease effect on bout time (72/28/0) (Figure 4).

Discussion

The present study is the first to investigate the acute metformin intake in athletes on performance and physiological parameters during a typical high-intensity exercise series. We found that metformin does improve performance only on the second bout in a swimming typical series. Physiological and neuromuscular results can explain and support other implications about metformin intake.

Metformin is mainly related to the maintenance of blood glucose (7), thus, it was expected that in pre-training conditions with healthy subjects there were no evident signs of the MET alterations for lactate and blood glucose (10). Although the present study evidences that metformin intake during rest may have a blood glucose lowering effect in swimmers, it is important to emphasize that this effect may have been due to an increase in the blood glucose depletion provided by the effort made in the warm-up.

Metformin intake showed a possible reduced effect on blood glucose followed by a sudden increase during the recovery time (Figure 1). Among the few studies investigating the metformin effects on exercise involving healthy participants, Johnson et al. (10) evidenced that during an aerobic exercise the blood glucose has a slight tendency to reduce when associated with metformin intake, however, this evidence was not significant.

During recovery, it was also possible to note that metformin promoted an "early" recovery behavior by blood glucose when compared to placebo (Figure 1), a situation possibly resulting from a higher uptake of glucose (2, 27, 28). Johnson et al. (10) investigated the metformin effects for healthy subjects, and showed that after an aerobic exercise the metformin had slightly lowered blood glucose levels than the placebo group, however, no difference was found. The difference between the responses for the two studies may be a consequence of the group training status, since athletes have a capillary and blood flow greater than sedentary ones facilitating glucose transport and consequently uptake (29, 30).

Metformin capability to increase the glycolysis rate, consequently increasing lactate production, has already been demonstrated in studies using cell cultures and rats (4, 31). However, as found in the study of Learsi et al. (8), which involved the metformin and exercise in healthy subjects, the present study did not present alterations of this parameter during the rest period.

Despite its behavior at rest, during the typical series, metformin showed a tendency to reduce lactate production from the 2nd to the 8th bout followed by an expressive increase at the end of the series (Figure 2),corroborated by the study of Johnson et al. (10) who verified a similar effect in healthy subjects performing high-intensity exercises after MET uptake. Considering that [Lac^-] after supramaximal efforts are associated with glycolysis index (8, 32) and that the typical series model was composed of maximal 50 m maximum efforts, the present study evidence suggest that there was glycolysis reduction during the efforts, contrasting a smaller use of the lactic anaerobic pathway (33).

Lower [Lac^-], that characterize discrete decrease of glycolysis (Figure 2) during the series, may indicate that another energetic pathway would have benefited from this metabolic change occurred with similar performance response (Figure 4). In this scenario, the absence of pathway analysis by oxygen consumption limits final conclusions regarding the relationship between the metabolic pathways triggered during and after the typical series. However, a study involving oxygen consumption analysis in healthy individuals after metformin intake evidenced that the anaerobic alactic pathway was potentialized during a supramaximal effort (8).

Learsi et al. (8) showed that metformin improves performance in a supramaximal effort without change in [Lac^-] when compared with placebo situation, suggesting the increase of the alactic anaerobic contribution. Despite the intensity of the bouts during the series, the only performance improvement occurred in the second bout accompanied by reduction of [Lac^-]. Analyzing this situation, and considering the aerobic participation in the series intervals, it is strongly hypothesized that metformin plays a different role in continuous efforts.

Another important consideration is that reducing the [Lac^-] has not been a facilitator for performance improvement, since acidosis increases the hydrogen ion accumulation (H^+) reducing the efficiency of the creatine-phosphate system (34). If metformin significantly increased ADP and phosphocreatine (35) resulting in increased creatine kinase, consequently favoring the alactic anaerobic pathway (8), the 50 m performance would have a discreet decrease during the series compared with placebo situation. This hypothesis would be confirmed for two reasons: due to the time of effort in agreement with the production of the energy pathway (50 m bout presented an average time of 30s in this study), and the rest between the bouts (3min) favored the complete resynthesizes of the alactic anaerobic pathway (34). Although these results indicate that the aerobic pathway may undergo alterations after metformin intake, Braun et al. (9) did not find differences in the parameters related to the oxidative pathway.

At the end of the series, a possible increase effect of [Lac^-] after metformin intake (1/5/94) was found, while in the 5th minute of the recovery the MET intake had a possible decrease effect on the same parameter (91/8/1). These responses contrast a different behavior after ingestion of the two compounds (evident in Figure 2), which the accumulation and removal process (until the 7th minute) of [Lac^-] became "faster" for metformin condition. This behavior becomes even more evident when it is observed that the peak of lactate accumulation after metformin intake occurred at the end of the typical series, whereas after the placebo intake the same physiological situation occurred at the 5th minute (Figure 2).

Although Johnson et al. (10) not investigating the [Lac^-] recovery in the short-term, it has been shown that after more than 30 min the [Lac^-] recovery is more efficient with the metformin intake. Emphasizing the metformin ability to mediate metabolic changes via AMPK (36), it is suggested that the [Lac^-] reduction at the end of an effort may be associated with an increase in the aerobic contribution, however, further investigations involving training in healthy people are required to study the [Lac^-] recovery kinetics after metformin intake (10).

Considering the [Lac^-],participants and the effort performed on the series (10 bouts with maximum intensity), it is possible that this scenario facilitates the establishment of neuromuscular fatigue (37). Due to the increased blood glucose uptake promoted by metformin, the present study had the complementary objective to verify if the metformin is able to promote better support of the efforts during the series (2). The results indicated that the TS_K parameter suffered a possible reduction effect, demonstrating a recovery from the central pathway or, as may have happened in this case, the metformin may have favored the central pathway before the series beginning (38).

In addition to the central pathway recovery, the typical series performed was not able to cause peripheral fatigue in the elbow extensor and knee extensor muscles, considering that the two parameters related to such fatigue (TC_{TRI} and TP_K respectively) did not present a significant effect size (38) (Table 1).

The literature lacks research on neuromuscular fatigue in swimming training models; however, studies have shown that a 100 m maximal bout on swimming can generate neuromuscular fatigue and modify the electrical response of upper limbs (39) as well as a 200 m maximum effort (40). There is evidence that the peripheral fatigue process affects lower limbs on a smaller scale in swimming, since much of the swimming propulsion is concentrated in upper limbs (40).

Instead to the above mentioned maximum continuous efforts, the absence of peripheral fatigue from the typical series can be explained by its intermittent characteristics, since the intermittent during a task that requires high-strength levels has the capacity to present lower levels of neuromuscular fatigue for muscles such as the triceps brachii when compared to continuous exercises (41).

The possible increase effect of VA_K corroborates the TS_K reduction parameter, indicating a facilitation by the metformin for post-activation potentiation of lower limbs, considering that lower limb are less required in swimming and consequently more spared of the fatigue process (40). In addition, metformin increases the recruitment of GLUT-4 (2, 3) which has the function of increasing intramuscular calcium during contraction (42), fundamental for post-activation potentiation to occur and increase muscle voluntary activation (43).

Another potential effect that has been studied for metformin is its ability to inhibit the Mammalian Target of Rapamycin (mTOR) pathway resulting in increased AMPK activity (44). The mTOR pathway plays an important role as a signal of the nervous system under chronic pain conditions, so it is possible that metformin may attenuate sensation of pain (44). Considering the sensations of performing a high-intensity exercise as in the typical series, it was evidenced in two possible (and slight) effects of this drug (after the 1st and 7th bout), indicating that the participants had different fatigue sensations. However, the representation of these scattered results in the general context of the series (two slight effects in 10 moments - Figure 3) are inconclusive and possibly this effect is not associated with fatigue sensations.

Finally, despite the possible effects found for neuromuscular fatigue, blood lactate and glucose, metformin promoted a single performance improvement within the typical series (2nd effort - Figure 4). Due to this was the first study involving metformin for athletes in a typical series in swimming, there is no ecological comparative scientific evidence. Even though the study of Learsi et al. (8) demonstrated a performance improvement in a high-intensity exercise, it is important to note that in the present study, which also used high-intensity efforts, used intermittent efforts with shorter durations. Therefore, the energetic metabolism involved in the task may be different.

Learsi et al. (8) evidenced that the anaerobic alactic pathway could be favored without altering the total anaerobic capacity, however, this would explain a possible improvement in the performance of high-

intensity and short duration efforts. In the present study the series used could be strongly favored by the anaerobic alactic pathway in the first efforts, however, no consistent results were found in a general view.

It is concluded that metformin alters metabolic functions in healthy individuals during intermittent exercise and recovery. The results showed that metformin was able to improve the central pathway response before the beginning of the exercise and to increase the voluntary activation; consequently, this neuromuscular alteration increases the post-activation potentiation of skeletal muscle. The neuromuscular mechanism in addition to metformin should still be studied in different situations with greater time-course.

Finally, it was possible to conclude that, besides not altering the fatigue sensation of the participants, the metformin improved performance only in the second bout, but not in the other series on a typical swimming training. In neuromuscular aspects, metformin has effects that can be interpreted as beneficial in a sports setting.

Declarations

- Ethics approval and consent to participate

All experiments were previously approved by the University Ethics Committee (Process number: 79144017.7.0000.5659) and conducted in accordance to the Declaration of Helsinki.

- Consent for publication

Not applicable.

- Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests or financial ties to disclose and have not received financial support from any companies or manufacturers.

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- Authors' contributions

The study was designed by MP and MSN; data were collected and analyzed by MSN, TBA; critical procedure support provided by VLA, JAC, GGA and MP; data interpretation and manuscript preparation were undertaken by MSN, GGA, and MP.

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References

1. Sterne J. Pharmacology and mode of action of hypoglycaemic guanidine derivatives. Oral hypoglycaemic agents. 1969:193-245.
2. Bailey CJ, Turner RC. Metformin. New England Journal of Medicine. 1996;334(9):574-9.
3. Hundal H, Ramlal T, Reyes R, Leiter L, Klip A. Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. Endocrinology. 1992;131(3):1165-73.
4. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, et al. Metformin improves healthspan and lifespan in mice. Nature communications. 2013;4:2192.
5. Chen SC, Brooks R, Houskeeper J, Bremner SK, Dunlop J, Viollet B, et al. Metformin suppresses adipogenesis through both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. Molecular and Cellular Endocrinology. 2016.
6. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature. 2013;494(7436):256-60.
7. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation. 2001;108(8):1167-74.
8. Learsi S, Bastos-Silva V, Lima-Silva A, Bertuzzi R, De Araujo G. Metformin improves performance in high-intensity exercise, but not anaerobic capacity in healthy male subjects. Clinical and Experimental Pharmacology and Physiology. 2015;42(10):1025-9.
9. Braun B, Eze P, Stephens BR, Hagobian TA, Sharoff CG, Chipkin SR, et al. Impact of metformin on peak aerobic capacity. Applied Physiology, Nutrition, and Metabolism. 2008;33(1):61-7.
10. Johnson S, Robert C, Bell G, Bell R, Lewanczuk R, Boulé N. Acute effect of metformin on exercise capacity in active males. Diabetes, Obesity and Metabolism. 2008;10(9):747-54.
11. Malin SK, Stephens BR, Sharoff CG, Hagobian TA, Chipkin SR, Braun B. Metformin's effect on exercise and postexercise substrate oxidation. International journal of sport nutrition and exercise metabolism. 2010;20(1):63-71.
12. Das S, Behera SK, Srinivasan A, Xavier AS, Selvarajan S, Kamalanathan S, et al. Effect of metformin on exercise capacity: A meta-analysis. Diabetes research and clinical practice. 2018;144:270-8.

13. Scalzo RL, Paris HL, Binns SE, Davis JL, Beals JW, Melby CL, et al. Ergogenic properties of metformin in simulated high altitude. Clinical and Experimental Pharmacology and Physiology. 2017;44(7):729-38.
14. Maglischo EW, do Nascimento FG. Nadando ainda mais rápido: Manole; 1999.
15. Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clinical science. 2012;122(6):253-70.
16. Ament W, Verkerke GJ. Exercise and fatigue. Sports Medicine. 2009;39(5):389-422.
17. Tucker G, Casey C, Phillips P, Connor H, Ward J, Woods H. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. British journal of clinical pharmacology. 1981;12(2):235-46.
18. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong J, et al. Clinical pharmacokinetics of metformin. Clinical pharmacokinetics. 2011;50(2):81-98.
19. Deminice R, Gabarra L, Rizzi A, Baldissera V. Série de treinamento intervalado de alta intensidade como índice de determinação da tolerância à acidose na predição da performance anaeróbia de natação. Revista Brasileira de Medicina do Esporte. 2007;13(3):185-9.
20. Lampropoulou SI, Nowicky AV, Marston L. Magnetic versus electrical stimulation in the interpolation twitch technique of elbow flexors. Journal of sports science & medicine. 2012;11(4):709.
21. Cheng AJ, Rice CL. Voluntary activation in the triceps brachii at short and long muscle lengths. Muscle & nerve. 2010;41(1):63-70.
22. Botter A, Oprandi G, Lanfranco F, Allasia S, Maffiuletti NA, Minetto MA. Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning. European journal of applied physiology. 2011;111(10):2461.
23. Milioni F. Associações entre, índices fisiológicos e fadiga neuromuscular com padrões de deslocamento e desempenho do chute de finalização no futsal. Rio Claro, SP: Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP; 2014.
24. Allen GM, Gandevia SC, McKenzie DK. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle Nerve. 1995;18(6):593-600.
25. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. International journal of sports physiology and performance. 2006;1(1):50-7.
26. Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Medicine+ Science in Sports+ Exercise. 2009;41(1):3.
27. Bailey C, Mynett K, Page T. Importance of the intestine as a site of metformin-stimulated glucose utilization. British journal of pharmacology. 1994;112(2):671-5.
28. Sambol NC, Chiang J, O'conner M, Liu CY, Lin ET, Goodman AM, et al. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. The Journal of Clinical Pharmacology. 1996;36(11):1012-21.
29. Ebeling P, Bourey R, Koranyi L, Tuominen JA, Groop LC, Henriksson J, et al. Mechanism of enhanced insulin sensitivity in athletes. Increased blood flow, muscle glucose transport protein (GLUT-4)

concentration, and glycogen synthase activity. The Journal of clinical investigation. 1993;92(4):1623-31.

30. Holmang A, Bjorntorp P, Rippe B. Tissue uptake of insulin and inulin in red and white skeletal muscle in vivo. American Journal of Physiology-Heart and Circulatory Physiology. 1992;263(4):H1170-H6.
31. Ouyang J, Parakhia RA, Ochs RS. Metformin activates AMP kinase through inhibition of AMP deaminase. Journal of Biological Chemistry. 2011;286(1):1-11.
32. Vytla VS, Ochs RS. Metformin increases mitochondrial energy formation in L6 muscle cell cultures. Journal of Biological Chemistry. 2013;288(28):20369-77.
33. Wasserman K. The anaerobic threshold measurement to evaluate exercise performance. American Review of Respiratory Disease. 1984;129(2P2):S35-S40.
34. Gaitanos GC, Williams C, Boobis LH, Brooks S. Human muscle metabolism during intermittent maximal exercise. Journal of applied physiology. 1993;75(2):712-9.
35. Wallimann T. Bioenergetics: Dissecting the role of creatine kinase. Current Biology. 1994;4(1):42-6.
36. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology. 2003;144(12):5179-83.
37. Figueiredo P, Zamparo P, Sousa A, Vilas-Boas JP, Fernandes RJ. An energy balance of the 200 m front crawl race. European journal of applied physiology. 2011;111(5):767-77.
38. Gandevia S. Spinal and supraspinal factors in human muscle fatigue. Physiological reviews. 2001;81(4):1725-89.
39. Stirn I, Jarm T, Kapus V, Strojnik V. Evaluation of muscle fatigue during 100-m front crawl. European Journal of Applied Physiology. 2011;111(1):101-13.
40. Figueiredo P, Rouard A, Vilas-Boas JP, Fernandes RJ. Upper-and lower-limb muscular fatigue during the 200-m front crawl. Applied Physiology, Nutrition, and Metabolism. 2013;38(7):716-24.
41. Bilodeau M. Central fatigue in continuous and intermittent contractions of triceps brachii. Muscle & nerve. 2006;34(2):205-13.
42. Wright DC. Mechanisms of calcium-induced mitochondrial biogenesis and GLUT4 synthesis. Applied Physiology, Nutrition, and Metabolism. 2007;32(5):840-5.
43. Sweeney H, Bowman BF, Stull JT. Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. American Journal of Physiology-Cell Physiology. 1993;264(5):C1085-C95.
44. Kiałka M, Doroszewska K, Janeczko M, Milewicz T. Metformin–new potential medicine in pain treatment? Przeglad lekarski. 2017;74(2):81-3.

Figures

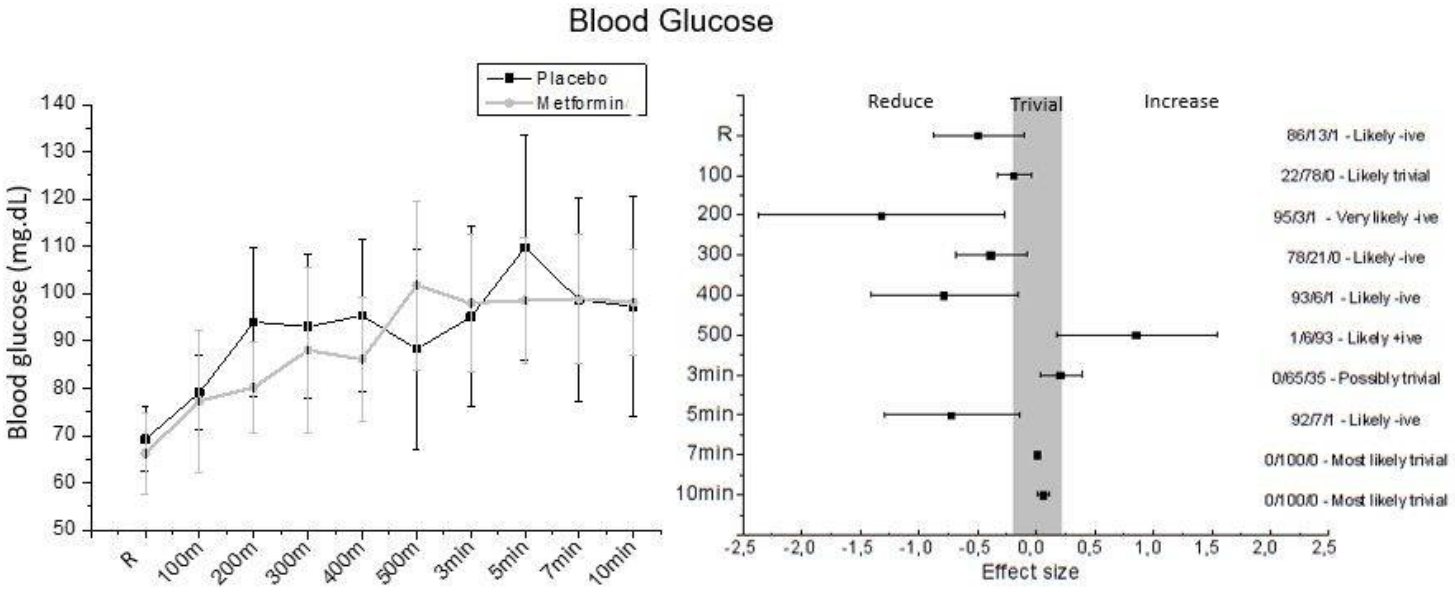
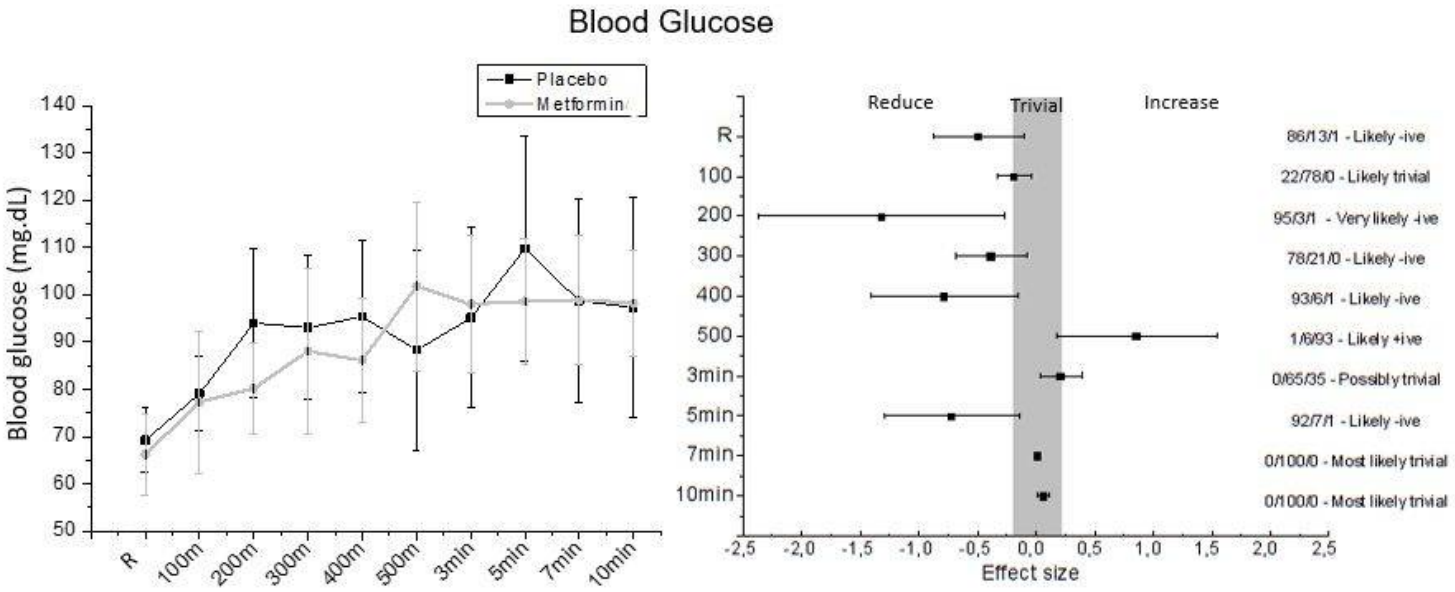


Figure 1

Left figure represents the real behavior of blood glucose; R - Rest. Right figure shows magnitude-based inference analysis between-conditions. Trivial area (grey) threshold is -0.2 to 0.2 and bars indicate ± 90% confidence limits.



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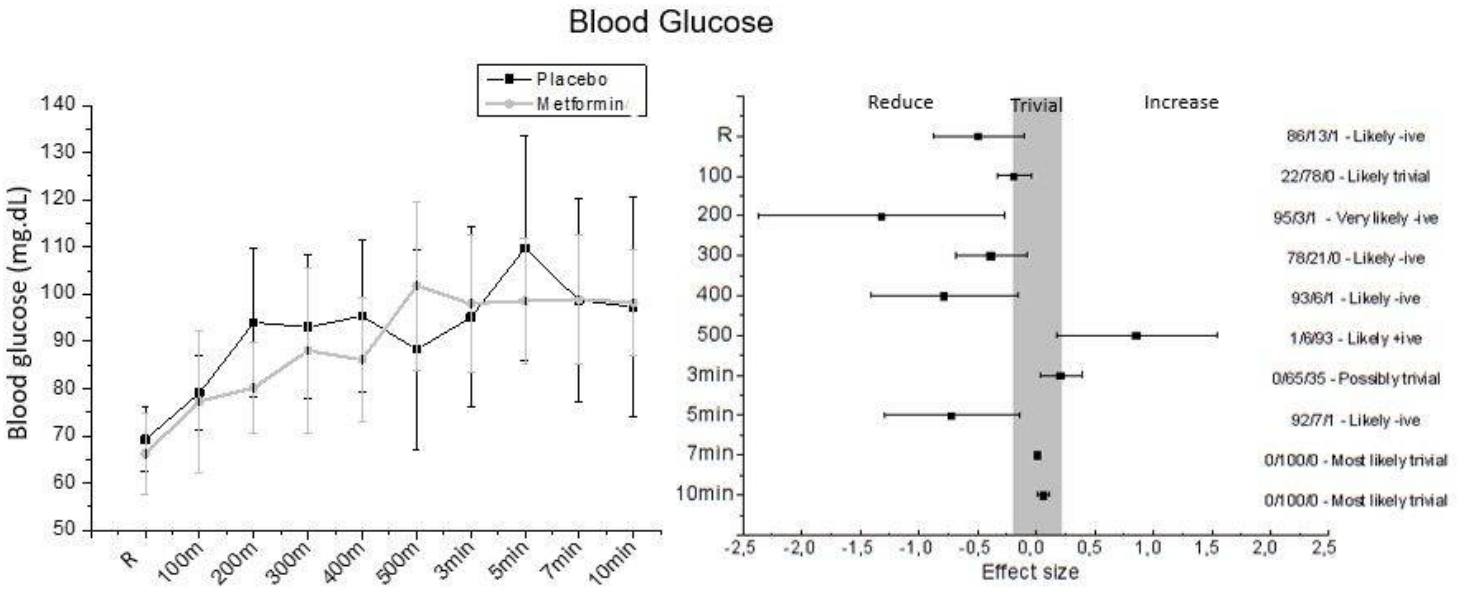

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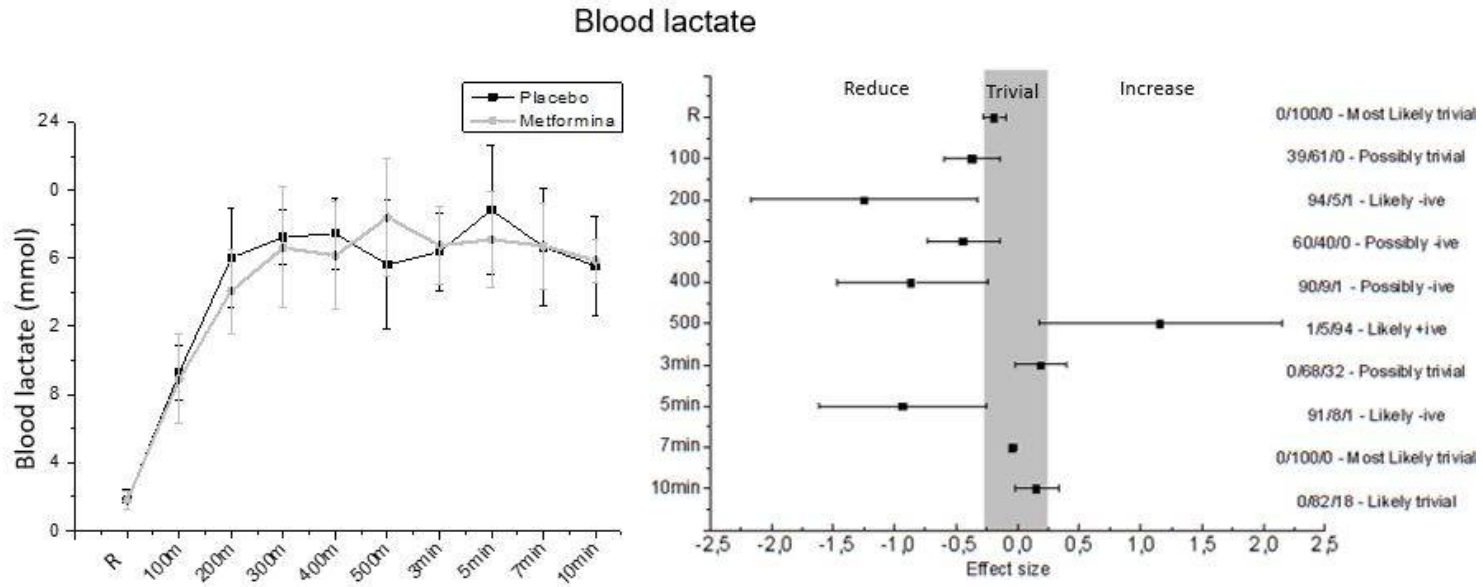
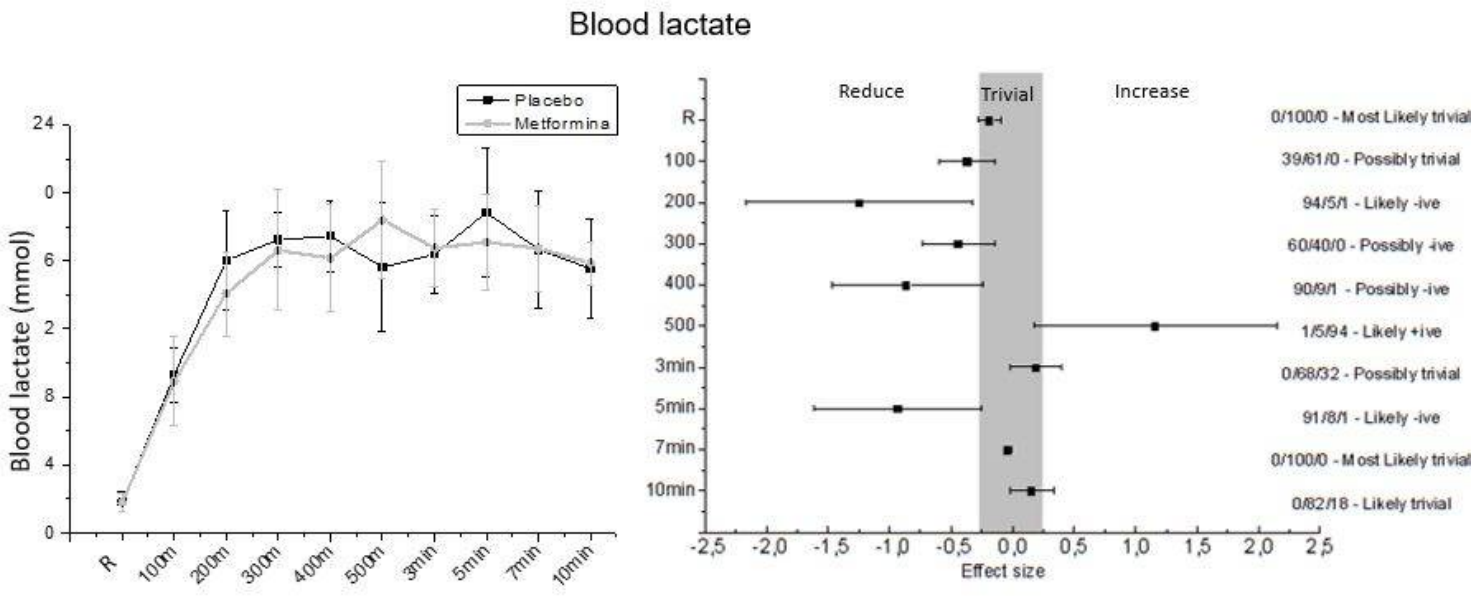


Figure 2

Left figure represents the real behavior of blood lactate; R - Rest. Right figure shows magnitude-based inference analysis between-conditions. Trivial area (grey) threshold is -0.2 to 0.2 and bars indicate ± 90% confidence limits.



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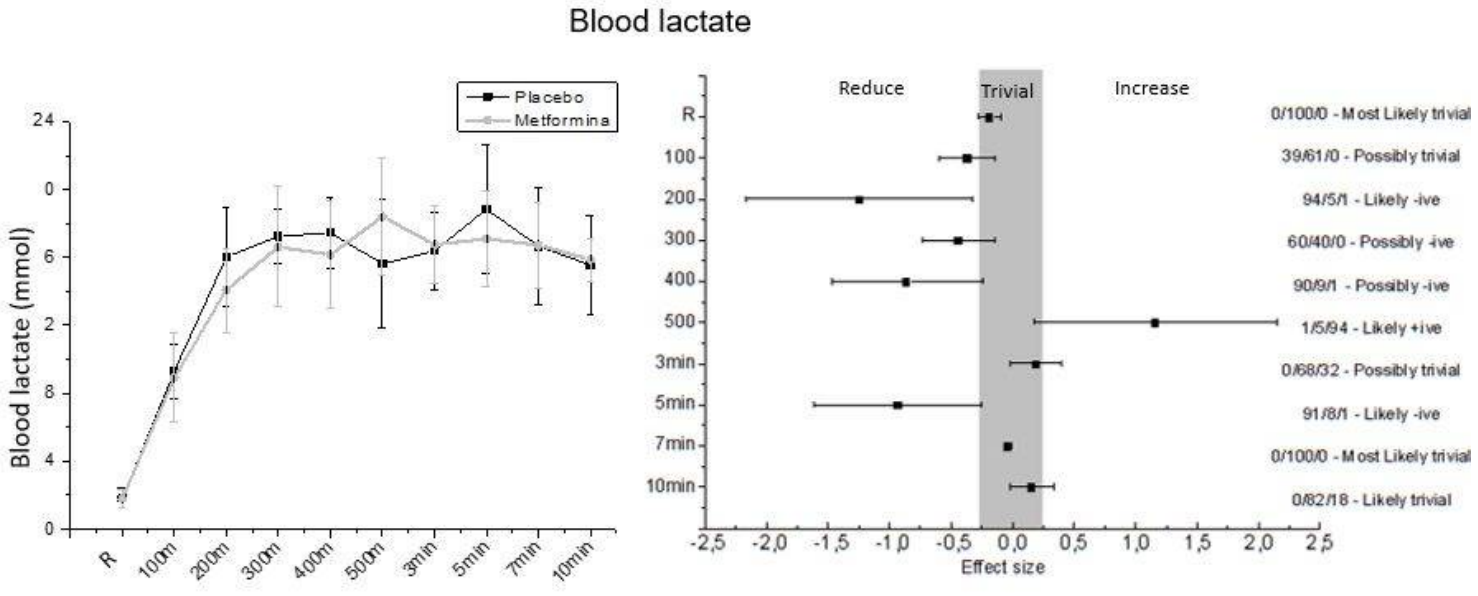

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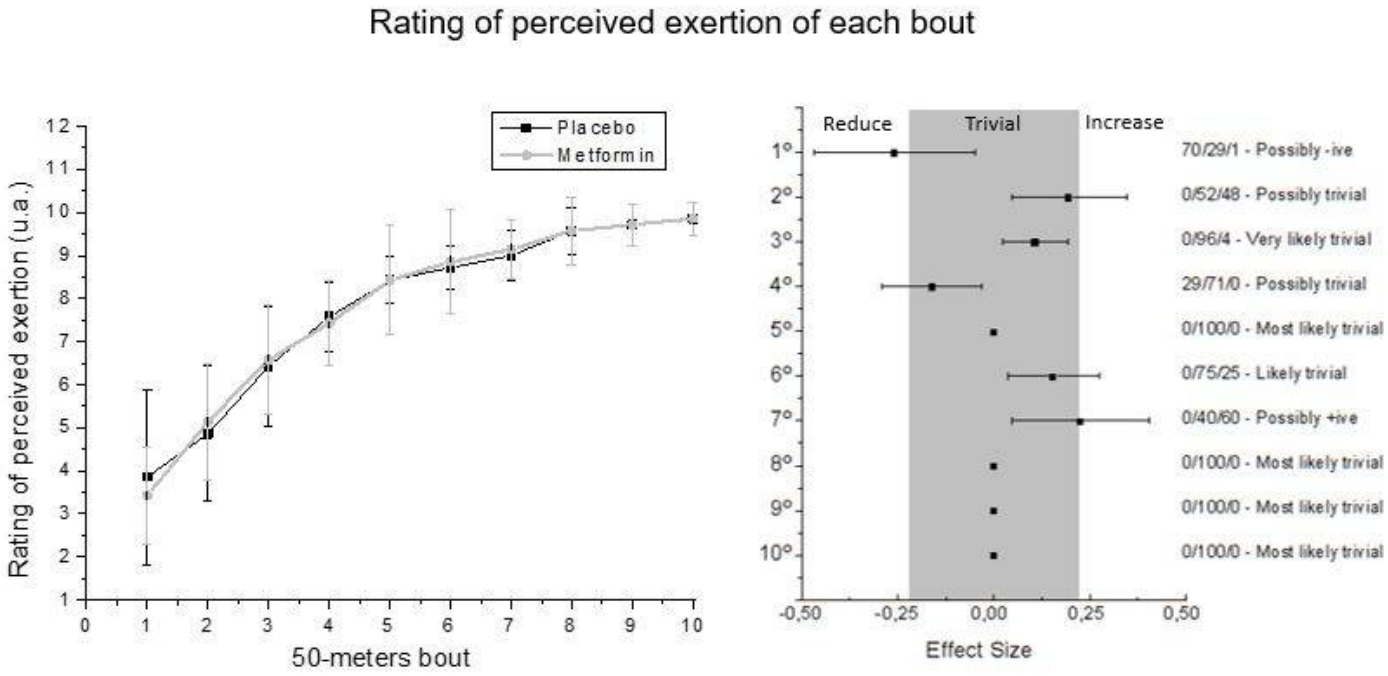
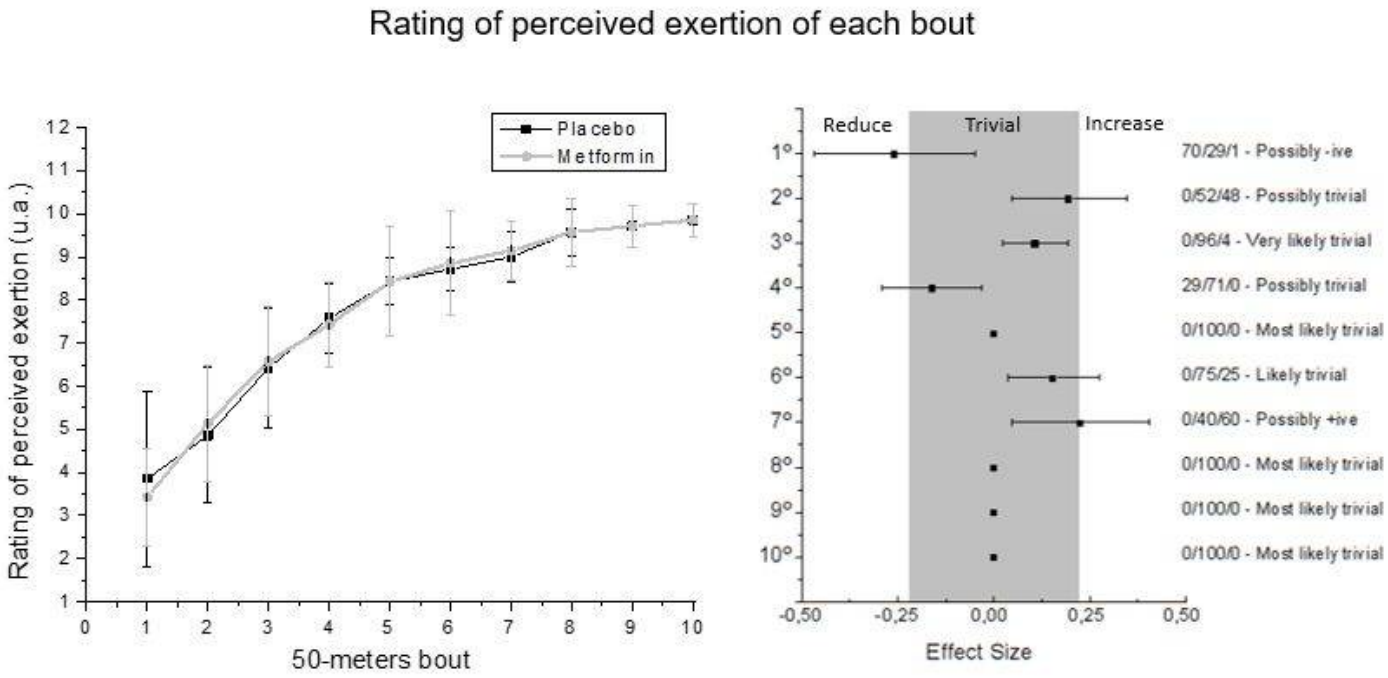


Figure 3

Left figure represents the real rating of perceived exertion behavior. Right figure shows magnitude-based inference analysis between-conditions. Trivial area (grey) threshold is -0.2 to 0.2 and bars indicate ± 90% confidence limits.



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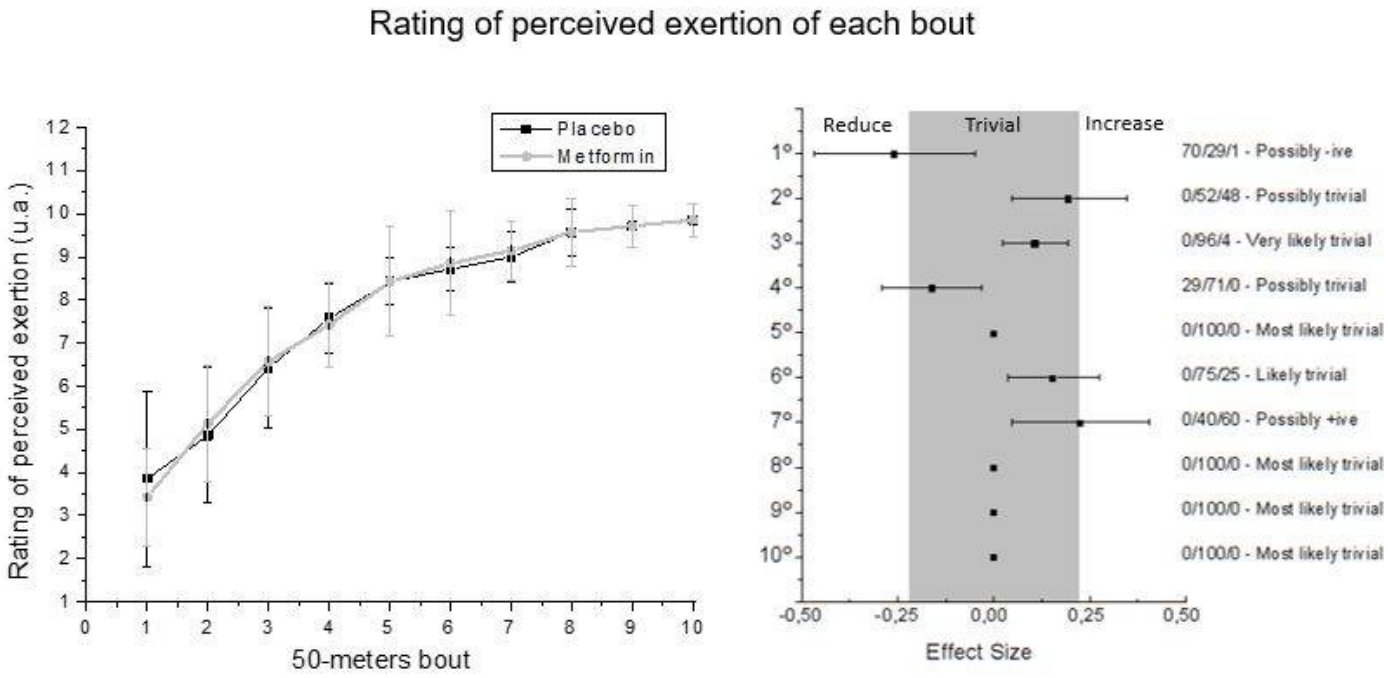

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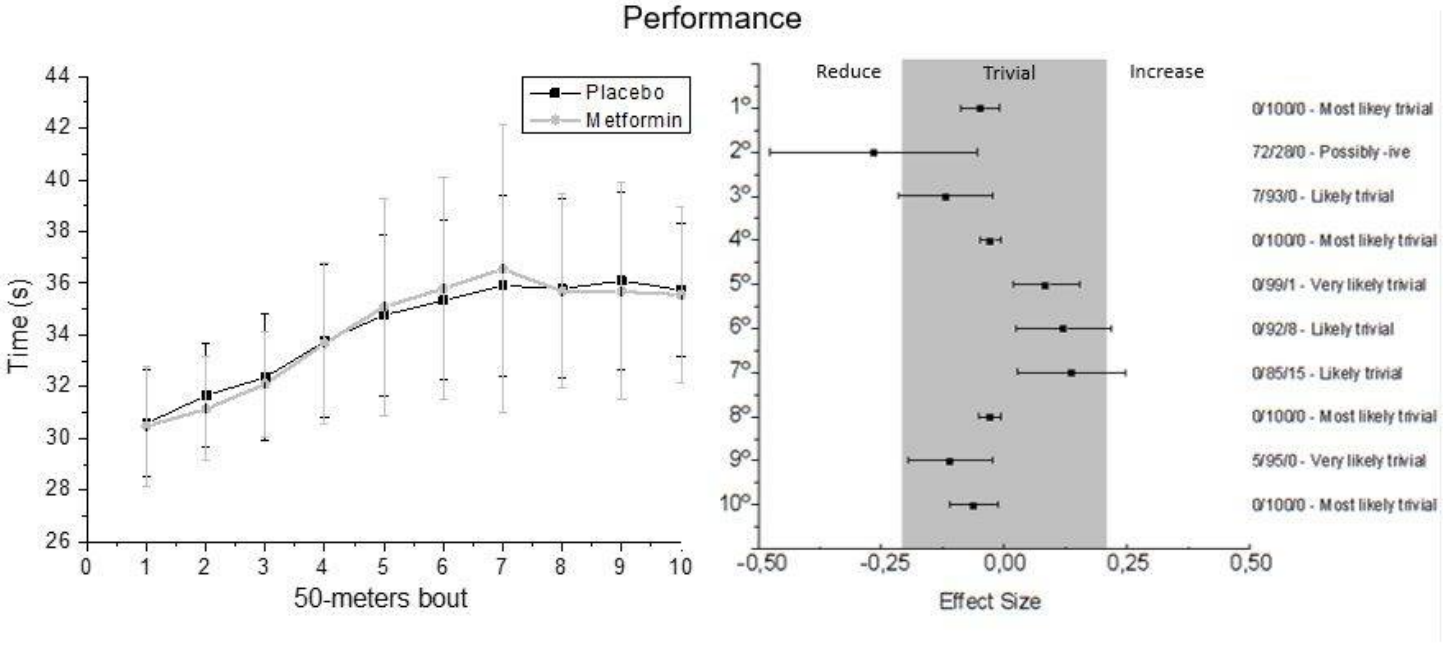
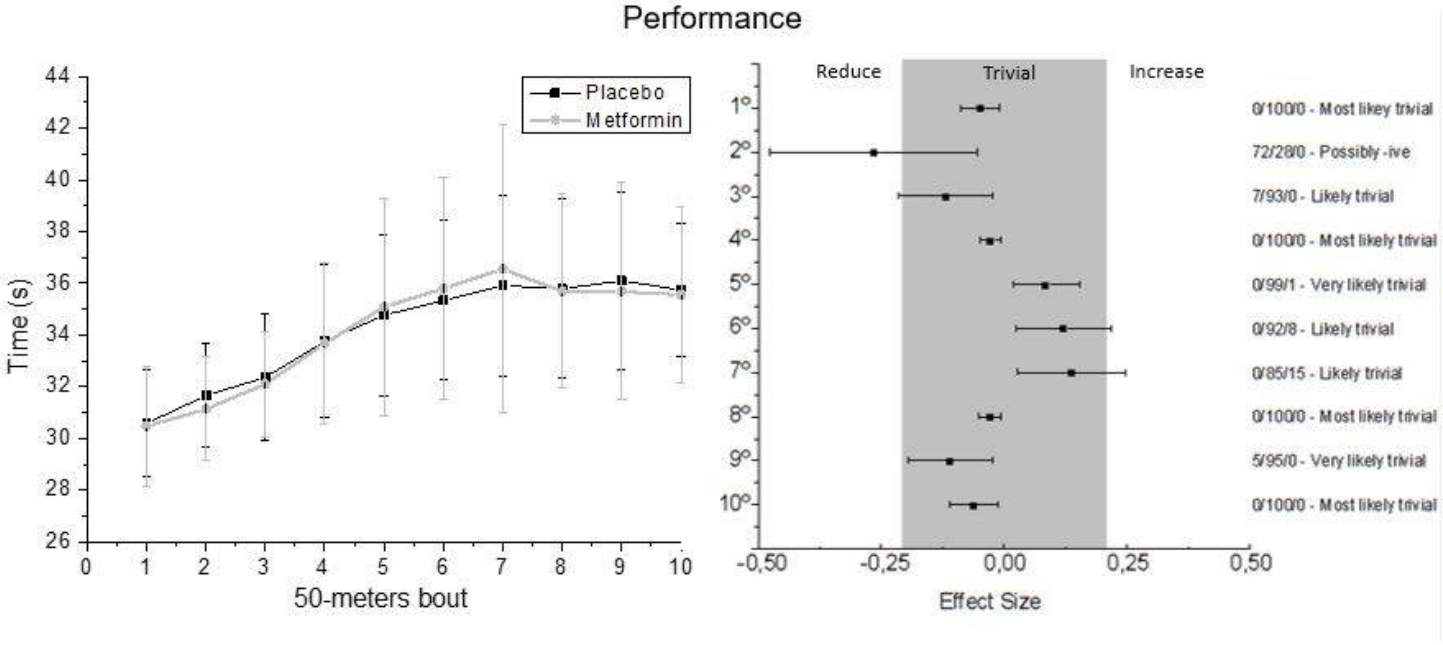


Figure 4

Left figure represents the real performance behavior. Right figure shows magnitude-based inference analysis between-conditions. Trivial area (grey) threshold is -0.2 to 0.2 and bars indicate ± 90% confidence limits.



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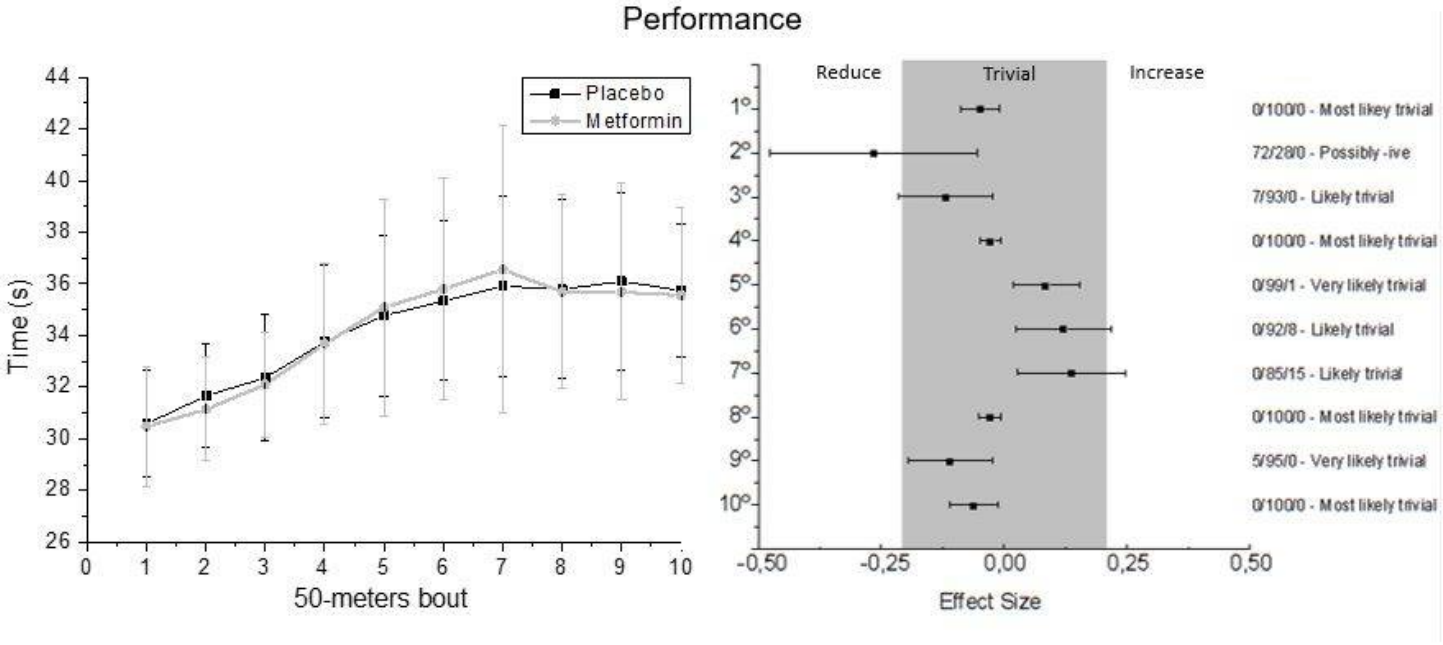

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