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Chronic metformin intake improves anaerobic but not aerobic capacity in healthy rats

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Running title: Metformin intake improves anaerobic capacity in rats
Abstract

The effect of chronic metformin intake on aerobic and anaerobic capacity was examined in healthy rats. Twenty rats completed 10 days of metformin ingestion (MET, 250 mg). After this period, the animals performed 4 high-intensity bouts until exhaustion at 9, 11, 13 and 15% of body weight (BW) in swimming, separated by 24h hours, with prior metformin (250 mg) or placebo (PL). The Critical Load (CL) and Anaerobic Work Capacity ($AWC - W'$) were calculated and considered aerobic and anaerobic capacity, respectively. There was no difference in CL between MET and PL group (p>0.05). The $AWC - W'$ was higher in MET than PL group (p=0.004). Time until exhaustion (s) at all bouts were higher (p<0.004) in MET (9% of BW= 434.5±267.3; 11% of BM= 269.6±214.2; 13% of BW=174.0±40.9; 15% of BW=146.6±15.9) compared to PL group (9% of BW= 96.4±22.3; 11% of BM= 65.5±13.4; 13% of BW=51.1±5.5; 15% of BW=40.8±7.5). Glucose concentration was higher at 90 and 120 min than 0 and 30 min for MET (intra-group) during Oral Glucose Test Tolerance; there was no difference between MET and PL for Area Under Curve (AUC). MET ingestion enhances $AWC - W'$ and times to exhaustion, but not aerobic capacity.

Keywords: drug; ergogenic; performance; time to exhaustion; glucose.
Introduction

The metformin (MET) has been extensively used to treat patients with obesity, Type 2 Diabetes and other diseases (Eriksson et al. 2007). The consumption of MET is associated with a reduction of peripheral resistance to insulin and consequent precaution of hyperinsulinemia, lipid control, reduction of vascular risk, increased glucose uptake and synthesis, and low risk of hypoglycemia during exercise (Eriksson et al. 2007). However, much less is known about the effects of MET and exercise in healthy subjects.

The acute effect of MET in healthy active males does not influence maximal oxygen consumption or ventilatory threshold (Johnson et al. 2008). A recent meta-analysis concluded that MET ingestion increases the aerobic capacity (as measured by the ventilatory anaerobic threshold), but not peak oxygen consumption in healthy volunteers (Das et al. 2018). On the other hand, the evidence of an improved anaerobic capacity after MET ingestion is scarce. Only one study reported that acute administration of MET (500 mg) improves the time to exhaustion during a high-intensity exercise and the fast component of the Excess Post-Exercise Oxygen Consumption (EPOC fast), representing an increased anaerobic alactic contribution (Learsi et al. 2015). Perhaps, the EPOC fast may be enhanced due to increased exercise time. Another study found increased in muscle glycogen content in the MET group after three days of treatment compared to placebo, but this short period was not enough to improve the endurance performance in healthy subjects (Scalzo et al. 2017). A common point in all studies above cited was an acute or short period of MET administration in healthy subjects (1-3 days). Thus, studies that explore measures exercise capacity, in particular, aerobic and anaerobic capacity during long-term of MET administration in healthy subjects are needed.

The Critical Power model may be a very convenient alternative evaluation method and has been used to predict the (1) maximum workload that can be sustained for a long period of time (i.e., 30 min or more) without fatigue (called Critical Power [CP]) and (2) Anaerobic Work Capacity (AWC – \( W' \)), without invasive procedures and expensive equipment (Monod and Scherrer 1965). Different adaptations of CP model have been used in the treadmill (Bergstrom et al. 2014; Housh et al. 1990; Smith et al. 2011), swimming (Wakayoshi et al. 1992) and even until for rodents submitted to swimming (Gobatto et al. 2013). The CP variables can be calculated from the hyperbolic relationship between power output or intensity and the time to exhaustion obtained in three or more high-intensities bouts with duration fixed between 1-15 min and a minimum interval of 24 hours (Jones and Vanhatalo 2017). Mathematically, the CP represents the power-asymptote of hyperbolic function \[ \text{Time} = \frac{W'}{\text{Power} - \text{CP}} \] and the studies has found correlation with anaerobic threshold (Monod and Scherrer 1965). On the other hand, the AWC - \( W' \) represents the curvature constant of hyperbole and the amount
of work during exercise above CP (Monod and Scherrer 1965; Jones and Vanhatalo, 2017). The AWC-W' is reported as a finite reserve of anaerobic energy in the muscle cell to be used in exercise intensities above the intensity corresponding to CP. This reserve is composed of creatine phosphate, oxygen-linked myoglobin and muscle glycogen (Bishop et al. 1998). The AWC - W' has been correlated with anaerobic tests (Jenkins and Quigley 1991) and anaerobic biochemical variables such as muscle ATP concentration and anaerobic ATP production in vitro (Green et al. 1994). Furthermore, AWC - W' is sensitive to training sessions (Jenkins and Quigley 1991) and creatine supplementation (Eckerson et al. 2005). Until the date, no studies investigated the effects of long-term MET administration on CP and AWC-W'. Studying the effects of chronic MET administration in healthy individuals on time to exhaustion and CP variables may better explain the possible ergogenic effect of this drug. However, differently of acute and short-term MET administration, the long-term may increase unnecessarily the risk of side effects in healthy individuals.

In this line, the use of laboratory animals has been expanded due to mimics the human stress responses provide a controlled environment (e.g., same temperature, food, age, same strain, light-dark cycle) and reproducibility of data. Thus, the chronic MET administration in healthy laboratory rats must be a primary and important strategy, before testing in healthy individuals, to understand its effect on performance, aerobic and anaerobic capacity. So, the purpose of the present study was to investigate the chronic administration of MET on time to exhaustion at high-intensity exercise, and on aerobic and anaerobic capacity in healthy rats. We hypothesized that chronic MET administration would increase the time to exhaustion, and both anaerobic and aerobic capacities.

Methods

Animals

All experiments involving animals were performed in accordance with the principles of laboratory animal care (NIH publication No. 86-23, revised 1985). The experimental protocol was approved by the local committee and specific resolutions on Bioethics in Experiments with Animals (number 018040/11-10).

Twenty 60-day-old healthy male Wistar rats (Rattus norvegicus Albinus) were used in this study. Animals were maintained in collective cages (5 rats/cage, 350 cm²/animal, 18 cm of height). Rats received water and commercial chow (23.5% protein, 6.5% fat, 70% carbohydrate, Purina 5008, St. Louis, MO) ad libitum and were housed at 22 ± 2°C with an inverted 12:12-h light–dark cycle (18:00–06:00 lights on).
**Experimental procedures**

The animals were randomly separated into placebo (PL, n = 10) and MET (n = 10) groups. The animals were adapted to the high-intensity swimming by performing water adaptation for 10 days. This period consisted of swimming exercise in cylindrical tanks (100 cm diameter × 100 cm depth), for 10-minute (31±1 °C), with a workload equivalent to 2% of body mass. During this period, MET (250 mg) was diluted in distilled water (1 ml) and administrated daily using the gavage method 1 h before each exercise adaptation session. In placebo group (PL), the animals received 1 mL of distilled water at the same periods.

After the 10 days of PL or MET administration, both groups started a series of four high-intensity exercises (24 h apart) for further determination of CL and AWC - $W'$ (Figure 1). The MET or PL were administered one hour before each test session.

“AINSERT FIGURE 1 HERE”

**Aerobic and Anaerobic Capacity – Critical Load Test (CL)**

The CP Model was adapted for rat swimming, so-called Critical Load (CL) (Marangon et al. 2002). The animals performed four high-intensity swimming loads test until exhaustion. The loads were applied randomly with a minimum interval of 24 hours and corresponded to 9, 11, 13 and 15% of the individual body mass (BM) in both metformin and placebo groups. These loads were chosen to induce exhaustion within 1-15 min and therefore to respect the principles of the CP Model (Gobatto et al. 2013). The exhaustion was assumed when the animal was unable to stay on the water surface for longer than 15 s. The mathematical model adopted was “1/time to exhaustion”, in accordance with Gobatto et al. (2013). The CL and Anaerobic Work Capacity ($AWC - W'$) corresponded to y-intercept and angular coefficient of the linear equation, respectively (Figure 2A).

**Oral Glucose Test Tolerance (OGTT)**

OGTT was performed after 12-h fasting and 48 hours after the last exercise session (Figure 1). A glucose solution (80%) was administered into the stomach of the rats through a gastric catheter at a dose of 2.0 g.kg$^{-1}$ BM. Blood samples (25µL) were collected from the distal tail for further determination of serum glucose. Blood samples were taken immediately before (0 min), and at 30, 60 and 120 min after the glucose administration. Blood glucose was determined by the glucose-oxidase method (Kit Labtest®, Brazil). The area under the blood glucose curve was calculated using the trapezoidal method on the software Origin 7.0®.
Statistics

All analyses were conducted using a statistical software package (Statistica, version 7.0, Tulsa, OK). The dependent variables were checked regarding normality using the W test of Shapiro-Wilk. The CL, $AWC - W'$ and time to exhaustion were compared between PL and MET groups using the unpaired *t*-test. The effect size was also calculated and interpreted as: below 0.20 *low effect*; between 0.20 and 0.50 *moderate effect*; above 0.51 *strong effect* (Cohen 1988). Two-way analysis of variance (ANOVA) was used to examine changes over time in OGTT (0, 30, 60, 90 and 120 min) and body weight (first, second and third week). When a significant interaction effect was found, a Tukey HSD post-hoc test was used to identify where the difference existed among groups. The significance level was set *a priori* $\alpha \leq 0.05$, and data are reported as mean ± standard deviation (SD).

Results

The BM (g) in MET group increased after 3rd week compared to the 1st week. In PL group, the BM (g) increased in the 2nd compared to the 1st week, and in the 3rd week compared to 2nd week (Table 1).

“INSERT TABLE 1 HERE”

The times to exhaustion at 9, 11, 13 and 15% of BM were higher (p<0.004) in MET group (9% of BW= 434.5 ± 267.3; 11% of BM= 269.6 ± 214.2; 13% of BW=174.0 ± 40.9; 15% of BW=146.6 ± 15.9) than PL group (9% of BW= 96.4 ± 22.3; 11% of BM= 65.5 ± 13.4; 13% of BW=51.1 ± 5.5; 15% of BW=40.8 ± 7.5), with a strong effect size for all exercise intensities (2.33, 1.79, 5.30 and 9.02, respectively). The Figure 2B shows the average of times to exhaustions at 9, 11, 13 and 15% of BW (1/time to exhaustion, s), the y-intercept and $AWC - W'$ by the coefficient angular of the linear regression for MET and PL. The linear regression showed high values of $R^2$ for the groups (MET = 0.90 ± 0.13; PL= 0.95 ± 0.03).

“INSERT FIGURE 2 HERE”

There was no difference for aerobic capacity (CL) between MET and PL groups (p = p>0.05, Figure 3A), although a strong effect size in favor of MET (1.00). The $AWC - W'$ in MET group was higher (p=0.0046) than PL group (Figure 3B), with a strong effect size (2.78).
Blood glucose concentration (mg.dL$^{-1}$) kinetics during 120 min was higher (p<0.05) at 90 and 120 min than 0 and 30 min for MET intra-group (0 min= 65.2 ± 4.9; 30 min= 62.1 ± 4.7; 60 min= 69.5 ± 7.1; 90 min= 76.1 ± 10.2; 120 min= 77.1 ± 9.9), but not different in comparison to PL (0 min= 64.3 ± 3.9; 30 min= 67.8 ± 7.8; 60 min= 70.3 ± 8.8; 90 min= 67.1 ± 5.5; 120 min= 69.2 ± 5.3). There were no differences (p>0.05) between MET and PL groups for Area Under Curve (MET= 8342± 378.6 (mg/dL/min.1000$^{-1}$); PL= 8158.5 ± 439.0 (mg/dL/min).1000$^{-1}$), peak concentration (MET=82.2 ± 7.6 mg.dL$^{-1}$; PL=75.7 ± 4.9 mg.dL$^{-1}$) and Peak – Rest (Δ) (MET= 16.9 ± 10.4 mg.dL$^{-1}$; PL=11.3 ± 6.8 mg.dL$^{-1}$).

Discussion

The present study is the first to compare the chronic MET and PL ingestion in healthy rats on performance during a high-intensity exercise on aerobic and anaerobic capacity. We found no improvement in the aerobic capacity with MET (Fig. 3A) but MET increased considerably the AWC - $W''$ (Fig. 3B). During the first and second weeks, there was no weight gain in MET group in contrast to PL (time effect intra-group, Table 1). Therefore, the body weight gain was lower in MET group throughout the intervention period instead of no difference inter-group (Table 1). Probably, in consequence to higher anaerobic supply and less weight gain in MET group, the time until exhaustion was higher in MET than PL group for all intensities performed.

No previous study evaluated the optimal MET dose to improve performance exercise in healthy animals, making it difficult a reference value. Chien et al. (2008) reported peak plasma MET between 1-2 hours after administration using a dose equivalent to 450 mg/kg in insulin-resistant Wistar rats weighing between 120-150 g (300 mg/ml). In our study, the animals of MET group started the experiment with a BM equivalent to 276.0 ± 27.8 g and the fixed dose equivalent to 250 mg was lower than proposed by Chien et al. (2008). This fixed dose associated with 10 days of administration was efficient to increase AWC and reduce BM. On the other hand, data not yet published by our group showed that 500 mg chronically for rats did not show ergogenic effects, and may have been toxic as reported by Martin-Montalvo et al. (2013). Thus, other studies need to be conducted to investigate the optimal dose of MET to improve performance in healthy rats.
We did not find an increase in aerobic capacity in healthy rats, although the effect size has been classified as “strong”. MET has been reported as an inhibitor of mitochondrial energy formation, specifically in complex I of the respiratory chain (Brunmair et al. 2004; Carvalho et al. 2008). However, MET stimulates fatty acid oxidation in liver and muscle cells (Ouyang et al. 2011), and recent studies showed that MET neither inhibit nor reduce mitochondrial function, VO₂ and VO₂peak in the healthy population (Das et al. 2018; Ouyang et al. 2011).

The anaerobic capacity increased after chronic MET ingestion (Fig. 3B). This result corroborates with other studies that showed improvements in anaerobic parameters after acute MET use such as greater phosphocreatine recovery (PCr) and free AMP in vitro (Vytla and Ochs 2013) and higher EPOCFAST indicating larger alactic contribution (Learsi et al. 2015). Unfortunately, we cannot inform which anaerobic pathways (e.g., ATP-CP or glycolysis) most contributed to increasing AWC - W' in the present study. However, with the best of our knowledge, no study in healthy volunteers found change in glycolysis products as lactate and pyruvate concentrations (Johnson et al. 2008; Learsi et al. 2015; Scalzo et al. 2017).

After 14 days, we could not to see weight loss in MET rats; however, there was weight stabilization in the second week compared to PL (Table 1). MET is associated with weight loss in diabetes treatment (Golay 2008). The MET-induced body weight reduction might be because MET increases fat oxidation (Ouyang et al. 2011), decreases hepatic glucose output, inhibits gluconeogenesis and decreases intestinal glucose absorption, providing less glucose for energy storage in the adipose tissue. On the other hand, the glucose concentration in MET group did not reduce in relation to PLA group, probably because OGTT was performed after 12-h fasting and 48 hours after the last exercise session without MET administration. In obese patients, weight loss is the main factor responsible for the decrease in glucose concentration, while the effects of MET are considered marginal (Damsbo et al. 1998). In a healthy organism, MET may have even less influence on glycemic control, but other studies are necessary. Besides that, MET leads to a decrease in appetite (Schultes et al. 2003) and was suggested that it could increase anorectic properties after 12 days of administration (Rouru et al. 1995). The reduction in leptin levels was also observed after MET ingestion (Mick et al. 2000; Morin-Papunen et al. 1998), and increase in GLP-1 levels, promoting weight loss (Mannucci et al. 2001; 2004). Some studies reported, however, that weight loss is limited in populations without diabetes (Golay 2008). The rats in MET group may have fed less than PL, but unfortunately, we cannot confirm that because this was not controlled.

The comparative physiology of exercise has been using for decades the forced swim protocols by the addition of weight load attached to the rat body to decrease the time to exhaustion (McArdle and Montoye 1966;
Gobatto et al. 2001; de Araujo et al. 2007). Forced swimming based in rats’ BM has similar organic responses to exercise/tests protocols of humans (Booth et al. 2010) as well as can be an important method to assess the exercise capacity and prescribe individually the workload (Gobatto et al. 2001; de Araujo et al. 2007; Gobatto et al. 2013). Our study shows that the CP model adapted for rat swimming, called CL, can be used to evaluate aerobic and anaerobic capacity since it presented excellent mathematical adjustments (R²) and was sensitive to drug intervention. Gobatto et al. (2013) reported the viability of CL model to estimate aerobic and anaerobic capacity by mathematical models in swimming rats. On the other hand, it is evident that animals with less density have an intrinsic advantage to buoyancy and further studies should not ignore the animal’s body density for swimming protocols (dos Reis et al. 2011; dos Reis et al. 2018). Reductions in body weight for MET group may have induced changes in fat mass, fat-free mass, buoyancy, and as a consequence the time to exhaustion and exercise capacity (dos Reis et al. 2018).

The higher supply of anaerobic energy associated with weight stabilization allowed a longer time to exhaustion in all exercise intensities in MET group. This is consistent with the previous study that showed improved time to exhaustion after acute MET ingestion in healthy volunteers (Learsi et al. 2015). In contrast, recently a 12.5 km time-trial performance (~25 min) was not affected by MET ingestion neither in normoxia nor hypoxia environments (Scalzo et al. 2017). However, participants ingested a high-carbohydrate breakfast (1225 kcals, 70% carbohydrate) in both situations, 3.5 h before completed a 12.5 km time-trial on a cycle ergometer (Scalzo et al. 2017). This rich-CHO meal ingested 3.5 h before 12.5 km time-trial probably provided the energy required to exercise (Chryssanthopoulos and Williams 1997) and this could be the reason for the absence of difference between conditions. Furthermore, the anaerobic contribution during 12.5 km time-trial is lower than supramaximal exercise at 110% of maximal oxygen uptake (Learsi et al. 2015) and time to exhaustion at 9, 11, 13 and 15% of BW. Thus, the MET may be useful for exercises with the high anaerobic component.

Some limitations of the present study must be recognized. The mathematical model applied to calculate the energy systems supply did not provide information about ATP-CP and glycolysis systems, but our results provide evidence that MET may be an ergogenic source for high-intensity exercise. The fixed-dose equivalent to 250 mg in our study may have been high and further studies on different doses/effects and physiological responses are needed to establish an optimal ergogenic concentration in a healthy organism. However, we do not encourage the ingestion of MET by healthy people to improve performance. Rather, these findings reinforce the importance to include MET in anti-doping list. Other studies are needed in high-performance athletes to confirm this suggestion.
In conclusion, metformin does not affect aerobic capacity but increases anaerobic capacity and time to exhaustion during high-intensity swimming exercise in healthy rats.

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Conflicts of Interest

The authors declare no conflict of interest.
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**Legends**

**Table 1.** Mean ± standard deviation and variation values (Δ) of body mass (g) gain during the 3 weeks in Metformin (MET) and Placebo (PL) groups.

**Figure 1.** Experimental design. CL: Critical Load Test; BM: body mass; OGTT: Oral Glucose Tolerance Test. MET and PL were ingested before adaptation period during 10 days and CL test at 9, 11, 13 and 15% of BM. The adaptation period lasted 10 days and was performed during 10 min, 2% of BM.

**Figure 2.** (A) Individual example of determination of Critical Load (CL). Critical Load (CL) and Anaerobic Work Capacity (AWC - \( W' \)) were equivalent to y-intercept and angular coefficient, respectively, from linear regression between load (% of BM) and reverse time (1/time to exhaustion, s), at 9, 11, 13 and 15% of body mass (BM). (B) Average values of 1/time to exhaustion (s) and equations of linear regression for MET and PL groups.

**Figure 3.** Aerobic and Anaerobic Capacity of the metformin (MET) and placebo (PL) groups. (A) Average ± Standard Deviation calculated from y-intercept (Critical Load-CL) of linear regression (X axis= 1/time (s); Y axis= % of body mass-BM). (B) Average ± Standard Deviation calculated from angular coefficient (Anaerobic Work Capacity-AWC - \( W' \)) of linear regression (X axis= 1/time (s); Y axis= % of BM). *different in relation to PL (p=0.0046).
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<tr>
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