61 - CORRELATION BETWEEN SPEED AND OXYGEN CONSUMPTION (VO₂) IN RATS SUBMITTED TO MAXIMUM EXERCISE TEST.

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INTRODUCTION
The measurement of the maximum consumption of oxygen (VO₂max) is an excellent parameter to quantify the cardiorespiratory capacity, as well as the level of physical conditioning of any individual, from athletes to sick individuals (Lange et al., 2001). VO₂max represents the highest consumption of oxygen achieved during increments of physical effort. The reduction of this parameter can be influenced by many factors, such as, cardiac output limitations, reduced peripheral blood flow and problems in the skeletal muscle metabolism (Francis et al., 2000).

Exercise tests and gas exchanges analysis have become an important tool in the assessment of individuals that participate in physical qualification programs and cardiac rehabilitation. In addition, exercise tests are also used to establish standards for cardiopulmonary responses to maximum and submaximum exercises.

It is important to point out that a great part of what is known today about health was acquired by doing research with small animals. Therefore, the assessment of cardio-respiratory responses to acute and chronic exercise, just like it is done with human beings, has been a common practice in the investigation of animal models with different pathologies. Like clinical practice, VO₂max measurement is a non invasive tool that has great validity in the study of the functional capacity of animals. Besides being used for physical training prescription, it is also used for the assessment of such approach. However, VO₂max measurements are still little used in animal experimentation because of the high costs for the gas analyzer. That is why few research laboratories use this technology. Maximum exercise test (ET), which consists of a test with graded loads on a treadmill until the exhaustion of the animal, has been used by our group as an alternative evaluation method to assess physical capacity and physical training prescription in rats. These tests are considered truthful and cheap. However, the relation between the speed of the ET and the VO₂ in rats is still unclear. Therefore, the objective of present study was to investigate the correlation between the speed of the exercise tests and the consumption of oxygen in control rats.

MATERIALS AND METHODS
Male Wistar rats (n=8) were used, weighing between 200 and 250g, from the biotery of the Medicine College of the Universidade de São Paulo. The experimental procedures were conducted in accordance with the rules of the National Committee of Ethics in Research (Resolution CNS, 196/96). Water and food were offered unrestrictedly. Their diet contained normal amounts of protein.

The ET was applied to evaluate the rats’ maximum effort physical capacity. The ET consists of a test with graded loads on a treadmill, with 3m/min speed increments every 3 minutes until the exhaustion of the animal (time >50% at the end of the treadmill lane). The oxygen consumption metabolic determination was performed in accordance with the method previously described by Brooks & White (1976). The VO₂ was analyzed by a metabolic cage connected to an oxygen sensor (Ametek N-22M-S-371). After this, the VO₂ values for each rat were calculated using the mathematical formula described bellow:

\[ \text{VO}_2 = \frac{\text{WF} \times (\text{FiO}_2 - \text{FeO}_2)}{\text{BW}} \]

where, WF = Withdrawal pump flow (ml/min); \( \text{FiO}_2 \) = Inspiration O₂ fraction; \( \text{FeO}_2 \) = Expiration O₂ fraction; BW = Animal body weight (g).

The animals were adapted to the treadmill (10 min/day; 0.3 Kmh/h) for three days prior to the TE and the VO₂ measurements.

The statistical analysis was performed using Windows software SPSS 12.0. All the results are presented as mean standard error (SEM). Correlations between variables were determined by linear regression. The differences were considered significant when p<0.05.

RESULTS
The VO₂ max and VO₂ reserve (VO₂ max - VO₂ reserve) were 81±2 ml/kg/min and 48±2 ml/Kg/min, respectively in normal rats. The average of maximum speed running in ET obtained by control animals was 21±0.8 m/min. The VO₂ of studied rats increased with increasing treadmill speed (3-24 m/min) until reached the VO₂ max. Confirming this observation, using a linear regression test, a positive correlation was obtained (r=0.8, p<0.05) between ET running speeds and VO₂ in control rats (Figure 1A).

A positive correlation was also obtained between the ET maximum speed of running and the VO₂ max. (r=0.7, p<0.05), demonstrating that rats with better ET performance presented greater VO₂ max. (Figure 1B).

![Figure 1. Positive correlation obtained by linear regression between: A. exercise test (ET) and oxygen consumption (VO₂); B. ET maximum speed and VO₂ maximum (VO₂ max); C. % of ET maximum speed and % of VO₂ reserve. Considering that 21m/min was ET 100%, the corresponding values of 50% (10 m/min), 60% (12 m/min), 75% (16 m/min) and 85% (18m/min) of the ET maximum speed were calculated. The ET percentiles and the respective values of ET speed, VO₂ % of VO₂ max, VO₂ reserve, % of VO₂ reserve were shown in Table 1. The running speeds (m/min) calculated for each percent ET were applied in the control rats’ linear regression equation (VO₂ =1.55* ET speed + 52.34) (Figure 1A), which correlated ET speed and VO₂. In this manner, the equivalent VO₂ for each ET speed was obtained. So, these calculated VO₂ values by the regression equation](image-url)
DISCUSSION

Laboratory rats are constantly used in exercise physiology research. However, the measurement of oxygen consumption in small animals is a limitation factor for research in this area because of the high costs of gas analysis systems. Moreover, it must be highlighted that the VO2 measurement must be carried out in a separate place from the rest of the laboratory to prevent alterations in the atmosphere and the recording of data acquisition. Another important consideration is that resting VO2 stabilization can take some time in rats. Therefore, the present study investigated the correlation between ET speed and VO2 in healthy adult rats, with the goal to be able to estimate VO2 from ET results. This can guide physical training prescription, as well as, detect differences in the cardio-respiratory capacity of these animals. The maximum VO2 values obtained in the present work are in accordance with previous findings for normal adult rats (Wisloff et al., 2001). Likewise, the maximum speed values obtained in the effort test are considered adequate values for rats and mice (De Angelis et al., 2004, Irigoyen et al., 2005, Parente Costa et al., 2004, Scheffer & Talan, 1996).

The VO2 results of the present study are given for adult Wistar male rats in different racing speeds on the treadmill. The VO2 increases gradually with the speed increment between 0-24 min of ET. This can be expressed by a simple equation obtained by linear regression (VO2=1.55*ET speed + 52.34). This equation allows VO2 estimation for one definitive work load. It also gives the VO2max from the maximum ET speed. The search for indirect forms to estimate VO2max has been widely investigated in both human beings and animals (Astrand & Ryhming, 1954, Pasquins et al., 1970, Scheffer & Talan, 1996). In 1954, Astrand & Ryhming published a nomogram to predict VO2max from the maximal pulse rates (120 to 170 bpm). Some years later this nomogram was modified and it is still being used nowadays (Astrand, 1960). It is known that there is a correlation between VO2 or maximum work load per minute with HR between 150 to 170 bpm and VO2max. (Strandell, 1964). In 1959 Balke and Weir developed a new protocol and established a formula to calculate VO2 based on the speed and inclination of the treadmill (Vivacqua & Hespampa, 1992). Nowadays, VO2max, for both men and women, can be estimated according to the formulas adopted by the American College of Sports Medicine (ACSM, 1996).

In exercise physiology, it is common and appropriate to characterize the work intensity as a percentage of VO2max. In this study, it was observed that a speed of 10 m/min (50% of the maximum ET speed) corresponded to 50% of the VO2max or 60% of the VO2 reserve in adult rats. In animal models, a correlation between ET speed and VO2 also was established for adult and old mice C57BL/6J, in which a speed of 12 m/min (~50% of the maximum ET speed) produced a VO2 equivalent to 76% and 89% of the VO2max, in the adult and old animals, respectively (Scheffer & Talan, 1996). Wisloff et al. (2001) demonstrated linear relationship between VO2 and HR in sedentary or trained normal rats according to the increment of ET speed. These authors emphasize, however, that the main factor determining VO2 and max HR is the anaerobic threshold. Another interesting result is that it is reached in intensities above VO2max. Moreover, this work evidences that values of 90% of the maximum HR correspond to ~80% of the VO2max.

It is important to highlight the positive correlation (r=0.7) obtained between maximum ET speed and maximum VO2 in adult rats in the present study. This correlation demonstrates that the rats with better ET performance presented greater VO2max. This finding supports that simple ET test is a valid tool to detect differences in the cardio-respiratory capacity of both strains. Since it is assumed that both the increase of VO2max and exercise capacity observed after training animals and human beings, it is possible to determine the improvement of cardio-respiratory performance (VO2max) after a period of physical training by high maximum ET speed. Noakes et al. (1990) demonstrated that the highest speed reached in maximum ET is a better predictor of performance in laboratory rats, %VO2max, VO2max during racing speed at exercise lactate threshold among marathon athletes. In our group we have used effort tests as indicators of improvement in the exercise capacity. Recently, we have demonstrated that female rats had were submitted to the bilateral withdrawal of ovaries, an experimental model of menopause, presented a 38% improvement in the maximum ET speed after 8 weeks of physical training, which was also confirmed by other markers of physical conditioning, such as bradycardia at rest and the reduction of the body weight (Irigoyen et al., 2004). Likewise, we have verified a greater maximum ET speed in diabetic rats, hypertensive rats and normal mice after physical training in treadmill (Parente Costa et al., 2004; De Angelis et al., 2004; De Angelis et al., 1999).

The results of the present study suggest that VO2 can be estimated from the results of ET using equations obtained by linear regression between ET speed and VO2. The correlation between ET speed and VO2 demonstrates that physical training prescription based on ET in normal rats is adequate and that the differences of aerobic performance can be detected by ET since the maximum speed achieved in the ET was correlated with the maximum VO2 in the evaluated rats. Therefore, the present study demonstrated that ET, a methodology of easy application and reduced cost, can be used as an indicator of cardio-respiratory capacity in future studies that investigate the physiological effect of acute or chronic exercise in normal male rats.

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**CORRELATION BETWEEN SPEED AND OXYGEN CONSUMPTION (VO2) IN RATS SUBMITTED TO MAXIMUM EXERCISE TEST.**

**SUMMARY.**

The objective of the present study was to investigate the correlation between the speed during maximum exercise test (ET) and the consumption of oxygen (VO2) in normal rats. Male Wistar rats (n=8) were submitted to a maximum exercise capacity evaluation with the use of metabolic gases analysis measurements using a respiremetric system. Maximum VO2 and VO2 reserve were 80±2 and 47±2 ml/Kg/min in normal rats. There was a positive correlation between ET speed and VO2 (r=0.8) in studied animals. Linear regression analysis was used to obtain the equation for the correlation between ET and VO2 (VO2=1.55 * speed ET + 52.34). The 50% and 85% of the maximum speed of ET was calculated from these equations and it corresponded to 68% and 95% of VO2 reserve, respectively.

The maximum speed of ET was correlated with maximum VO2 (r=0.7). Conclusion: 1) VO2 can be estimated by using linear regression equations obtained from correlations between ET speed and VO2 in control animals; 2) the correlation obtained between ET speed and VO2 demonstrates that physical training prescriptions based on ET in normal rats is adequate 3) the improvement of aerobic performance can be detected by ET since maximum speed was correlated with maximum VO2 among the animals studied.

**Key-words:** effort test, oxygen consumption, rats.

**CORRÉLATION ENTRE VITESSE DE L’ÉPREUVE D’EFFORT MAXIMAL ET CONSOMMATION D’OXYGÈNE (VO2) DANS LES SOURIS; RÉSUMÉ**

L’objectif du présent travail était d’enquêter sur la corrélation entre la vitesse de l’effort maximal (TE) et la consommation d’oxygène (VO2) dans les souris normales. Les souris viriles ont été utilisées Wistar normales (n=8). Les animaux ont été soumis à une évaluation de capacité physiologique maximum par moyen d’une respirométrie ouverte et test d’effort. Le TE et la détermination de VO2 a été effectuées 60 jours, VO2 maximal et réserve VO2, soit 80±2 et 47±2 ml/kg/min, respectivement. Il y avait une corrélation positive de la vitesse du TE et VO2 (r=0.8). Commencer des équations de retour en arrière linéaire entre TE et VO2 a été obtenu pour dans les souris normales (VO2=1.55 * Vitesse TE + 52.34) il a été démontré que 50% et 85% de la vitesse maximale de TE a correspondu TE à 68% et 90% de VO2 de réserve. La vitesse de maximale de l’effort correspond à VO2 maximal (r=0.7). Conclusion: 1) VO2 peut être considéré VO2 qui commence des résultats du TE utiliser elle donner l’équation de retour en arrière linéaire entre VO2 et TE proposition; 2) la corrélation entre la vitesse du TE et VO2, démontre que la prescription de l’éducation physique qui commence du TE dans les souris normales sont dignes de confiance; 3) l’amélioration d’aérobic de la performance peut être détectée par le TE une fois la vitesse maximale a obtenu dans l’effort correspondant avec VO2 maximal dans les animaux estimés.

**La mot ciep: éprouve d’effort, consommation d’oxygène, souris**

**EM RATONES.

RESUMEN**

El objetivo del presente trabajo fue investigar la correlación entre la velocidad del test del esfuerzo máximo (TE) y el consumo del oxígeno (VO2) en ratones normales. Fueron utilizados ratones machos Wistar normales (n=8). Los animales fueron submetidos a la evaluación de la capacidad física máxima por medio de respirometría abierta y test de esfuerzo. El TE y la determinación VO2, a été effectuées 60 jours, VO2 maximal y VO2 de reserva, soit 80±2 et 47±2 ml/kg/min, respectivement. Hubo una correlación positiva entre la velocidad del TE y el VO2. A partir de la ecuación de regresión lineal entre TE y VO2, obtenida para el ratones (VO2=1.55Velocidad TE + 52.34) se demostró que el 50% y 85% de la velocidad máxima del TE correspondía al 68% y 90% del VO2 de la reserva. La máxima velocidad del TE fue correlacionada con el VO2 maximal, (r=0.7). Conclusión: 1) se puede estimar el VO2 a partir de los resultados del TE aprovechando la ecuación de regresión lineal entre VO2 y TE propuestas; 2) la correlación entre la velocidad del TE y el VO2, demuestra que la prescripción del entrenamiento físico a partir del TE en ratones normales es fidedigna; 3) la mejora del la performance aeróbica puede ser detectada para el TE una vez que la velocidad máxima obtenida en el TR fue correlacionada con el VO2 máximo en los animales avilados. **Palabras-chave: test de esfuerzo, consumo del oxígeno, ratones**

**CORRELACIÓN ENTRE VELOCIDAD DEL TEST DE EFUERZO MÁXIMO Y CONSUMO DEL OXIGÉNIO (VO2) EM RATONES.

RESUMEN**

O objetivo do presente trabalho foi investigar a correlação entre a velocidade do teste de esforço máximo (TE) e o consumo de oxigênio (VO2) em ratos normais. Ratos machos Wistar controles (n=8) foram submetidos a uma avaliação da capacidade física máxima durante a qual foi realizada uma análise de gases utilizando-se um sistema de respirometria. Houve uma correlação positiva entre a velocidade do TE e o VO2 (r=0.8). A partir da equação de regressão linear entre TE e VO2, (VO2=1.55Velocidade TE + 52.34) demonstrou-se que o 50% e 85% da velocidade máxima do TE corresponde a 68% e 90% de VO2 de reserva. A máxima velocidade do TE foi correlacionada com o VO2 maximal, (r=0.7). Conclusão: 1) pode-se estimar o VO2 a partir dos resultados do TE utilizando-se a equação de regressão linear entre VO2 e TE; 2) a correlação entre a velocidade do TE e o VO2, demuestra que a prescrição do treinamento físico a partir do TE em ratos normais é fidedigna; 3) a melhora de performance aeróbica pode ser detectada pelo TE uma vez que a velocidade máxima obtida no TE foi correlacionada com o VO2 máximo nos animais avilados. **Palavras-chave: teste de esforço, consumo de oxigênio, ratos.**

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