61 - CORRELATION BETWEEN SPEED AND OXYGEN CONSUMPTION (Vo_2) IN RATS SUBMITTED TO MAXIMUM EXERCISE TEST.

BRUNO RODRIGUES[1], MARIA CLAUDIA IRIGOYEN[1], KATIA DE ANGELIS[2]
[1]Unidade de Hipertensão, Instituto do Coração (InCor), Faculdade de Medicina, Universidade de São Paulo, Sao Paulo, Brazil & [2]Laboratório do Movimento Humano, Universidade São Judas Tadeu, Sao Paulo, SP, Brazil.

INTRODUCTION

The measurement of the maximum consumption of oxygen (VO_2máx.) it is an excellent parameter to quantify the cardio-respiratory capacity, as well as the level of physical conditioning of any individual, from athletes to sick individuals (Lange et al., 2001). VO_2max. 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, VO_2max. 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, VO_2max. 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 VO_2 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 Sao 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 speedy increments each 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 previous described by Brooks & White (1978). The VO_2 was analyzed by a metabolic cage connected to an oxygen sensor (Ametek N-22M-S-3^A/I). After this, the VO_2 values for each rat were calculated using the mathematic formula described bellow:

$$\mathbf{VO_2\ (ml O_2 . kg^{-1} . min^{-1})} = WF\ (FiO_2\ \ FeO_2)/BW$$

where, WF = Withdrawal pump flow (ml/min); FiO_2 = Inspiration O_2 fraction; FeO_2 = Expiration O_2 fraction; BW = Animal body weight (g).

The animals were adapted to the treadmill (10 min/day; 0.3 Km/h) for three days prior to the TE and the VO_2 measurements.

The statistical analysis was performed with 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 VO_2 max. and VO_2 reserve (VO_2max. - $VO_{2\,rest}$) 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 VO_2 of studied rats increased with increasing treadmill speed (3-24 m/min) until reached the VO_2 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 VO_2 in control rats (Figure 1A).

A positive correlation was also obtained between the ET maximum speed of running and the VO_2 max. ($r=0.7$, $p<0.05$), demonstrating that rats with better ET performance presented greater VO_2 max. (Figure 1B).

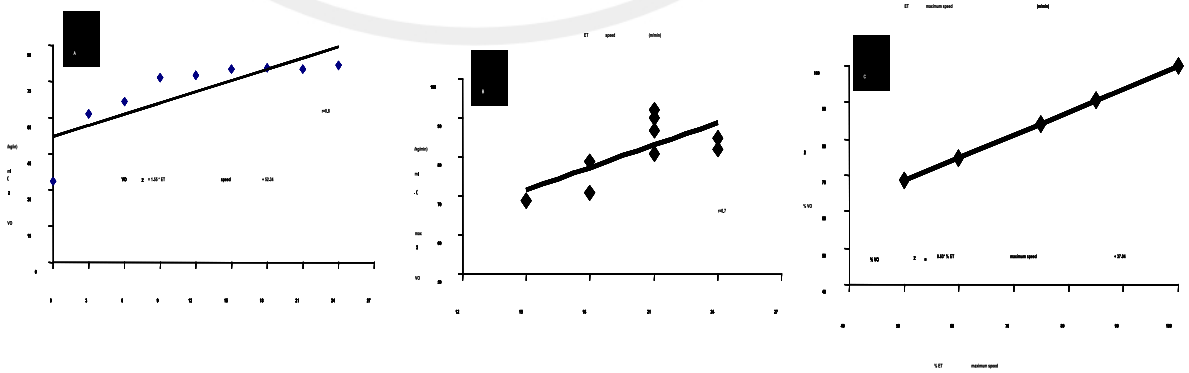

Figure 1. Positive correlation obtained by linear regression between: **A.** exercise test (ET) and oxygen consumption (VO_2); **B.** ET maximum speed and VO_2 maximum (VO_2max.); **C.** % of ET maximum speed and % of VO_2 reserve.

Considering that 21m/min was ET 100%, the corresponding values of 50% (10 m/min), 60% (12 m/min), 75% (16m/min) and 85% (18m/min) of the ET maximum speed were calculated. The ET percents and the respective values of ET speed, VO_2, % of VO_2max., VO_2 reserve, % of VO_2 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 (VO_2 =1.55* ET speed + 52.34) (Figure 1A), which correlated ET speed and VO_2. In this manner, the equivalent VO_2 for each ET speed was obtained. So, these calculated VO_2 values by the regression equation

were used to calculate the VO_2 reserve for each pre-determined speed running. Finally, the %VO_2max. and the % VO_2 reserve were calculated for each VO_2 and VO2 reserve obtained at 50, 60, 75, 85 and 100% of the ET maximum speed (Table 1). Figure 1C shows the relation between the % ET maximum speed and the % VO_2 reserve. This correlation between the percents evidences, for example, that 60% and 85% of ET maximum speed running were equivalent to 74 and 90% of VO_2 reserve, respectively (Table 1, Figure 1C).

Table 1 – Exercise test percents (%ET) and equivalent oxygen consumption percents (%VO2).

% ET maximum speed	50%	60%	75%	85%	100%
ET speed (m/min)	10	12	16	18	21
VO_2 (ml/Kg/min)	68	71	76	79	81
% VO_2 maximum	**80**	**85**	**90**	**95**	**100**
VO_2 (ml/Kg/min)	35	38	43	46	47
% VO_2 reserve	**68**	**74**	**84**	**90**	**100**

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 VO_2 measurement must be carried out in a separate place from the rest of the laboratory to prevent alterations in the concentration of gases around the environment during data acquisition. Another important consideration is that resting VO_2 stabilization can take some time in rats. Therefore, the present study investigated the correlation between ET speed and VO_2 in healthy adult rats, with the goal to be able to estimate VO_2 from ET results. This can guide physical training prescription, as well as, detect differences in the cardio-respiratory capacity of these animals.

The maximum VO_2 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 VO_2 results of the present study are given for adult Wistar male rats in different racing speeds on the treadmill. The VO_2 increased gradually with the speed increment between 0-24 min of ET. This can be expressed by a simple equation obtained by linear regression (VO_2=1,55*ET speed + 52.34). This equation allows VO_2 estimation for one definitive work load. It also gives the VO_2max. from the maximum ET speed.

The search for indirect forms to estimate VO_2max. has been widely investigated in both human beings and animals (Astrand & Ryhming, 1954, Pasquis et al., 1970, Scheffer & Talan, 1996). In 1954, Astrand & Ryhming published a nomogram to predict VO_2max. from submaximal pulse rates (120 to 170 bpm). Some years later this nomogram was modified and it is still being used nowadays (Astrand, 1960). Studies have also confirmed that there is a correlation between VO_2 or maximum work load per minute with HR between 150 to 170 bpm and VO_2max. (Strandell, 1964). In 1959 Balke and Were developed a new protocol and established a formula to calculate VO_2 based on the speed and inclination of the treadmill (Vivacqua & Hespanha, 1992). Nowadays, VO_2max., 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 VO_2max. In this study, it was observed that a speed of 10 m/min (50% of the maximum ET speed) corresponded to 80% of the VO_2max. or 68% of the VO_2 reserve in adult rats. In animal models, a correlation between ET speed and VO_2 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 VO_2 equivalent to 76% and 89% of the VO_2max. in the adult and old animals, respectively (Scheffer & Talan, 1996). Wisloff et al. (2001) demonstrated linear relationship between VO_2 and HR in sedentary or trained normal rats according to the increment of ET speed. These authors emphasize, however, that the maximum HR is not reached along with VO_2max. Instead it is reached in intensities above VO_2max. Moreover, this work evidences that values of 90% of the maximum HR correspond to ~80% of the VO_2max..

It is Important to highlight the positive correlation (r=0.7) obtained between maximum ET speed and maximum VO_2 in adult rats in the present study. This correlation demonstrates that the rats with better ET performance presented greater VO_2max.. This finding suggests that simple ET is capable to detecting differences in the cardio-respiratory capacity of control rats. Since it is assumed that both the increase of VO_2max. and exercise capacity observed after-training animals and human beings, it is possible to determine the improvement of cardio-respiratory performance (VO_2max.) after a period of physical training by highest maximum ET speed. Noakes et al. (1990) demonstrated that the highest speed reached in maximum ET is a better predictor of performance in laboratory tests than %VO_2max., VO_2max. or racing speed at extreme lactate threshold among marathon athletes. In our group we have used effort tests as indicators of improvement in the exercise capacity. Recently, we have demonstrates that female rats hat 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 VO_2 can be estimated from the results of ET using equations obtained by linear regression between VO_2 and ET. Moreover, the correlation between ET speed and VO_2 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 VO_2 in the evaluated rats. Therefore, the present study demonstrated that ET, a methodology of easy application and reduced cost, can be used as indicator of cardio-respiratory capacity in future studies that investigate the physiological effect of acute or chronic exercise in normal male rats.

REFERENCES

AMERICAN COLLEGE OF SPORTS MEDICINE. Cálculos metabólicos. In: Manual para o Teste de Esforço e Prescrição do Exercício. 5ª ed. Rio de Janeiro: Revinter, 237-50, 2000.

ASTRAND I. Aerobic work capacity in men and women with special reference to age. Acta Physiol. Scand, 49(suppl. 169):1960.

ASTRAND PO & RYHMING. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J Appl Physiol. 7:218, 1954.

BROOKS GA & WHITE TP. Determination of metabolic and heart rate responses of rats to treadmill exercise. J Appl Physiol 45(6): 1009-1015, 1978.

DE ANGELIS K, GADONSKI G, FANG J, DALL'AGO P, ALBUQUERQUE V, PEIXOTO L, FERNANDES T, IRIGOYEN MC. Exercise reverses peripheral insulin resistance in trained L-NAME hypertensive rats. Hypertension. EUA: , v.34, n.2, p.768 - 772, 1999.

DE ANGELIS K, WICHI R, MOREIRA ED, MORRIS M, KRIEGER EM, IRIGOYEN MC. Exercise training changes autonomic cardiovascular control balance in mice. J Appl Physiol. EUA: v.96, n.6, p.2174 - 2178, 2004.

FRANCIS DP, SHAMIM W, CERI DAVIES L, PIEPOLI MF, PONIKOWSK P, ANKER SD, COATS AJS. Cardiopulmonary

exercise testing for prognosis in chronic heart failure: continuous and independent prognostic value from VE/CO_2 slope and peak VO_2. Eur. Heart J, 21: 154-161, 2001.
IRIGOYEN MC, PAULINI J, FLORES LJ, FLUES M, MOREIRA ED, CONSOLIM-COLOMBO F, BELLO-KLEIN A, DE ANGELIS K. Exercise training improves baroreflex sensitivity associated with oxidative stress reduction in ovariectomized rats. Hypertension, 46(2):1-6, 2005.
LANGE B, BRUY T. Exercise spirometry in medical practice. Rev. Med Liege, 56(4); 228-32, 2001.
NOAKES TD, MYBURGH KH, SCHALL R. Peak treadmill running velocity during the VO_2 max test predicts running performance. J Sports Sci. 1990; 8(1):35-45.
PARENTE COSTA L, HARTHMANN A D, ARRUDA P, DE ANGELIS K, IRIGOYEN MC. Treinamento físico melhora a disfunção quimiorreflexa em ratos diabéticos. Rev. Bras. Educ. Física, v.18, n.13, p.293 - 301, 2004.
PASQUIS P, LACAISSE A, DEJOURS P. Maximal oxygen uptake in four species of small mammals. Resp. Physiol, 9(2); 298-309, 1970.
SCHEFER V, TALAN MI. Oxygen consumption in adult and aged C57BL/6J mice during acute treadmill exercise of different intensity. Exp.Gerontol., 31(3); 387-392, 1996.
STRANDELL T. Circulatory studies on healthy old men with special reference to the limitation of the maximal physical working capacity. Acta Med Scand, 175 (Suppl 414), 1964.
VIVACQUA R, HESPANHA R. Introdução Histórico Perspectiva. In: Ergometria e Reabilitação em Cardiologia. Rio de Janeiro: Medsi, 1-6, 1992.
WISLøFF U, HELGERUD J, JOHAN KEMI O, ELLINGSEN. O Intensity-controlled treadmill running in rats: $VO_{2\,max}$ and cardiac hypertrophy. Am J Physiol (Heart Circ Physiol) 280: H1301-H1310, 2001.

Corresponding author: Kátia De Angelis, PhD, Universidade São Judas Tadeu.
Rua Taquari,546, Sao Paulo, SP, Brazil,03166-000,
phone: 55 11 6699 1909, e-mail:prof.kangelis@usjt.br

CORRELATION BETWEEN SPEED AND OXYGEN CONSUMPTION (VO_2) 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 (VO_2) in normal rats. Male Wistar rats (n=8) were submitted a maximal exercise capacity evaluation with gases analyses measurements using a respirometry system. Maximum VO_2 and VO_2 reserve were 80±2 and 47±2 ml/Kg/min in normal rats. There was a positive correlation between ET speed and VO_2 (r=0.8) in studied animals. Linear regression analysis was used to obtain the equation for the correlation between ET and VO_2 (VO_2= 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 VO_2 reserve, respectively. The maximum speed of ET was correlated with maximum VO_2 (r=0.7). Conclusion: 1) VO_2 can be estimated by using linear regression equations obtained from correlations between VO_2 and ET in control animals; 2) the correlation obtained between ET speed and VO_2 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 VO_2 among the animals studied.

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

CORRÉLATION ENTRE VITESSE DE L'ÉPREUVE D'EFFORT MAXIMAL ET CONSOMMATION D'OXYGÈNE (VO_2) DANS LES SOURIS.

RÉSUMÉ

L'objectif du présent travail était enquêter sur la corrélation parmi la vitesse de l'épreuve d'effort maximal (TE) et la consommation d'oxygène (VO_2) dans les souris normales. Les souris virilles ont été utilisées Wistar normales (n=8). Les animaux ont etaient soummis a une évaluation de capacité physique maximum par moyen d'une respirometrie ouverte et test d'effort. Le TE et la détermination de VO_2 avez été accomplies 60 jours. VO_2 maximal et réservation VO_2 soit 80±2 et 47±2 ml/Kg/min. il y avait une corrélation positive parmi la vitesse du TE et VO_2 (r=0,8). Commencer des équations de retour en arrière linéal entre TE et VO_2 a obtenu pour dans les souris normales (VO_2 =1,55*Vitesse TE + 52,34) il a été démontré que 50% et 85% de la vitesse maximale de l'il a correspondu TE à 68% et 90% de VO_2 de réservation. La vitesse de la maxime de l'il TE a correspondus avec VO_2 maximal (r=0,7). Terminer: 1) il peut être considéré VO_2 qui commence des résultats du TE utiliser elle le équation de retour en arrière linéal entre VO_2 et TE proposition; 2) la corrélation parmi la vitesse du TE et VO_2 démontrez que la prescription de l'éducation physique qui commence du TE dans les souris normales sont dignes de confiance; 3) l'amélioration d'aeróbia de la performance peut être détectée par le TE une fois la vitesse maximale a obtenu dans l'il TE a correspondus avec VO_2 maximal dans les animaux estimés.

La mot clef: épreuve d'effort, consommation d'oxygène, souris

CORRELACIÓN ENTRE LA VELOCIDAD DEL TEST DE ESFUERZO MAXIMO Y CONSUMO DEL OXÍGENO (VO_2) EM RATONES

RESUMEN

El objetivo del presente trabajo fue investigar la correlación entre la velocidad del test del esfuerzo maximo (TE) y el consumo del oxígeno (VO_2) em ratones normales. Fueron utilizados ratones machos Wistar normales (n=8). Los animales fueron submetidos a la avaliación de la capacidad fisica maxima por medio de respirometria abierta y test de esfuerzo. El TE y la determinación do VO_2 fueron realizados en 60 días. El VO_2 maximo y el VO_2 de reserva fue 80±2 e 47±2 ml/Kg/min, respectivamente. Hubo una correlación positiva entre la velocidad del TE y el VO_2. A partir de la equación de regresión linear entre TE y VO_2 obtenida para el ratones (VO_2 = 1,55*Velocidad TE + 52,34) se demonstrou que el 50% y 85% de la velocidad maxíma del TE correspondia al 68% y 90% del VO_2 de la reserva. Lá maxima velocidad del TE fue correcionada con el VO_2 maximo (r=0,7). Conclusión: 1) se puede estimar el VO_2 a partir de los resultados del TE aprovechando la equación de regresión linear entre VO_2 y TE propuestas; 2) la correlación entre la velocidad del TE y el VO_2 demuestra que la prescrición del treinamiento físico a partir del TE en ratones normales és fidedigna; 3) la mejora del la performance aerobia puede ser detectada para el TE una vez que la velocidad maxima obtida en el TR fue correlacionada con el VO_2 maximo en los animales avaliados. **Palabras-chave**: test del esfuerzo, consumo del oxígeno, ratones

CORRELAÇÃO ENTRE VELOCIDADE DO TESTE DE ESFORÇO MÁXIMO E CONSUMO DE OXIGÊNIO (VO_2) EM RATOS.

RESUMO

O objetivo do presente trabalho foi investigar a correlação entre a velocidade do teste de esforço máximo (TE) e o consumo de oxigênio (VO_2) em ratos normais. Ratos machos Wistar controles (n=8) foram submetidos a uma avaliação da capacidade física máxima durante a qual foi realizada a análise de gases utilizando-se um sistema de respirometria. Houve uma correlação positiva entre a velocidade do TE e o VO_2 (r=0,8). A partir da equação de regressão linear entre TE e VO_2 (VO_2= 1,55*Velocidade TE + 52,34) demonstrou-se que 60% e 85% da velocidade máxima do TE correspondia a 74% e 90% do VO_2 de reserva. A máxima velocidade do TE foi correlacionada com o VO_2 máximo (r=0,7). Concluindo: 1) pode-se estimar o VO_2 a partir dos resultados do TE utilizando-se a equação de regressão linear entre VO_2 e TE; 2) a correlação entre a velocidade do TE e o VO_2 demonstra que a prescrição do treinamento físico a partir do TE em ratos normais é fidedigna; 3) a melhora de performance aeróbia pode ser detectada pelo TE uma vez que a velocidade máxima obtida no TE foi correlacionada com o VO_2 máximo nos animais avaliados.

Palavras-chave: teste de esforço, consumo de oxigênio, ratos.