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ORIGINAL

ACUTE EXERCISE EFFECT ON GLOMERULAR FILTRATION IN THE ELDERLY

EFFECTO DEL EJERCICIO AGUDO SOBRE LA FILTRACIÓN GLOMERULAR DE ADULTOS MAYORES

Trejo Trejo, M.¹; Pineda Espejel, H.¹; Villalobos Molina, R.²; Ramos Jiménez, A.³; Vázquez Jiménez, J.G.¹; Machado Contreras, J.R.¹; Mejía-León, M.E.⁷; Arrayales Millán, E.⁴

¹ Profesor de Tiempo completo, Facultad de Deportes, Universidad Autónoma de Baja California (México) marina.trejo@uabc.edu.mx, antonio.pineda@uabc.edu.mx, gustavo.vazquez@uabc.edu.mx, rene.machado@uabc.edu.mx, esther.mejia86@uabc.edu.mx

² Profesor de Carrera Titular C, Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala y Facultad de Medicina U.N.A.M. (México) rafaelvillalobosmolina@gmail.com

³ Profesor investigador en el Departamento de Ciencias de la Salud del Instituto de Ciencias Biomédicas de la Universidad Autónoma de Ciudad Juárez (México) aramos@uacj.mx

⁴ Profesor, Facultad de Deportes, Universidad Autónoma de Baja California (México)
earrayales@uabc.edu.mx

Spanish-English translator: Dr. Rafael Villalobos Molina,
rafaelvillalobosmolina@gmail.com

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ABSTRACT

Introduction: There is a decrease in Glomerular Filtration Rate (GFR) in older adults and the exercise practice may lead to changes in renal function.

Purpose: To evaluate the effect of different intensities of acute exercise on elderly population GFR. **Methods:** 20 apparently healthy older adults ($69,8 \pm 4$ years) performed 3 exercise tests a week apart: one maximal and 2 submaximal (80% and 60%). Blood samples were collected to determine serum creatinine and to estimate GFR, before and after each exercise test. **Results:** A

significant post-exercise decline in GFR, estimated from serum creatinine values, was observed in the maximal and submaximal 80% tests ($p < 0,05$).

Conclusion: Maximum and sub-maximum acute physical exercise at 80% intensity causes a decrease in glomerular filtration in apparently healthy older adults.

KEYWORDS: Glomerular filtration rate, sub-maximal test, exercise, maximal exercise, elderly.

RESUMEN

Introducción: Existe una disminución de la Filtración Glomerular (FG) en adultos mayores y la práctica del ejercicio puede causar cambios en la función renal.

Objetivo: Evaluar el efecto de diferentes intensidades de ejercicio agudo sobre la filtración glomerular en adultos mayores. **Método:** 20 adultos mayores aparentemente sanos de $69,8 \pm 4$ años realizaron 3 pruebas de ejercicio físico: máxima & 2 sub-máximas (80% & 60%). Se colectaron muestras de sangre venosa para estimar la filtración glomerular por creatinina, antes y después del ejercicio. **Resultados:** Se observó una disminución significativa post-ejercicio en la filtración glomerular, estimada a partir de creatinina sérica en las pruebas máxima y sub-máxima a 80% ($p < 0,05$). **Conclusión:** El ejercicio físico agudo máximo y sub-máximo al 80% de intensidad en adultos mayores aparentemente sanos, provoca una disminución de la filtración glomerular.

PALABRAS CLAVE: Filtración glomerular, ejercicio sub-máximo, ejercicio máximo, adulto mayor.

INTRODUCTION

Glomerular filtration (GF) is considered the best indicator of kidney function and global health (Stevens & Levey, 2009), and can be estimated from serum creatinine concentration using equations developed in the last 30 years, which include age, gender and body size (Cirillo, 2010). One of these equations, commonly used is that simplified to predict GF, i.e., $FG = 186 \times (Cr/88.4)^{1.154} \times age^{-0.203} \times 0.742$ if is a female (Levey et al., 2000).

In the elderly a diminished GF, as well as the ultrafiltration coefficient with increase in glomerular capillary pressure, and altered sensitivity to vasoactive substances occur that reduce the auto-regulatory capacity and the functional renal reserve (Young, 1997). Diminished GF is more notorious in men (Xin, Dinesh, Dinesh, Ramesh & Nostratola, 2008; Pannarale, Carbone, Del Mastro, Gallo, Gattullo & Natalicchio, 2009), reaching about 66% at 70 years of age (Poortmans & Ouchinsky, 2006). Even though kidney function diminishes with age, it is preserved unless homeostasis is not challenged by extreme conditions such as high intensity exercise (Anderson et al., 2011).

Since the positive effects of practicing exercise on human health and retarding aging are evident (Aparicio García-Molina, Carbonell-Baeza & Delgado-

Fernández, 2010; Gálvez González, Caracuel Tubío & Jaenes Sánchez, 2011; Lera-López, Garrues, Ollo-López, Sánchez, Cabasés & Santos, 2017; Hall López, Ochoa, Alarcón. Moncada, García & Martín Dantas, 2017), the practice of exhausting exercise may cause kidney function modifications, such as reducing renal circulation and GF (Poortmans & Ouchinsky, 2006), as well as microalbuminuria (Robinson, Fisher, Forman & Curhan, 2010; Trejo et al., 2018). These occur because exhausting exercise increases perfusion to active muscles, while in other organs, like kidneys, perfusion can decrease up to 25% of resting values (Poortmans, 1984; Poortmans & Vanderstraeten, 1994).

It has been hypothesized that diminished blood flow to renal system, as well as decreased circulatory plasma volume, could attenuate kidney function while inducing ischemic kidney stress and even a ‘temporary’ kidney damage (Bonventre, 1988; Lippi, Schena, Salvagno, Tarperi, Montagnana, Gelati, & Guidi, 2008). Moreover, some studies suggest that exercise-induced dehydration, heat stress, inflammation and oxidative stress may also influence kidney function and induce renal stress (Otani, Kaya, & Tsujita, 2013; Hewing et al., 2015).

Farquhar & Kenney (1999) showed that GF diminished after 1 hour of moderate intensity physical exercise in people older than 64 years (57% of $\text{VO}_{2\text{max}}$). Whereas Hawkins, Sevick, Richardson, Fried, Arena, & Kriska (2011), suggest that systematic physical activity either moderate or severe, positively affect health, it is important to examine its relationship with kidney function. In addition, Touchberry, Ernsting, Haff, & Kilgore (2004), mention that it is necessary to establish the mechanisms that modify kidney function due to exercise, because they found a diminished GF in elite athletes and this effect could occur in different populations. On the other hand, Poortmans & Ouchinsky (2006), suggest that in the elderly the biological stress due to repetitive exercise could result in cumulative damage on GF, if exercise is not properly regulated.

It is known that exercise helps preserve and improve kidney health, it is necessary to define exercise intensity and duration in order to diminish or avoid possible kidney damage. Few studies that determined the effect of acute maximal and sub-maximal exercise on kidney function in the elderly have been reported. Then, we aimed to determine the effect of different intensities of physical exercise on glomerular filtration in apparently healthy older adults.

MATERIAL AND METHODS

Study sample

The design of the study is quasi-experimental, longitudinal, prospective and comparative. Twenty volunteers aged ≥ 65 years participated in the study; they were informed about the protocol procedures and signed an informed consent. Inclusion criteria were: to be sedentary, able to practice physical exercise as determined by medical interview and clinical and laboratory data. The study observed the General Health Law normative to human research in México as well as the Helsinki declaration.

Experimental design

Four sessions were set, in the first one the study sample was categorized for to know the health status of participants, in which blood chemistry, blood count (SPIN 120, Girona Spain), clinical records and basal electrocardiography (Mortara x scribe 5, WI, USA) were determined. In addition, anthropometrics measurements like body weight (Tanita SC 331S, Arlington Heights, IL, USA), height (SECA 213, Hamburg, Germany), Body Mass Index calculation (BMI, kg/m²) and body fat percentage (Tanita SC 331S) were determined. In the next three sessions physical exercise a maximal intensity with graded increments, as well as 80% and 60% sub-maximal intensities of reserve cardiac frequency (CF) found at maximal intensity test, were performed, according to:

$[(CF_{max/peak}^a - CF_{rest}) \% \text{ designed intensity} + CF_{rest}]$ (American College of Sports Medicine, 2018). The sequence of sub-maximal tests were randomly assigned and were conducted 1 week apart of each other.

Physical exercise tests

All physical exercise tests were conducted on a cycloergometer (Monark model 128 E, Vasnbro, SW) with an established pedaling frequency of 50 rpm. The incremental exercise test started with a load of 0,5 kp (kiloponds), augmenting the load in 0,5 kp each 2 min. The following parameters were considered as indicators of maximal test: unable to keep pedaling frequency at 50 rpm in the last 15 seconds and CF equal or higher than 90% of CF predicted for age, according to Karvonen formula (Karvonen, Kentala, & Mustala, 1957). Cardiac frequency was monitored through a CF transmitter (Polar RS 400, Oulu, Finland), and the electrocardiographic recordings with a 12 derivations electrocardiograph (Mortara x scribe 5). Maximal oxygen consumption ($VO_{2\text{Max}}$) was estimated according to the formula $Y = 10.51(W) + 6.35(\text{kg}) - 10.49(\text{yr}) + 519.3 \text{ ml} \cdot \text{min}^{-1}$; $R = 0.939$, $\text{SEE} = 212 \text{ ml} \cdot \text{min}^{-1}$.

Females: $Y = 9.39(W) + 7.7(\text{kg}) - 5.88(\text{yr}) + 136.7 \text{ ml} \cdot \text{min}^{-1}$; $R = 0.932$, $\text{SEE} = 147 \text{ ml} \cdot \text{min}^{-1}$ validated by the American College of Sports Medicine (Storer, Davis, & Caiozzo, 1990). Sub-maximal exercise tests were pedaling 20 min without rest, while CF was recorded continuously.

Estimating Glomerular Filtration

To determine serum creatinine to estimate GF, immediately before and after each one of the physical exercise tests blood samples were withdrawn from the antecubital vein. From these serum creatinine concentrations (Cr), FG was estimated in $\text{ml}/\text{min} \times 1.73 \text{ m}^2$, using above equation adjusted to mg/dL of creatinine: $FG = 1.86 \times (Cr)^{-1.154} \times \text{age}^{-0.203} \times 0.742$ if the subject is a woman (Gracia, Montañés, Bover, Cases, Deulofeu, Martín de Francisco & Orte 2006).

Statistical Analysis

To know the statistics to be used, first we made descriptive analysis. Data distribution was analyzed according to Z of Kolmogorov-Smirnov; once normal distribution was tested, the following parametrics were done: two way ANOVA to analyze difference between times (basal and final) and protocols (exercise type), and Student's *t* test of related samples for differences among pairs. Table 1 shows mean \pm standard deviation ($x, \bar{x} \pm SD$) and figure 1 in $x, \bar{x} \pm 95\%$ confidence interval. A value of $p < 0,05$ was considered significant.

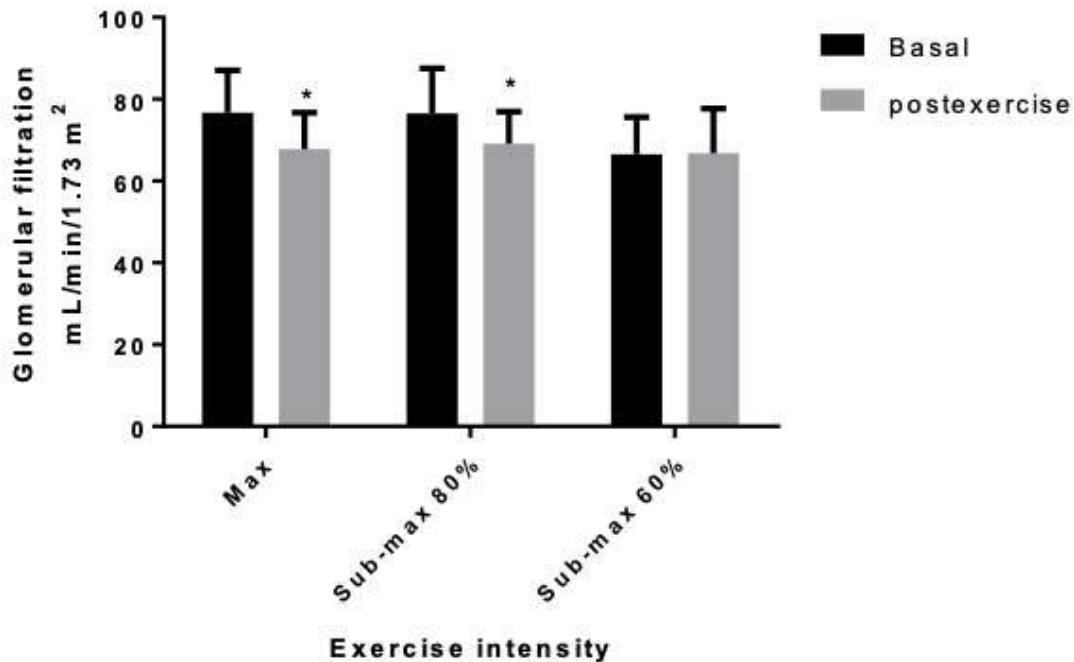
RESULTS

Table 1 describes participants' characteristics, where healthy values for BMI and fat % according to age are shown.

Table 1. General characteristics of participants, $n= 20$.

Variable	$x, \bar{x} \pm SD$
Age (years)	$69,8 \pm 4$
Weight (kg)	$70,6 \pm 11,7$
Height (cm)	$162,3 \pm 9,1$
BMI	$26,8 \pm 3,5$
% Fat	$24,6 \pm 6,2$

As shown in figure 1, GF was not affected at 60% sub-maximal exercise intensity, but at 80% sub-maximal and maximal exercise it diminished in average 8.3 mL/min/1.73 m² at the end of exercise bout ($p < 0,05$). Between exercise protocols significance was observed in the basal values at 60% sub-maximal exercise compared to the other groups.



EJES: Glomerular Filtration ($\text{mL}/\text{min} \times 1.73 \text{ m}^2$) vs Exercise Intensity Basal post-exercise
Figure 1. Glomerular filtration before and after physical exercise, $n= 20$ ($\bar{x}, \pm SD$). * $p < 0,05$.

DISCUSSION

In this study participants showed a GF ($73.6 \pm 6.3 \text{ ml}/\text{min}/1.73 \text{ m}^2$) lower to that reported for young adults of both genders ($128.82 \pm 29.28 \text{ ml}/\text{min}/1.73 \text{ m}^2$), due mainly to the aging process (Xin, Dinesh, Xueqing, Ramesh, & Nostratola, 2008). All 3 determinations of GF before maximal and sub-maximal physical exercise tests were significantly different, suggesting that estimating GF through seric creatinine concentration in older adults is not a good index due to its variability, as has been suggested by Swedko et al. (2003) where serum creatinine is inadequate to detect renal failure in old adults. Currently new markers have been proposed to determine GF, like cistatin C that could be better indicators of GF (Ferguson & Waikar, 2012).

Maximal exercise physical test provoked a significant diminution in GF estimated via serum creatinine, and the same decrease was obtained after 80% sub-maximal exercise test in agreement with Poortmans & Ouchinsky (2006), who reported 12% decrease in GF from old adults subjected to acute exhausting exercise, which might indicate that the physiological mechanisms of GF function similarly at these two physical exercise intensity in these persons. Whereas the 60% sub-maximal test did not modify GF. In another study on ultramarathon bikers a diminished GF was recorded immediately after exercise ($85 \pm 19 \text{ mL}/\text{min}$), compared with basal values ($114 \pm 27 \text{ mL}/\text{min}$), and returning to basal in the next 24h after exercise ($113 \pm 28 \text{ mL}/\text{min}$) (Neumayr, Pfister, Hoertnagl, Mitterbauer, Getzner, Ulmer, ... & Joannidis, 2003). In our study we only monitored the acute effect of physical exercise on GF, without determining if the effect was kept 24h after exercise.

Other authors observed that both exercise intensity as well as duration influence the decrease of GF (Poortmans, Blommaert, Baptista, De Broe, & Nouwen, 1997; Poortmans & Ouchinsky 2006; Banfi, Del Fabbro, & Lippi, 2009). In this work we corroborated their results, since acute maximal and 80% sub-maximal exercise showed GF changes determined by serum creatinine concentration when the last test lasted 20 min; while the maximal test was 11 min as average.

CONCLUSION

Even though GF is diminished, there was lower risk for participants since their GF did not decrease, indicating that physical exercise of moderate intensity lasting 20 min and those tests of maximal exercise did not impact kidney function in apparently healthy old adults.

This study reports that in acute maximal and 80% sub-maximal exercise A diminished GF occurs; while at 60% sub-maximal intensity GF is not modified and the physiological kidney response in healthy old adults is not unfavorable.

Study Limitations

The low number of participants in the sample is due to a low prevalence of clinical healthy old adults, since most individuals in this age group bear pathologies that influence kidney function, like hypertension, diabetes, cardiovascular disease and even kidney failure. This panorama guarantees to make studies on kidney response to acute physical exercise in subjects with those pathologies.

REFERENCES

- American College of Sports Medicine. (2018) ACSMs Guidelines for exercise testing and prescription, Thenth edition. *American College of Sports Medicine*. p150.
- Anderson, S., Eldadah, B., Halter, J. B., Hazzard, W. R., Himmelfarb, J., Horne, F. M., ... & Schmader, K. E. (2011). Acute kidney injury in older adults. *Journal of the American Society of Nephrology*, 22(1), 28-38. <https://doi.org/10.1681/ASN.2010090934>
- Aparicio García-Molina, V.A.; Carbonell-Baeza, A. & Delgado-Fernández, M. (2010). Beneficios de la actividad física en personas mayores. *Revista internacional de Medicina y Ciencias de la Actividad Física y del Deporte*, 10(40), 556-576.
- Banfi, G., Del Fabbro, M. & Lippi, G. (2009). Serum creatinine concentration and creatinine-based estimation of glomerular filtration rate in athletes. *Sports Medicine*, 39(4), 331-337. <https://doi.org/10.2165/00007256-200939040-00005>
- Bonventre J. (1988). Mediators of ischemic renal injury. *Annual Review of Medicine*, 39:531–544. <https://doi.org/10.1146/annurev.me.39.020188.002531>

- Cirillo M. (2010). Evaluation of glomerular filtration rate and of albuminuria/proteinuria. *Journal of Nephrology*, 23(2), 125-132.
- Douville, P., Martel, A. R., Talbot, J., Desmeules, S., Langlois, S., & Agharazii, M. (2008). Impact of age on glomerular filtration estimates. *Nephrology Dialysis*. <https://doi.org/10.1093/ndt/gfn473>
- Farquhar, W. B., & Kenney, W. L. (1999). Age and renal prostaglandin inhibition during exercise and heat stress. *Journal of Applied Physiology*, 86(6), 1936-1943. <https://doi.org/10.1152/jappl.1999.86.6.1936>
- Ferguson, M. A. & Waikar, S. S. (2012). Established and emerging markers of kidney function. *Clinical chemistry*, 58(4), 680-689. <https://doi.org/10.1373/clinchem.2011.167494>
- Gálvez González, J.; Caracuel Tubío, J.C. & Jaenes Sánchez, J.C. (2011). Práctica de Actividad física y velocidad de procesamiento cognitivo en mayores. *Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte*, 11(44), 803-816.
- Gracia S, Montañés R, Bover J, Cases A, Deulofeu R, Martín de Francisco A & Orte L. (2006). Documento de consenso: Recomendaciones sobre la utilización de ecuaciones para la estimación del filtrado glomerular en adultos. *Nefrología*. 26(6), 658-665.
- Gekle M. (2017). Kidney and aging: a narrative review. *Experimental Gerontology*, 87, 153-155. <https://doi.org/10.1016/j.exger.2016.03.013>
- Hall López, J.A., Ochoa Martínez, P.Y., Alarcón Meza, E.I., Moncada-Jiménez, J.A., García Bertruy, O. & Martín Dantas, E.H. (2017). Programa de entrenamiento de hidrogimnasia sobre las capacidades físicas de adultas mayores. *Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte*, 17(66), 283-298. <https://doi.org/10.15366/rimcafd2017.66.005>
- Hawkins, M. S., Sevick, M. A., Richardson, C. R., Fried, L. F., Arena, V. C., & Kriska, A. M. (2011). Association between physical activity and kidney function: National Health and Nutrition Examination Survey. *Medicine and Science in Sports and Exercise*, 43(8), 1457-1464. <https://doi.org/10.1249/MSS.0b013e31820c0130>
- Hewing, B., Schattke S., Spethmann S., Sanad W., Schroechk S., Schimke I., et al. (2015). Cardiac and renal function in a large cohort of amateur marathon runners. *Cardiovascular Ultrasound*, 13:13. <https://doi.org/10.1186/s12947-015-0007-6>
- Karvonen, M. J., Kentala, E., & Mustala, O. (1957). The effects of training on heart rate; a longitudinal study. *Annales Medicinae Experimentalis et Biologiae Fenniae*, 35(3), 307.
- Lera-López, F., Garrues Irisarri, M.A., Ollo-López, A., Sánchez Iriso, E., Cabasés Hita, J.M. & Sánchez Santos, J.M. (2017). Actividad física y salud autopercebida en personas mayores de 50 años. *Revista Internacional de Medicina y Ciencias de la Actividad Física y Deporte*, 17(67), 559-571. <https://doi.org/10.15366/rimcafd2017.67.011>
- Levey, AS, Greene T, Kusek JW, et al. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol*, 2000, vol. 11pg. 155A
- Lippi G., Schena L., Salvagno C., Tarperi M., Montagnana M., Gelati M, Guidi GC. (2008). Acute variation of estimated glomerular filtration rate following

- a half-marathon run. *Internacional Journal of Sports Medicine*, 29, 948–951. <https://doi.org/10.1055/s-2008-1038745>
- Neumayr, G., Pfister, R., Hoertnagl, H., Mitterbauer, G., Getzner, W., Ulmer, H., ... & Joannidis, M. (2003). The effect of marathon cycling on renal function. *International Journal of Sports Medicine*, 24(02), 131-137. <https://doi.org/10.1055/s-2003-38205>
- Nyengaard, J. & Bendtsen, T. (1992). Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *The Anatomical Record*, 232(2), 194-201. <https://doi.org/10.1002/ar.1092320205>
- Otani, H., Kaya M., & Tsujita J. (2013). Effect of the volume of fluid ingested on urine concentrating ability during prolonged heavy exercise in a hot environment. *Journal of Sports Science Medicine*, 12, 197–204.
- Pannarale, G., Carbone, R., Del Mastro, G., Gallo, C., Gattullo, V., Natalicchio, L. & Tedesco, A. (2009). The aging kidney: structural changes. *Journal of Nephrology*, 23, S37-40.
- Poortmans, J. R. (1984). Exercise and renal function. *Sports Medicine*, 1, 125–153. <https://doi.org/10.2165/00007256-198401020-00003>
- Poortmans J. R. & Vanderstraeten J. (1994). Kidney function during exercise in healthy and diseased humans. *Sports Medicine*, 18, 419–437. <https://doi.org/10.2165/00007256-199418060-00006>
- Poortmans J. R., Blommaert E., Baptista M., De Broe M. E., & Nouwen E. J. (1997). Evidence of differential renal dysfunctions during exercise in men. *European Journal of Applied Physiology and Occupational Physiology*, 76(1), 88-91. <https://doi.org/10.1007/s004210050217>
- Poortmans J. R., & Ouchinsky M. (2006). Glomerular filtration rate and albumin excretion after maximal exercise in aging sedentary and active men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 61(11), 1181-1185. <https://doi.org/10.1093/gerona/61.11.1181>
- Robinson, E. S., Fisher, N. D., Forman, J. P., & Curhan, G. C. (2010). Physical activity and albuminuria. *American Journal of Epidemiology*, 171(5), 515-521. <https://doi.org/10.1093/aje/kwp442>
- Stevens, L.A. & Levey, A.S. (2009) Measured GFR as a confirmatory test for estimated GFR. *Journal American Society Nephrology*, 20(11), 2305-2313. <https://doi.org/10.1681/ASN.2009020171>
- Storer, T. W., Davis, J. A., & Caiozzo, V. J. (1990). Accurate prediction of VO₂max in cycle ergometry. *Medicine and Science in Sports and Exercise*, 22(5), 704-712. <https://doi.org/10.1249/00005768-199010000-00024>
- Suzuki, M. (2015). Physical exercise and renal function. *The Journal of Physical Fitness and Sports Medicine*, 4(1), 17-29. <https://doi.org/10.7600/jpfsm.4.17>
- Swedko, P.J., Clark, H.D., Paramsothy, K., & Akbari, A. (2003). Serum creatinine is an inadequate screening test for renal failure in elderly patients. *Archives of Internal Medicine*, 163, 356-360. <https://doi.org/10.1001/archinte.163.3.356>
- Touchberry Ch, Ernsting M, Haff G, Kilgore J. (2004). Training alterations in elite cyclists may cause transient changes in glomerular filtration rate. *Journal of Sport Science & Medicine*, 3, 28-36.

- Trejo M, Díaz F, Kornhauser C, Macias M, Najera M, Arce R, Pineda-Espejel H. (2018). Efecto del ejercicio máximo y submáximo sobre excreción de albúmina en adultos mayores, *RETOS*, 33, 238-240.
- Xin J, Dinesh R, Xueqing Y, Ramesh S, y Nostratola D. (2008). The aging kidney. *Kidney International*, 74, 710-720. <https://doi.org/10.1038/ki.2008.319>
- Young, A. (1997). Aging and physiological functions. *Philosophical Transactions of the Royal Society B: Biological*, 352, 1837-1843. <https://doi.org/10.1098/rstb.1997.0169>

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