

Effects of caffeine (3 mg) on maximal oxygen consumption, plasmatic lactate and reaction time after maximum effort*.

Efectos de la utilización de 3 mg de cafeína sobre los niveles de potencia aeróbica máxima, de lactato plasmático y del tiempo de reacción simple, ambos después de la realización de esfuerzo máximo*.

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Abstract

The stimulants caffeine effects on sport performance have been widely investigated. The Maximal Oxygen Uptake (MOU) has been used in recent researches which aim to elucidate mechanisms of caffeine during maximal effort. As a physiological pattern to evaluate the effect of caffeine during the effort and after it (recovery), plasmatic lactate is presented in many studies. In this context, the present study aimed to investigate physiological changes: VO₂ MAX on an ergometric device (speed and grade on a treadmill); plasmatic lactate (L) and modification of cognitive and motor performance (Reaction Time Test - RTT) produced by caffeine.

Five apparently healthy volunteers (26 ± 5 years; 67 ± 12.5 kg) were submitted twice to the following routine: plasmatic lactate at rest (L 0), reaction time test at rest RTT (R), maximum effort test on treadmill, plasmatic lactate concentrations at minute 1 (L 1), 2 (L 2) and 4 (L 3) after effort, and RTT (1). They were given either one placebo capsule (400 mg corn starch) or caffeine (3 mg/kg of body weight). Two-way ANOVA with repetition was used to compare variables at placebo (P) and caffeine (C) moments. The caffeine moment presented non-significant reduction in RRT, non-significant increase in plasmatic lactate and non-significant modification in VO₂ MAX, when compared to placebo moment. Thus, one can conclude that 3 mg/kg/bw of caffeine with 12 h of abstinence, presented non-significant effects in maximal oxygen uptake, plasmatic lactate and in simple reaction time.

Resumen

Los efectos estimulantes de la cafeína en el desarrollo atlético vienen siendo ampliamente investigados. El consumo Máximo de Oxígeno (VO₂ MAX) ha sido empleado en estudios recientes que buscan elucidar los mecanismos de la cafeína durante el esfuerzo máximo a través de métodos neurológico así como fisiológicos. En este contexto, este estudio objetiva analizar las variaciones generadas por la cafeína en respuestas ergoespirométrica (VO₂ MAX), plasmática (Lactato-L) y motora (Test del Tiempo de Reacción - TTR). Para eso, 5 individuos hígidos, todos hombres, (26 ± 5 años, 67 ± 12,5 kg) se sometieron dos veces a la siguiente rutina: dosaje de lactato plasmático (L0), TTR (0), test de esfuerzo máximo en estera ergométrica, dosaje de lactato (L1), (L2), (L3), y TTR(1). Les fue administrada una cápsula de 400 mg de placebo (almidón de maíz) o 3 mg/kg/peso corpóreo (pc) de cafeína. Fue empleada la ANOVA de dos factores con repetición para la comparación de las variables en los momentos C, P. El momento cafeína presentó reducción no significativa en el tiempo de reacción, en el lactato plasmático y en VO₂ MAX. Así, se puede concluir que cafeína 3 mg/kg/pc con abstinencia de 12 h. no presenta efectos significativos en el consumo máximo de oxígeno, así como en la concentración de lactato plasmático y en el tiempo de reacción simple.

Palabras clave: cafeína, VO₂ MAX, lactato plasmático, tiempo de reacción.

Key Words: caffeine, VO₂ MAX, plasmatic lactate, reaction time.

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Introduction

The ergogenic aids can be classified as nutritional, mechanical, pharmacological, physical and psychological, including legal and safe procedures as carbohydrate supplementation, or even illicit and unsafe ones like anabolic steroids and blood infusion (Thein *et al.*, 1995).

In this sense, caffeine has frequently been used, in an acute way, previously to physical exercises, with the intention to delay fatigue and consequently improve athletic performance, especially in endurance activities (Delbeke, 1984; Jacobson, 1989; Spriet, 1995).

There are three mechanisms that may explain the caffeine action at cellular level (calcium mobilization by the sarcoplasmic reticulum, inhibition of the enzyme phosphodiesterase and the adenosine receptor's antagonism). However, the main mechanism of caffeine's action at cellular level is undoubtedly the antagonism to the adenosine receptors, since it's the only mechanism observed *in vivo*. It's well defined that caffeine is an antagonist competitor of the adenosine receptors, acting in these receptors in a lot of varied areas, like the whole body peripheral circulation and at the brain cortex (Cauli and Morelli, 2005).

A study using caffeine dose of 9 mg/kg/bw increased run time to exhaustion at 85% of $\text{VO}_2 \text{ MAX}$ in elite runners and cyclists in 44% and 51% respectively (Graham *et al.*, 1991). Other authors accept that caffeine is an ergogenic aid in doses from 3 to 9 mg/kg and that it can prolong significantly the time to exhaustion in high intensity (80% $\text{VO}_2 \text{ MAX}$) endurance exercises (Graham, 1994; Nehlig, 1994; Sinclair, 2000).

Nevertheless, Pasma *et al.* (1995) examined the effect of different dosages of caffeine (0-5-9-13 mg.kg body weight⁻¹) on endurance performance, and a significant increase in endurance performance was found for all caffeine tests compared to placebo (endurance time 47 +/- 13, 58 +/- 11, 59 +/- 12 and 58 +/- 12 min for 0, 5, 9 and 13 mg.kg⁻¹ body weight, respectively). There is no relationship between the dosages of caffeine and the improve in the performance.

Anselme *et al.* (1992) and Collomp *et al.* (1992) speculated that the increase in catecholamines, during intense exercise, increases glycogenolysis and this lead to a greater anaerobic metabolism, resulting in a higher lactate production and muscular strength. The combination of caffeine and ephedrine prolonged significantly the time to exhaustion, compared to placebo. While neither caffeine nor ephedrine treatments alone significantly changed time to exhaustion. The improved performance was attributed to increased central nervous system stimulation (Bell *et al.*, 1998).

* The present study has considered the Norms for the Research Performance in Human Beings, Resolution 196/96 from the Health National Council of 10/10/1996 and has been approved by the Ethics Committee – UCB – RJ.

* Los aspectos éticos fueron observados de acuerdo con la Resolución N° 196/96 del Consejo Nacional de Salud, sobre investigación con seres humanos en el Brasil em 10/10/96 y con aprobación de la Comisión de Ética de la Universidad Castelo Branco – RJ.

Lactate is the final product of the anaerobic glycolysis that occurs in hypoxic tissues, although, well oxygenated tissues can, in certain conditions, originate lactate through aerobic glycolysis. The normal lactate production is of 1 mmol/kg/hour and it occurs mainly in the skeletal muscle, intestine, brain and red globules. To Calderón (2006), the determination of released metabolites in plasma is of fundamental importance on training intensity determination, and lactate is the most relevant and used to such control.

Some studies (Bridge e Jones, 2006; Doherty *et al.*, 2004) mention the increase of concentration of plasmatic lactate in association to caffeine consumption after physical effort. On the other hand, others (Haller *et al.*, 2004; Collomp *et al.*, 2002) narrate no significant difference in lactate concentration in association with caffeine use. The belief diffused that intense exercise leads to “lactic acid” production, which contributes to acidosis, is questionable. In the stepwise breakdown of a molecule of glucose in two pyruvate ones, three reactions release the total of four protons and one reaction consumes two protons. Thus, the lactate production should retard instead of contribute to acidosis (Robergs *et al.*, 2004).

Oxygen uptake is considered the standard to measure the physiological intensity of exercise. $\text{VO}_2 \text{ MAX}$ may be defined as the highest oxygen (O_2) uptake accomplished by an individual breathing air at sea level (Astrand, 1952).

But physical performance is not the only scope concerned by researchers using caffeine. Some authors (Smith, 2002; Lorist, 2003; Daniel, 2005) inquiry the effects of caffeine in psychomotor responses. To evaluate possible effects of caffeine (3 mg/kg/bw) on cognition and motor responses, this study adopted the simple Reaction Time Test (RTT).

Juliano and Griffiths (2004) undertook a comprehensive review of the literature about human caffeine withdrawal; they found that 10 symptoms were validated for caffeine withdrawal: headache, fatigue, drowsiness, decreased contentedness, depressed mood, difficulty on concentrating, irritability, and foggy/not clearheaded. Also, flu symptoms like, nausea/vomiting, and muscle pain/stiffness were judged likely to represent valid symptom categories. In this manner, this study aim to evaluate the effect of 3 mg/kg/bw of caffeine on simple reaction time, $\text{VO}_2 \text{ MAX}$ and plasmatic lactate after 12 h of caffeine abstinence avoiding possible discomfort due to the syndrome of abstinence, presented in previous data.

Material and method

The study took place in the Brazilian Army Institute of Research and Fitness, Rio de Janeiro, and it could count on its support for its development.

This study was approved by the Ethic Committee Research of the Castelo Branco University, Rio de Janeiro, according to the rules of the National Council of Health's Resolution 196/96 on researches involving human beings. After being previously clarified about the purposes of the investigation and the related procedure which they would be submitted to, each participant signed a free and clarified consent term.

All the participants were volunteers. As the initial criteria to the inclusion, all the participants should practice everyday physical activity and present no health limitations. Smokers weren't accepted to take part in the investigation.

Subjects

5 apparently healthy subjects (men, 26 ± 5 y. o.), non smokers, physically active and abstinent from caffeine and alcohol for at least 12 hours, took part in the experiment. Their average stature and weight were 174.8 ± 14.1 cm and 75.7 ± 5.3 kg, respectively. They were not following any treatment with psychotropic or psychoactive drugs. Their average consumption of caffeine was under 400 mg/day.

Procedures

The subjects received one capsule (placebo or caffeine 3 mg/kg/bw) in two different days with at least 4 days of interval between them. The procedures consisted of two visits to the laboratory following the routine: plasmatic lactate dosage (L_0), execution of the reaction time test $RTT_{(0)}$, capsule ingestion (placebo or caffeine). The maximum effort test on treadmill occurred after 60 min of the ingestion of the capsule and it was followed by lactate dosages at first (L_1), second (L_2) and third minutes (L_3) and $RTT_{(1)}$.

Plasmatic lactate

The plasmatic lactate was collected before and 1 (L_1), 2 (L_2) and 4 min (L_3) after maximum treadmill test. The analysis method applied was automatized enzymatic colorimetric (Cobas Mira Plus – Roche[®]), with the Katal[®] kit. Results from subjects at day one (placebo treatment – 400 mg corn starch) were compared with their results at day two (caffeine treatment – 3 mg/kg/bw).

Reaction Time Test

Reaction time was used as an index of individuals' motor performance. Volunteers responded to the target stimulus by pressing a button in a joystick. The joystick was used to measure individuals' reaction time at each trial. Reaction times (100 stimuli)

were subsequently averaged to yield a final value for each subject. Missed stimuli were not considered.

The software was developed at the Brain Mapping and Sensorimotor Integration Laboratory (Rio de Janeiro – Brazil). The first RTT took part at the time the volunteer arrived at the laboratory at rest, RTT_(R), before the capsule ingestion (placebo or caffeine). Soon after collecting of the last blood sample for lactate analysis, volunteers executed the last RTT₍₁₎.

Maximal Aerobic Power Test

For the direct VO_{2 MAX} measurement, the gas analyzer system used was Cpx-d Medical Graphics (Minnesota – USA) at treadmill model Super ATL – Inbrasport (Porto Alegre - RS, 1997). The maximal aerobic test (MAT) was applied individualized to each volunteer following the ramp protocol in treadmill. It is a continuous protocol, without probations, where the load increment occurs continuously and gradually during the time effort. The load rate was increased in accordance to each volunteer's fit state and it started at 1% inclination at 7.5 km/h velocity.

A neoprene mask with a specific valve to ergoespirometry was attached to the subject previously and the test procedure was explained to them. There was a warm up period of 4 min, walking or running comfortably until the protocol starts, followed by progressive and continuous incremental velocity and grade of inclination until the subject attained his maximal oxygen consumption predetermined to be reached in 12 min at most (Wasserman *et al.*, 1999). Their VO_{2 MAX} at placebo day was compared to caffeine day.

Results

Preliminary descriptive analysis indicated that none of the analyzed variables presented normal distribution. One-way ANOVA was applied and statistical analyses indicated a no significant difference between treatments (placebo and caffeine). The one-way ANOVA presented significant difference between samples based on F values to VO_{2 MAX}. F test for two samples (placebo and caffeine) was used to variance analysis. T test to two samples presuming equivalent variance showed no meaningful difference.

Concentrations of plasmatic lactate were analyzed by enzymatic-colorimetric method (table 1). Two-way ANOVA was used without repetition with $\alpha \leq 0.05$.

Table 1. Concentrations of plasmatic lactate in four different moments to placebo and caffeine (3 mg/kg/bw) days.

DAY	L0	L1	L2	L3
PLACEBO	1.5 ± 0.5	14.6 ± 3	14.8 ± 1.8	15 ± 1.7
3mg CAFFEINE	1.6 ± 0.4	14.9 ± 3.1	15.4 ± 3.1	16.5 ± 3.6

*Mean values to concentration (mmol/L).

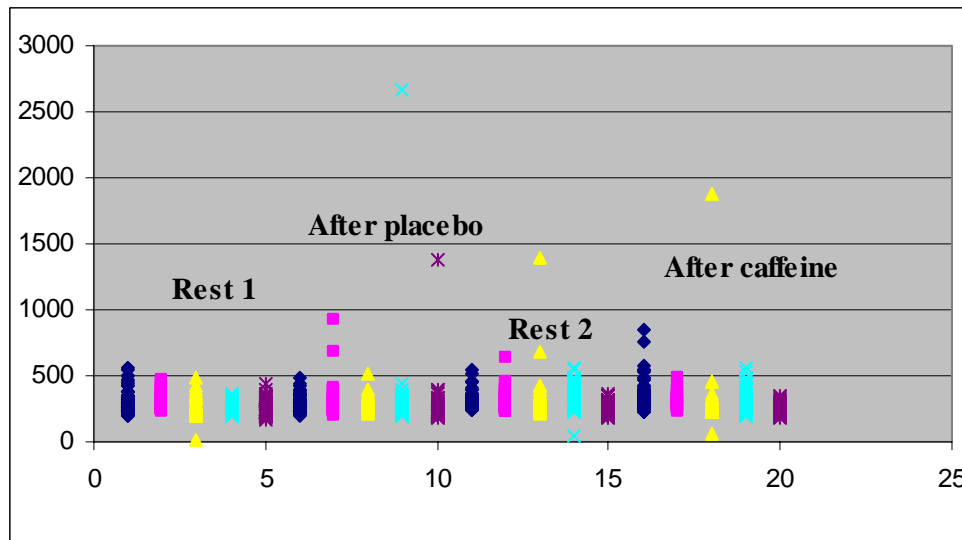
Each volunteer executed four RTT in total: (RTT_R) as soon as arrived at laboratory, at rest; RTT_{1P} after maximum effort with placebo; RTT_{R2} at rest on the second day as soon as arrived; RTT_{1C} after maximum effort with caffeine 3 mg/kg/bw (table 2).

Table 2. Average and standard deviation values of volunteers RTTs in four moments.

VOLUNTEER	RTT _R	RTT _{1P}	RTT _{R2}	RTT _{1C}
1	292.93 ± 71.41	273.49 ± 54.67	308.83 ± 58.20	337.92 ± 98.08
2	299.87 ± 52.79	292.26 ± 85.42	300.89 ± 59.57	303.23 ± 43.26
3	257.73 ± 36.82	288.66 ± 244.73	335.91 ± 78.16	305.16 ± 71.14
4	255.80 ± 55.35	279.68 ± 44.25	296.17 ± 125.67	287.58 ± 165.96
5	245.33 ± 46.39	242.98 ± 122.95	234.20 ± 37.97	232.14 ± 31.51

*Mean values in milliseconds.

One-way ANOVA was applied to evaluate the four moments in variance. There was significant difference among the four moments, but no significant difference among volunteers (table 3).



* Values in milliseconds/second.

Graph 1. Dispersion values graph of RTTs on two days tests.

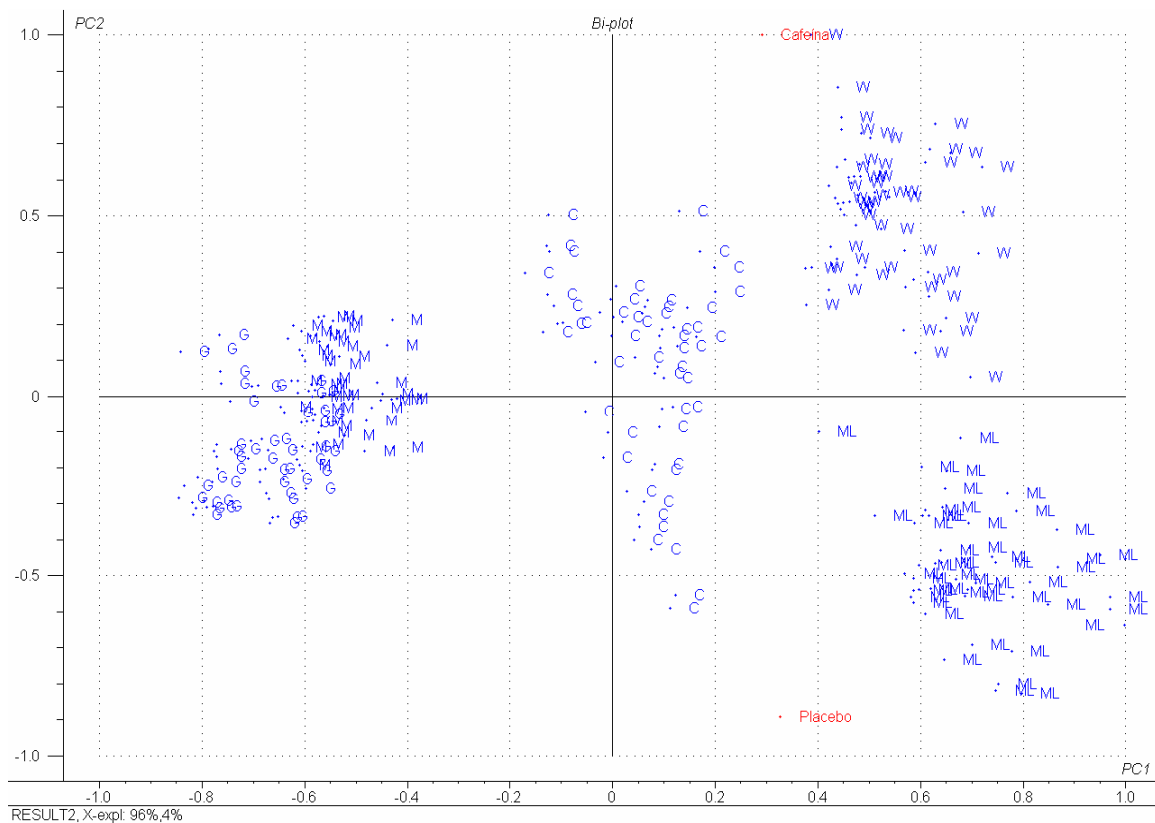
The results from effort test for VO₂ MAX were evaluated before the day volunteers had ingested caffeine (3 mg/kg/bw) (table 3).

Table 3. Average and standard deviation values of $VO_{2\text{MAX}}$ at maximum effort on treadmill (ramp protocol).

VOLUNTEER	$VO_{2\text{MAX}}$ PLACEBO	$VO_{2\text{MAX}}$ CAFFEINE
1	39.70 ± 0,72	39.69 ± 0.66
2	45.01 ± 1.36	45.20 ± 1.47
3	38.65 ± 0.81	37.47 ± 1.01
4	48.46 ± 1.08	50.26 ± 0.83
5	52.96 ± 1.42	48.89 ± 1.01

*Mean and standard deviation values of $VO_{2\text{MAX}}$ (mL/kg/min)

Through principal components analysis of $VO_{2\text{MAX}}$ data, it was verified a dispersion of the results showing a great difference among volunteers pointing out ineffective effect of 3 mg/kg/bw of caffeine on $VO_{2\text{MAX}}$ after maximum effort on ramp protocol (The Unscrambler® v 8.0).



Graph 2. Principal component analysis of $VO_{2\text{MAX}}$ with caffeine and placebo, after maximum effort.

* The letters presented in the graph (G, C, ML, W and M) mean the initials of the names of the volunteers.

Discussion

The purpose of this study was to evaluate possible caffeine (3 mg/kg/bw) and placebo effects on neuromotor function (reaction time), on plasmatic lactate and on $\text{VO}_2 \text{ MAX}$ after maximum effort at treadmill (ramp protocol).

To evaluate possible caffeine effects on sports performance during training routine, this study aimed to evaluate caffeine's action with a short abstinence time, avoiding, this way, possible discomforts reported in abstinence syndrome. Juliano (2004) reported that 50% of caffeine consumers, who abstained from caffeine for 12 to 24 h, presented at least one of the four main symptoms: headache, fatigue and dysphoria, lethargy, bad temper, irritation, depression, difficulty of concentration; and 13% felt clearly sick. Van Thuyne and Delbeke (2005) showed, in doping analysis, that caffeine concentration in weightlifters urine is superior if compared to other sports modality. This study also presented that the number of caffeine positive sample in cyclists, increased after removal of caffeine from the WADA (World Anti Doping Agency) prohibited list.

Kovacs *et al.* (1998), using 150, 225, and 320 mg caffeine/L supplying amounts of caffeine of 2.1, 3.2, and 4.5 mg/kg (body weight), respectively, observed ergogenic benefits on the performance, however, the highest dosage of 320 mg/L caffeine did not result in a further improvement compared with the 225 mg/L dosage.

Anselme *et al.* (1992) and Collomp *et al.* (1990; 1991; 1992) have frequently found an increase in concentration of plasmatic lactate during intense exercise with caffeine; and in other studies Collomp *et al.* (1991; 1990) showed an increase in power output. This could indicate that raised lactate production by active muscles, could even be due to decrease in its clearance. Besides that, increased plasmatic epinephrine, which is suggested as the stimulus to increase plasmatic fat free acid, is not always associated to better endurance performance (Laurent *et al.*, 2000). To Anselme *et al.* (1992) and Collomp *et al.* (1991) the epinephrine increase can be counter productive on endurance performance due to its stimulant effects on the glucose breakdown and on the plasmatic and muscular lactate. But plasmatic lactate studies are controversial (Robergs *et al.*, 2004).

Plasmatic lactate concentrations after effort with caffeine or placebo, showed no significant difference.

Volunteers $\text{VO}_2 \text{ MAX}$ presented no significant difference between caffeine and placebo treatments. Future studies, with larger sample groups, are necessary to reach significant statistical results.

As expected, it was observed a significant increase in lactate concentration after effort, even in subjects who have ingested placebo. Significant difference between plasmatic lactate concentrations from placebo and caffeine (3 mg/kg/bw) weren't found. Such results suggested that 3 mg/kg/bw of caffeine in this situation didn't present ergogenic effect on $\text{VO}_2 \text{ MAX}$, plasmatic lactate or reaction time after maximum effort at treadmill, following the ramp protocol.

Many studies have presented improvement in both physical performance and mood state with caffeine ingestion, usually with more than 24 h abstinence from caffeine previous to study. But the question that remains unclear is whether the improvement is due to caffeine's ergogenic effect or is due to the reverse effect of abstinence syndrome.

Although caffeine is widely perceived to have beneficial psychostimulant effects, appropriately controlled studies show that its apparent beneficial effects on performance and mood are almost wholly attributable to reversal of the withdrawal effects that occur after fairly short periods of abstinence (e.g. overnight) (James and Rogers, 2005).

No abstinence syndrome symptoms were recorded by volunteers in this study.

Conclusion

In accordance with Yeomans et al. (2002) after been analyzed the variables (plasmatic lactate, reaction time and $VO_{2\text{ MAX}}$) with placebo and caffeine (3 mg/kg/bw), there was no significant difference among tests results, opposing some previous studies that, in different circumstances, evaluated the same variables individually with this same dose. As a limitation of the study, the group of volunteers was small and presented great data variance. Larger group could result in more robust results.

References

- Anselme, F., Collomp, K., Mercier, B., ahmaidi, S., Prefaut, C. (1992) Caffeine increases maximal anaerobic power and blood lactate concentration. *Eur. J. Appl. Physiol. Occup. Physiol.* 65: 188–191.
- Astrand, P. O. (1952) Experimental studies of physical work capacity in relation to sex and age. Copenhagen: Ejnar Munksgaard.
- Bridge, C. A., Jones, M. A. (2006) The effect of caffeine ingestion on 8 km run performance in a field setting. *J. Sports Sci.* 24(4): 433-9.
- Calderón, J., Benito, P., Meléndez, A., González, M. (2006). Control biológico del entrenamiento de resistencia. *Revista Internacional de Ciencias del Deporte*. 2 (2), 65-87.
- Cauli, O., Morelli, M. (2005) Caffeine and the dopaminergic system. *Behav. Pharmacol.* 16(2): 63-77.
- Collomp, K., C. Caillaud, M. Audran, J.-L. Chanal, C. Prefaut (1990) Influence de la prise aigue ou chronique de cafeine sur la performance et les catecholamines au cours d'un exercice maximal. *C. R. Soc. Biol.* 184: 87–92.

- Collomp, K., S. Ahmaidi, M. Audran, J.-L. Chanal, C. Prefaut (1991) Effects of caffeine ingestion on performance and anaerobic metabolism during the Wingate test. *Int. J. Sports Med.* 12: 439–443.
- Collomp, K., Ahmaidi, S., Chatard, J. C., Audran, M., Prefaut, C. (1992) Benefits of caffeine ingestion on sprint performance in trained and untrained swimmers. *Eur. J. Appl. Physiol. Occup. Physiol.* 64: 377–380.
- Collomp, K., Candau R, Millet, G, Mucci, P., Borrani, F., Prefaut, C., de Ceaurriz, J. (2002) Effects of salbutamol and caffeine ingestion on exercise metabolism and performance. *Int J Sports Med.* 23(8):549-54.
- Dalvi, R.R. (1986) Acute and chronic toxicity of caffeine: A review. *Vet. Hum. Toxicology*, 28:144-150.
- Delbeke, F.T., Debachere, M. (1984) Caffeine: use and abuse in sports. *International Journal of Sports Medicine* 5(4): 179-82.
- Doherty, M., Smith, P., Hughes, M., Davison, R. (2004) Caffeine lowers perceptual response and increases power output during high-intensity cycling. *J. Sports Sci.* 22 (7): 637-43.
- Gaesser, G. A., Poole, D. C. (1996) The slow component of oxygen uptake kinetics in humans. *In: Exercise and Sport Sciences Reviews*, ed. Holloszy O, William & Wilkins, Baltimore, 24: 35–70.
- Graham, T. E., Spriet, L. L. (1991) Performance and metabolic responses to a high caffeine dose during prolonged exercise. *J. Appl. Physiol.* 71: 2292-2298.
- Graham, T. E., Rush, J. W. E., Van Soeren, M. H. (1994) Caffeine and exercise: metabolism and performance. *Can. J. Appl. Physiol.* 19: 111–138.
- Haller, CA., Jacob, P. 3RD, Benowitz, NL. (2004) Enhanced stimulant and metabolic effects of combined ephedrine and caffeine. *Clin Pharmacol Ther.* 75(4):259-73.
- Jacobson, B.H., Kulling, F.A. (1989) Health and ergogenic effects of caffeine. *British Journal of Sports Medicine* 23(1): 34-40.
- Juliano, L. M. Groffiths, R. R. (2004) A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology (Berl)*. 176 (1):1-29.
- James, J. E.; Rogers, P. J. (2005) Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation *Psychopharmacology* 182: 1–8
- Kovacs, E., Steagen, M. R., Jos, H. C. H., Brouns, F. (1998) Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *J. Appl. Physiol.* 85(2): 709-715.
- Laurent, D., Schneider, K. E., Prusaczyk, W. K., Franklin, C., Vogel, S. M., Krssak, M., Petersen, K. F., Goforth, H. W., Shulman, G. I. (2000) Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J Clin Endocrinol Metab* 85: 2170–2175.

- Myers, J., walsh, D., Sullivan, M., Froelicher, V. F. (1989) Can maximal cardiopulmonary capacity be recognized by a plateau in oxygen uptake? *Chest.*, 96: 1312-1316.
- Myers, J., Walsh, D., Sullivan, M., Froelicher, V. F. (1990) Effects of sampling on variability and plateau in oxygen uptake. *Journal of Applied Physiology* 68: 404-410
- Nehlig, A., Debry, G. (1994) Caffeine and sports activity: a review. *Int. J. Sports Med.* 15: 215–223.
- Noakes, T. D. (1988) Implications of exercise testing for prediction of athletic performance: A contemporary perspective. *Medicine and Science Sports Exercise.* 20: 319-330.
- Pasman, W. J., Baak, M. A., Jeukendrup, A. E., Haan, A. (1995) The effect of different dosages of caffeine on endurance performance time. *Int. J. Sports Med.* 16(4): 225-330.
- Poole, D. C., Schaffartzik, W., Knight, D. R., Derion, T., Kennedy B., Guy, H. J., Prediletto, R., Wagner, P. D. (1991) Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. *J. Appl. Physiol.* 71: 1245–1260.
- Robergs, A. R.; Ghiasvand, F.; Parker, D. (2004) Biochemistry of exercise-induced metabolic acidosis. *Am. J. Physiol. Comp. Physiol.* 287: 502-516.
- Shinohara, M., Moritani, T. (1992) Increase in neuromuscular activity and oxygen uptake during heavy exercise. *Ann. Physiol. Anthropol.* 11: 257–262.
- Sinclair, C. J., Geiger, J. D. (2000) Caffeine use in sports. A pharmacological review. *J. Sports Med. Phys. Fitness* 40: 71–79.
- Spriet, L. L. (1995) Caffeine and performance. *International Journal of Sport Nutrition* 5: 84-99.
- Thein, L. A., Thein, J. M., Landry, G. L. (1995) Ergogenic aids. *Physiol. Appl. Ther.* 75(5): 426-439.
- Van Thuyne, W., Delbeke, F. T. (2005) Distribution of Caffeine Levels in Urine in Different Sports in Relation to Doping Control Before and After the Removal of Caffeine from the WADA Doping List. *Int J Sports Med.* 26: 714-718.