

# Prevalence of caffeine use in elite athletes following its removal from the World Anti-Doping Agency list of banned substances

Juan Del Coso, Gloria Muñoz, and Jesús Muñoz-Guerra

**Abstract:** The aim of this investigation was to determine the use of caffeine by athletes after its removal from the World Anti-Doping Agency list. For this purpose, we measured the caffeine concentration in 20 686 urine samples obtained for doping control from 2004 to 2008. We utilized only urine samples obtained after official national and international competitions. Urine caffeine concentration was determined using alkaline extraction followed by gas chromatography–mass spectrometry. The limit of detection (LOD) was set at  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ . The percentage of urine samples below the LOD was 26.2%; the remaining 73.8% of the urine samples contained caffeine. Most urine samples (67.3%) had urinary caffeine concentrations below  $5 \mu\text{g}\cdot\text{mL}^{-1}$ . Only 0.6% of urine samples exceeded the former threshold for caffeine doping ( $12 \mu\text{g}\cdot\text{mL}^{-1}$ ). Triathlon ( $3.3 \pm 2.2 \mu\text{g}\cdot\text{mL}^{-1}$ ), cycling ( $2.6 \pm 2.0 \mu\text{g}\cdot\text{mL}^{-1}$ ), and rowing ( $1.9 \pm 1.4 \mu\text{g}\cdot\text{mL}^{-1}$ ) were the sports with the highest levels of urine caffeine concentration; gymnastics was the sport with the lowest urine caffeine concentration ( $0.5 \pm 0.4 \mu\text{g}\cdot\text{mL}^{-1}$ ). Older competitors ( $>30$  y) had higher levels of caffeine in their urine than younger competitors ( $<20$  y;  $p < 0.05$ ); there were no differences between males and females. In conclusion, 3 out of 4 athletes had consumed caffeine before or during sports competition. Nevertheless, only a small proportion of these competitors (0.6%) had a urine caffeine concentration higher than  $12 \mu\text{g}\cdot\text{mL}^{-1}$ . Endurance sports were the disciplines showing the highest urine caffeine excretion after competition.

**Key words:** caffeine, methylxanthine, doping control, endurance, intermittent sports, exercise.

**Résumé :** Cette étude se propose d'évaluer la consommation de caféine chez les athlètes après son retrait de la liste de dopage. À cette fin, on évalue la concentration de caféine dans 20 686 échantillons d'urine prélevés dans le contexte du contrôle antidopage entre 2004 et 2008. On analyse seulement les échantillons d'urine prélevés lors de compétitions nationales et internationales. La détermination de la concentration urinaire de caféine se fait par extraction alcaline suivie d'une chromatographie en phase gazeuse – spectrométrie de masse. La limite de détection (LOD) est fixée à  $0,1 \mu\text{g}\cdot\text{mL}^{-1}$ . Le pourcentage des échantillons d'urine sous la LOD est de 26,2 %; les autres échantillons (73,8 %) contiennent donc de la caféine. La plupart des échantillons d'urine (67,3 %) présentent une teneur en caféine inférieure à  $5 \mu\text{g}\cdot\text{mL}^{-1}$ . Seulement 0,6 % des échantillons d'urine présentent une teneur en caféine supérieure au seuil défini antérieurement comme celui du dopage, soit  $12 \mu\text{g}\cdot\text{mL}^{-1}$ . On observe les plus hauts taux urinaires de caféine au triathlon ( $3,3 \pm 2,2 \mu\text{g}\cdot\text{mL}^{-1}$ ), au cyclisme ( $2,6 \pm 2,0 \mu\text{g}\cdot\text{mL}^{-1}$ ) et à l'aviron ( $1,9 \pm 1,4 \mu\text{g}\cdot\text{mL}^{-1}$ ) et les plus faibles taux à la gymnastique ( $0,5 \pm 0,4 \mu\text{g}\cdot\text{mL}^{-1}$ ). Les concurrents les plus âgés ( $>30$  ans) présentent de plus hauts taux urinaires de caféine que les plus jeunes ( $<20$  ans;  $p < 0,05$ ); on n'observe pas de différences entre les femmes et les hommes. En conclusion, trois athlètes sur quatre consomment de la caféine avant ou pendant la compétition. Toutefois, une faible proportion de concurrents (0,6 %) présente un taux urinaire de caféine supérieur à  $12 \mu\text{g}\cdot\text{mL}^{-1}$ . C'est dans les sports d'endurance qu'on observe les plus importantes excrétions urinaires de caféine après la compétition.

**Mots-clés :** caféine, méthylxanthine, contrôle antidopage, endurance, sports intermittent, exercice physique.

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## Introduction

According to the World Anti-Doping Code, the use of a substance before or during sports competition should be banned when, derived from its consumption, individuals meet 2 of the following 3 criteria: benefit from an increase in sports performance; put his–her health at stake; or violate the spirit of the sport (World Anti-Doping Agency 2010).

The use of caffeine (1,3,7-trimethylxanthine) is one of the most controversial behaviors to be evaluated by sports organizations and anti-doping authorities. On the one hand, there is abundant scientific evidence regarding the ergogenic effects of caffeine in a wide variety of sports specialties (Doherty and Smith 2004; Burke 2008; Davis and Green 2009; Ganio et al. 2009), while health-related problems derived from caffeine ingestion are negligible in adults (Roti et al. 2006; Del

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Coso et al. 2009). On the other hand, caffeine is a naturally occurring compound in many foods and drinks (e.g., chocolate, coffee, tea), or it may be added to many beverages, dietary supplements, and over-the-counter medications. Therefore, sports competitors may ingest caffeine as a part of their habitual diet (social use), but also with the aim of obtaining a physical advantage during competition (doping attempts), which may represent a violation of the spirit of sport.

Due to the reported abuse of caffeine in sports competition (Delbeke and Debackere 1984), this substance was listed as a doping agent in 1984. To differentiate the social use of caffeine from doping attempts, anti-doping authorities set a threshold of caffeine in urine of  $15 \mu\text{g}\cdot\text{mL}^{-1}$ ; this limit was reduced to  $12 \mu\text{g}\cdot\text{mL}^{-1}$  in 1985. This urinary cut-off was controversial because caffeine doses from 3 to  $6 \text{mg}\cdot\text{kg}^{-1}$  body mass had been shown to increase performance, while urine caffeine concentration derived from the ingestion of these doses was well below  $12 \mu\text{g}\cdot\text{mL}^{-1}$  (Graham and Spriet 1991; Pasma et al. 1995; Bruce et al. 2000; Cox et al. 2002). Van Thuyne et al. (2005) measured the caffeine content in 11 361 urine samples tested for doping control from 1993 to 2002. They found that 8361 samples (74% of the total) contained caffeine, but only 16 samples (0.1%) surpassed  $12 \mu\text{g}\cdot\text{mL}^{-1}$ . The inefficacy of the urinary threshold to distinguish between the social use and the abuse of caffeine in sports forced the World Anti-Doping Agency (WADA) to remove caffeine from the prohibited list in January 2004.

Currently, athletes can freely consume caffeine before or during competition without fear of violating the WADA Code or being penalized by anti-doping organizations. Van Thuyne and Delbeke (2006) measured the caffeine concentration in 4633 urine samples obtained for doping testing in 2004, just after caffeine was removed from the doping list. They found that only 6 samples (0.1%) contained caffeine in a concentration higher than  $12 \mu\text{g}\cdot\text{mL}^{-1}$ , suggesting that the use of caffeine in sports did not change after its removal from the WADA list. It can be argued, however, that 1 year is not long enough to modify the use of caffeine in sports; therefore, the urine samples gathered in 2004 may not have been representative of the use of caffeine in competition after its removal from the list. The aim of this study was to describe the current use of caffeine in sports. For this purpose, we measured the caffeine concentration in urine samples received by the Doping Control Laboratory in Madrid (WADA certified) from 2004 to 2008. We hypothesized that, due to the permissiveness of caffeine use in sports, urinary caffeine concentration would have increased progressively after 2004.

## Materials and methods

### Samples

For this study, we measured the urine caffeine concentration in a total of 20 686 urine samples, corresponding to the specimens received in the Spanish Anti-Doping Laboratory for doping control from 2004 to 2008. A total of 3262 samples were received in 2004, 4911 were received in 2005, 4710 were received in 2006, 4960 were received in 2007, and 2843 were received in 2008. All samples corresponded to the urine collected after official national and international competitions, since urine specimens collected during training

are not routinely analyzed for caffeine detection. Information about the sex, age, sport discipline and the use of other medications (included on the anti-doping form) were integrated into a database for the analysis. The study conforms to the Declaration of Helsinki and was approved by the local Hospital Research Ethics Committee.

### Urine analysis

The methodology to quantify the urine caffeine concentration was based on gas chromatography–mass spectrometry (GC–MS), and it was validated according to ISO 17025. The measurement of each batch of urine samples was preceded by a calibration process, using a solution with an established caffeine concentration ( $6 \mu\text{g}\cdot\text{mL}^{-1}$ ). Caffeine (dissolved in GC grade methanol), diphenylamine, sodium hydroxide, and sodium sulphate were analytical grade, and were purchased from Sigma–Aldrich (Madrid, Spain). High-performance liquid chromatography grade methyl tert-butyl ether was obtained from Scharlau (Barcelona, Spain).

### Sample preparation

A portion (5 mL) of each urine sample was poured into a 15-mL screw-capped glass tube. Then,  $50 \mu\text{L}$  of internal standard (diphenylamine  $100 \mu\text{g}\cdot\text{mL}^{-1}$ ) was added to the sample. After that,  $100 \mu\text{L}$  of sodium hydroxide  $10 \text{mol}\cdot\text{L}^{-1}$  and 0.5 g of sodium sulphate were added to increase the transfer of analytes from the aqueous to the organic phase. Alkaline extraction was performed by adding 5 mL of methyl tert-butyl ether and centrifuging the sample at  $60 \text{r}\cdot\text{min}^{-1}$  for 20 min. After that, the sample was frozen in a cryogenic bath, and the organic phase (upper phase and not frozen) was transferred to a clean vial. The extract was concentrated with nitrogen, and  $\sim 2 \mu\text{L}$  of the remaining extract was injected into the system for caffeine quantification.

### Chromatographic conditions

GC–MS analysis was performed using a 6890N Gas Chromatograph (Agilent Technologies) coupled to a 5973N Mass Selective Detector (Agilent Technologies). All chromatograms were obtained in the scan mode range. The GC was equipped with a fused silica capillary column OV-1 (J & W Scientific Inc., Folsom, Calif.), and the carrier gas was helium. The analysis was carried out at a constant pressure of 15 psi. To facilitate separation, the initial column temperature was set at  $90 \text{ }^\circ\text{C}$  and the final column temperature was set at  $300 \text{ }^\circ\text{C}$ . The temperature on the injector port was set at  $275 \text{ }^\circ\text{C}$ .

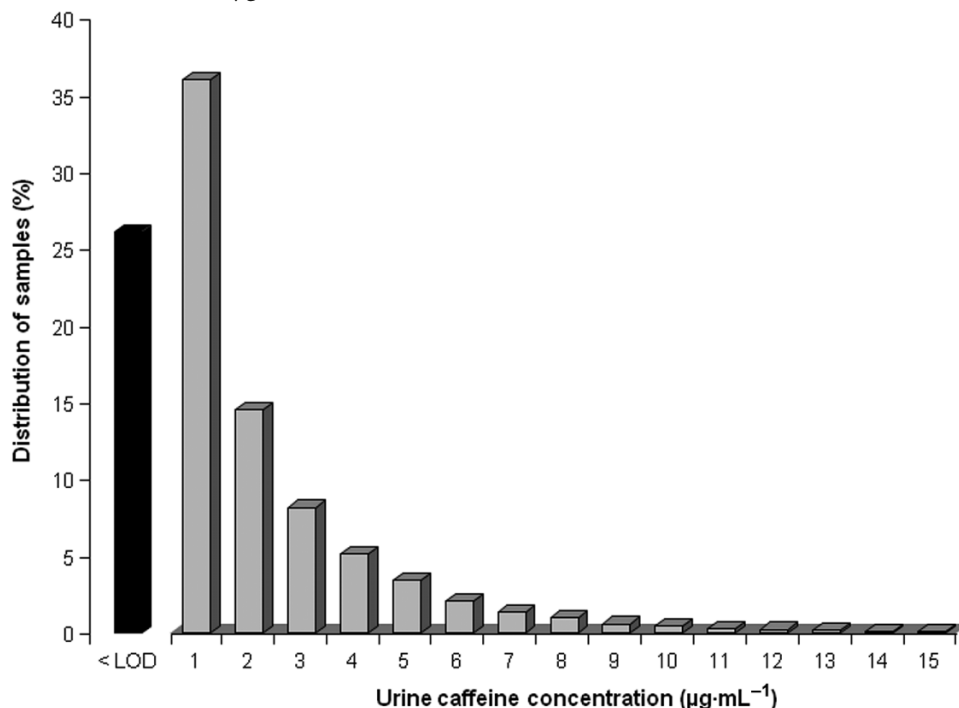
### Validation procedure

The between-days reproducibility was evaluated using 20 measurements of the calibration solution obtained over 2 months. The between-days coefficient of variation (at  $6 \mu\text{g}\cdot\text{mL}^{-1}$ ) was 7%. Accuracy was calculated in terms of the recovery factor (experimental value/theoretical value, expressed as a percentage). The value obtained was 105%, and no tendencies were observed. Uncertainty was calculated using accuracy and reproducibility, with a  $k$  value of 2. 21%. The limit of detection (LOD) was  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ .

### Statistical analysis

All samples with a urinary caffeine concentration below the LOD ( $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ ) were considered to be specimens

**Fig. 1.** Distribution of urinary caffeine levels in 20 686 samples from 2004 to 2008. The limit of detection (LOD) represents samples with urine caffeine concentrations lower than  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ .



without caffeine. The remaining samples were categorized into intervals of  $1 \mu\text{g}\cdot\text{mL}^{-1}$ , with a maximal caffeine concentration of  $15 \mu\text{g}\cdot\text{mL}^{-1}$ . Most of the samples had a urinary caffeine concentration between 0 and  $15 \mu\text{g}\cdot\text{mL}^{-1}$ , but 35 samples surpassed this limit. These samples were included in the statistical analysis, but they were not included in the graphical presentation of the data. To compare the urine caffeine concentrations among sports specialties, we selected only those sports with more than 200 samples analyzed from 2004 to 2008. The comparison of the most frequently tested sports, and the relationship between age, sex, and urinary caffeine concentration were determined using the Mann-Whitney rank-sum test (nonparametric statistical analysis). The statistical analysis was performed with the SPSS software package (version 17.0, SPSS Inc., Chicago, Ill.).

## Results

The average caffeine concentration in the urine samples was  $1.6 \pm 2.3 \mu\text{g}\cdot\text{mL}^{-1}$  in 2004,  $1.4 \pm 2.3 \mu\text{g}\cdot\text{mL}^{-1}$  in 2005,  $1.3 \pm 2.2 \mu\text{g}\cdot\text{mL}^{-1}$  in 2006,  $1.5 \pm 2.4 \mu\text{g}\cdot\text{mL}^{-1}$  in 2007, and  $1.5 \pm 2.2 \mu\text{g}\cdot\text{mL}^{-1}$  in 2008. The proportion of urine samples below the LOD (with respect to the number of samples in that year) was 28% in 2004, 29% in 2005, 27% in 2006, 26% in 2007, and 25% in 2008. These data suggest that the distribution of caffeine levels in sports was very similar from 2004 to 2008. For this reason, we merged all the data corresponding to urine samples obtained in this period and present them as a single group.

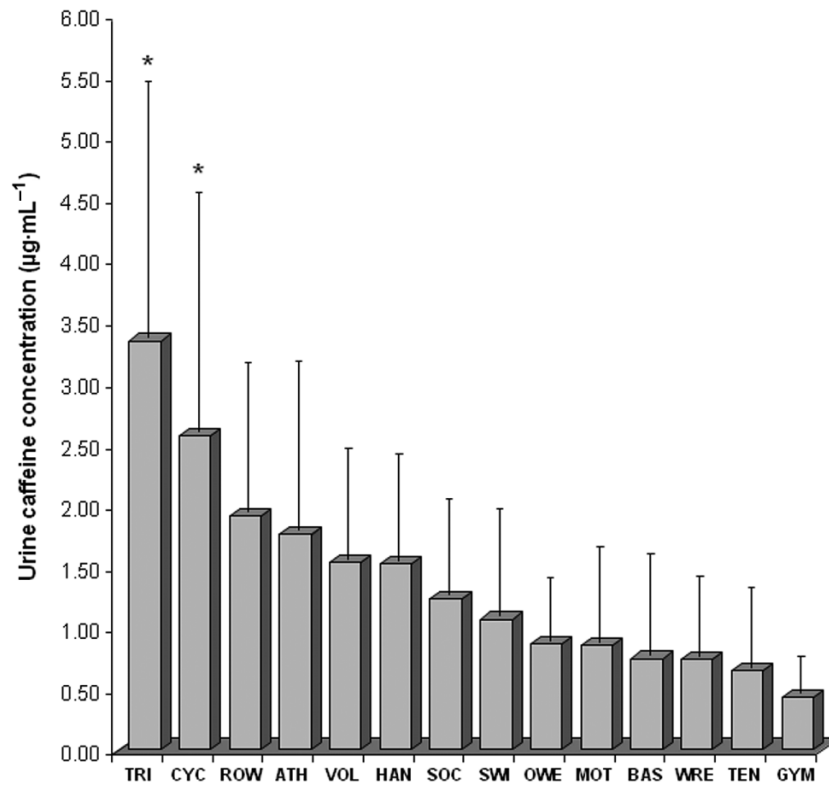
Figure 1 represents the distribution (in percentage) of all urine samples received from 2004 to 2008 in relation to the caffeine concentration measured. The percentage of urine samples below the LOD was 26.2%; the remaining 73.8% of the urine samples contained caffeine in a concentration

higher than  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ . Most urine samples (67.3%) had urinary caffeine concentrations below  $5 \mu\text{g}\cdot\text{mL}^{-1}$ , and only 1.2% of the samples had a urinary caffeine concentration above  $10 \mu\text{g}\cdot\text{mL}^{-1}$ . The proportion of samples with caffeine concentrations above the former WADA cut-off ( $12 \mu\text{g}\cdot\text{mL}^{-1}$ ) was 0.6%. The average urine caffeine concentration in males was  $1.43 \pm 2.20 \mu\text{g}\cdot\text{mL}^{-1}$ ; in females it was  $1.47 \pm 2.54 \mu\text{g}\cdot\text{mL}^{-1}$ . In addition, males and females had similar proportions of samples below the LOD (24.8% and 29.3%, respectively) and in the 0 to  $5 \mu\text{g}\cdot\text{mL}^{-1}$  range (68.9% and 63.3%, respectively). Therefore, the caffeine concentration in urine was very similar in males and females, although these results should be analyzed cautiously, since there were nearly 4 times as many samples from men as from women (16 494 vs. 4192).

Figure 2 represents the mean urinary caffeine concentration (including the samples below the LOD) in the most frequently tested sports. The number of samples was 200 from triathlon, 3950 from cycling, 228 from rowing, 2686 from athletics, 416 from volleyball, 578 from handball, 2691 from soccer, 747 from swimming, 382 from Olympic weightlifting, 660 from motor sports (including auto racing and motorcycling), 1140 from basketball, 222 from tennis, and 231 from gymnastics. The sports with the highest concentrations of caffeine were triathlon ( $3.3 \pm 2.2 \mu\text{g}\cdot\text{mL}^{-1}$ ), cycling ( $2.6 \pm 2.0 \mu\text{g}\cdot\text{mL}^{-1}$ ), and rowing ( $1.9 \pm 1.4 \mu\text{g}\cdot\text{mL}^{-1}$ ); the sports with the lowest concentrations were gymnastics ( $0.5 \pm 0.4 \mu\text{g}\cdot\text{mL}^{-1}$ ), tennis ( $0.7 \pm 0.9 \mu\text{g}\cdot\text{mL}^{-1}$ ), and wrestling ( $0.8 \pm 1.1 \mu\text{g}\cdot\text{mL}^{-1}$ ) (Fig. 2).

To test whether age affected the use of caffeine in sports, we grouped all the urine samples into 10-year intervals: competitors younger than 20 years of age (4294 samples), between 20 and 29 years of age (10 161 samples), between 30 and 39 years of age (4340 samples), and older than 40 years

**Fig. 2.** Urine caffeine concentrations in frequently tested sports. TRI, triathlon; CYC, cycling; ROW, rowing; ATH, athletics; VOL, volleyball; HAN, handball; SOC, soccer; SWI, swimming; OWE, Olympic weightlifting; MOT, motor sports; BAS, basketball; WRE, wrestling; TEN, tennis; GYM, gymnastics. \*Different from the remaining sports,  $p < 0.05$ .



of age (1877 samples) (Fig. 3). There were differences in the distribution of urine caffeine concentrations among all these age groups ( $p < 0.05$ ); while the youngest competitors (<20 years) had higher percentages of samples in the intervals from 0 to 1 µg·mL<sup>-1</sup>, the proportion of samples in intervals higher than 1 µg·mL<sup>-1</sup> was greater in older competitors (Fig. 3).

## Discussion

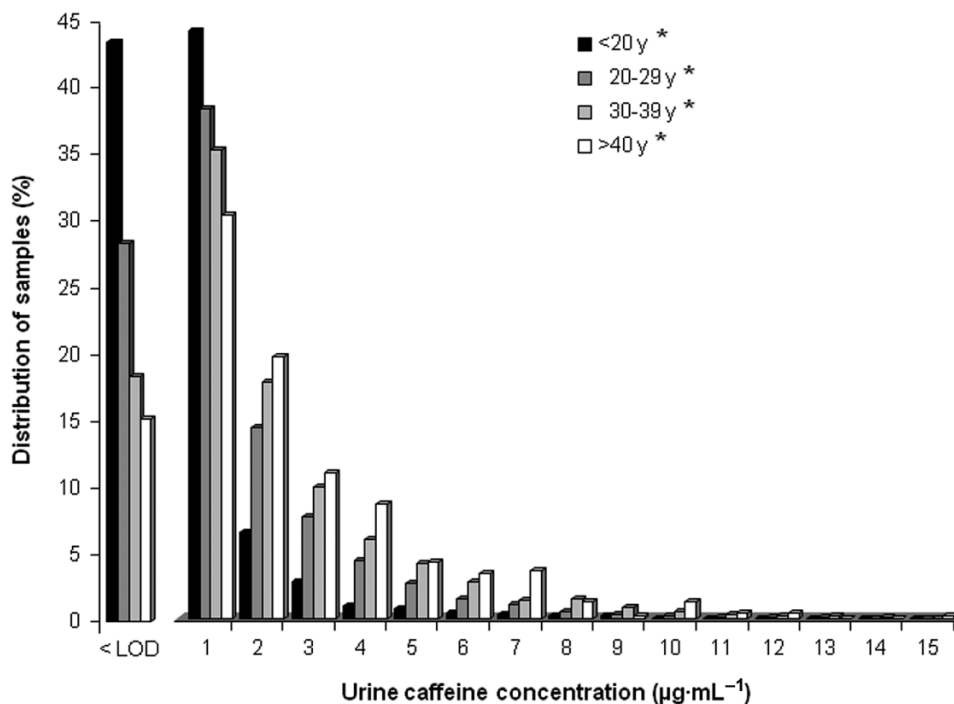
The aim of this study was to investigate the use and abuse of caffeine in sports competition. The abuse of caffeine in sports has been a concern for anti-doping institutions for several decades, and this substance was banned from 1984 to 2004, with a urinary cut-off of 12–15 µg·mL<sup>-1</sup>. However, the ineffectiveness of this limit to distinguish between the social use of caffeine (ingestion of foods containing small amounts of caffeine) and the ingestion of high doses of caffeine to deliberately enhance performance (doping attempts) forced WADA to remove caffeine from the list of prohibited substances. Therefore, since 2004, athletes can freely consume caffeine before or during competitions without penalization. Judging from the caffeine content of the 20 686 urine samples we analyzed, there are 3 main findings of this study: the use of caffeine in sports competitions from 2004 to 2008 was similar to the use during the period when it was banned; males and females had a similar use of caffeine, but older competitors had higher levels of urine caffeine concentrations; participants from endurance sports (triathlon, cycling, rowing) had higher urine caffeine concentrations than participants from sports with an intermittent nature (gymnastics,

tennis, wrestling). This information may help to understand the use of this stimulant as an ergogenic aid during exercise.

From 1993 to 2002, Van Thuyne and colleagues (2005) analyzed 11 361 urine samples collected after sports competitions. They found that 26.4% of the urine samples had a caffeine concentration below 0.1 µg·mL<sup>-1</sup> (their lower limit of quantification). Of the remaining 73.6% of the urine samples that contained caffeine, most of them were far below 12 µg·mL<sup>-1</sup>; only 16 samples (0.1% of the total) were declared to be positive samples. These authors considered the threshold for caffeine doping detection to be adequate, since it coincided with the average concentration  $\pm 4$  standard deviations ( $1.22 \pm 2.45 = 11.02$  µg·mL<sup>-1</sup>). Van Thuyne and Delbeke (2006) repeated the analysis on 4633 urine samples received in 2004 under the WADA monitoring program. These samples were obtained just after the removal of caffeine from the list of prohibited substances, and therefore could be considered adequate to determine whether caffeine use in sports varied with the elimination of caffeine as a doping agent. Those authors reported that urine samples with a caffeine concentration below 0.1 µg·mL<sup>-1</sup> represented 20.3% of the total; thus, ~80% of samples contained caffeine. However, only 6 samples (0.1%) contained a caffeine concentration higher than the former WADA threshold of 12 µg·mL<sup>-1</sup>.

From the investigations of Van Thuyne and colleagues (2005, 2006) before and just after the exclusion of caffeine as a doping agent, it can be concluded that the use of caffeine in sports changed minimally after its removal from the WADA list. It can be argued that 1 year is not long enough to modify the use of caffeine in sports; therefore, the urine

**Fig. 3.** Distribution of caffeine levels in urine, according to the age of the participants. LOD represents samples with urine caffeine concentrations lower than  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ . \*Different from the remaining age groups,  $p < 0.05$ .



samples collected in 2004 may not be representative of the current use of caffeine. Our study examined a longer period of time — the 5 years after caffeine was removed from the prohibited list (from 2004 to 2008). We found that the percentage of urine samples with a caffeine concentration below the LOD ( $0.1 \mu\text{g}\cdot\text{mL}^{-1}$ ) was 26.2%; therefore, 73.8% of the urine samples contained caffeine in some quantity. Most of the urine samples were below  $12 \mu\text{g}\cdot\text{mL}^{-1}$ , and only 126 (0.6%) samples surpassed this urinary cut-off. The distribution of urine samples in relation to the caffeine concentration we found (Fig. 1) is very similar to those previously reported by Van Thuyne and colleagues (2005, 2006). Therefore, we can safely confirm that the use of caffeine in sports has not varied with the removal of caffeine from the list of prohibited substances.

From a simplistic point of view, one might have expected an increase in the use of caffeine (and therefore an increase in the urinary caffeine concentration) after the removal of this substance from the WADA prohibited list. Although no reason explaining the absence of an increase in the use of caffeine is evident from our data, we hypothesize that the former limit was not restrictive enough to control the utilization of caffeine with doping attempts (to deliberately increase physical performance). Most of the classic and recent studies (Costill et al. 1978; Wiles et al. 1992; Graham and Spriet 1995; MacIntosh and Wright 1995; Pasman et al. 1995; Anderson et al. 2000; Bruce et al. 2000; Cox et al. 2002; Conway et al. 2003; Doherty et al. 2004a; Stuart et al. 2005; Schneiker et al. 2006; Green et al. 2007; Del Coso et al. 2008; Hulston and Jeukendrup 2008; McNaughton et al. 2008; O'Rourke et al. 2008; Foskett et al. 2009), but not all (Greer et al. 1998; Hunter et al. 2002; Lorino et al. 2006; Astorino et al. 2008) have demonstrated that the ingestion of 3 to  $9 \text{ mg}\cdot\text{kg}^{-1}$  body mass of caffeine increases physical and

cognitive performance in a wide variety of sports and exercise activities. In contrast, lower doses ( $<3 \text{ mg}\cdot\text{kg}^{-1}$ ) may not increase performance (Jenkins et al. 2008; Desbrow et al. 2009).

Nevertheless, a few studies have measured physical performance and urine caffeine concentration simultaneously. Graham and Spriet (1991) administered  $9 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine to individuals 1 h before exercising at 85% maximal oxygen consumption until fatigue, either running or cycling. This caffeine dose greatly increased the time to exhaustion ( $\sim 48\%$ ) compared with placebo, but the caffeine concentration in urine was  $\sim 9 \mu\text{g}\cdot\text{mL}^{-1}$ . Pasman and coworkers (1995) administered 5, 9, or  $13 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine to participants 1 h before cycling to exhaustion at 80% of their maximal work rate. They found a similar increase in endurance performance for all caffeine doses ( $\sim 23\%$  longer exercise duration, compared with placebo). Only when the cyclists ingested  $13 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine was their urine caffeine concentration in excess of  $12 \mu\text{g}\cdot\text{mL}^{-1}$  ( $\sim 5$ ,  $\sim 9$ , and  $\sim 15 \mu\text{g}\cdot\text{mL}^{-1}$  for 5, 9, and  $13 \text{ mg}\cdot\text{kg}^{-1}$ , respectively). Cox et al. (2002) administered  $6 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine either before or during 120 min of exercise, and then measured performance in a  $7 \text{ kJ}\cdot\text{kg}^{-1}$  time trial. They found that caffeine decreased the time to complete the trial by 3.3%, while the caffeine concentration in urine ranged from 4 and  $6 \mu\text{g}\cdot\text{mL}^{-1}$ . Finally, Bruce and colleagues (2000) administered 6 and  $9 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine to rowers before a 2000-m trial. They found that both doses reduced the time to complete the trial in a similar fashion, but only the higher dose ( $9 \text{ mg}\cdot\text{kg}^{-1}$ ) produced a urinary caffeine concentration that was above the WADA threshold ( $14.5 \mu\text{g}\cdot\text{mL}^{-1}$ ).

According to all these studies, the ingestion of 3 to  $6 \text{ mg}\cdot\text{kg}^{-1}$  of caffeine before exercise increases physical performance, even though the caffeine concentration in urine after exercise will be lower than  $12 \mu\text{g}\cdot\text{mL}^{-1}$ . In contrast, in-

gesting a higher dose (from 9 to 13 mg·kg<sup>-1</sup>) will not produce further benefits in performance, although it is possible that some subjects will excrete caffeine in a concentration higher than 12 µg·mL<sup>-1</sup>. It seems probable that, during the period in which caffeine was considered a doping agent (1984–2004), competitors benefited from caffeine ingestion (3–6 mg·kg<sup>-1</sup>) without exceeding the urine caffeine threshold proposed by WADA. In addition, the removal of caffeine from the doping list in 2004 did not modify its use in sports (as shown in this study), probably because high doses of caffeine do not seem to be more beneficial, in terms of performance, than moderate doses.

The samples we analyzed came from individuals from more than 62 sports specialties. Because of the difficulty in presenting data on all these sports, Fig. 2 presents data on sports for which more than 200 samples were analyzed. The sport with the highest excretion level of caffeine after competition was triathlon (3.3 µg·mL<sup>-1</sup>), whereas gymnastics was the sport with the lowest urine caffeine concentration (0.4 µg·mL<sup>-1</sup>). The differences in urine caffeine concentrations among sports suggest that the use and (or) misuse of caffeine before competition greatly depends on the specialty and its physical characteristics. For example, the sports with the highest caffeine concentration in urine (triathlon, cycling) are endurance specialties, whereas the sports with the lowest caffeine concentration in urine (tennis and gymnastics) are specialties with an intermittent nature (Fig. 2). If one considers that the use of caffeine in sports is only social (ingestion of food and drinks containing caffeine), one would expect all sports specialties to have similar urine caffeine concentrations. However, the differences in the urine caffeine concentration among sports specialties suggest that the use of caffeine and (or) caffeine derivatives in sports is related to an attempt to increase performance, mainly in endurance sports.

Figure 3 depicts the distribution of urine caffeine concentrations by age. Interestingly, the distribution of caffeine levels in urine was significantly different among the age groups set for this study ( $p < 0.05$ ). While the percentage of samples with a urine concentration below 1 µg·mL<sup>-1</sup> was greater in the youngest competitors (less than 20 years), there was a tendency for higher urinary caffeine levels in older competitors. These data strongly suggest that the use of caffeine in sports increases with age. Several reasons may explain the relation between caffeine and age. First, the ingestion of coffee, tea, and other caffeine-containing foods may increase with age, although, to our knowledge, there are no scientific data to confirm this nutritional tendency. Second, the age effect could be related to the sports specialty effect (triathlon and cycling vs. tennis and gymnastics). However, the mean age was not significantly different among these sports specialties. Finally, it is well known that several physiological capacities decline with age (McArdle et al. 2000). Since caffeine increases physical performance in a variety of exercise disciplines, higher caffeine consumption in older individuals may be related to the attempt to fight against age. All these explanations are speculative, and the true reason for a higher caffeine use in sports with age requires further investigation.

Caffeine is an ingredient in many nutritional products, including coffee, tea, energy drinks, and sport gels, but it is also included in several over-the-counter medications. Therefore, there is a wide variety of ways to use caffeine-contain-

**Table 1.** Co-utilization of caffeine with other substances, determined by the metabolites found in the urine samples.

| Substance                      | <i>n</i> | Percentage |
|--------------------------------|----------|------------|
| Not used with other substances | 1672     | 81         |
| Pseudoephedrine                | 133      | 6.4        |
| Tramadol                       | 77       | 3.7        |
| Ibuprofen                      | 56       | 2.7        |
| Paracetamol                    | 31       | 1.5        |
| Diclofenac                     | 19       | 0.9        |
| Cathine                        | 15       | 0.7        |
| Doxylamine                     | 14       | 0.7        |
| Aminophenazone                 | 10       | 0.5        |
| Methyl salicylate              | 10       | 0.5        |
| Benzocaine                     | 6        | 0.3        |

**Note:** The data ( $n = 2037$ ) correspond only to samples with urine caffeine concentrations higher than 4 µg·mL<sup>-1</sup> to select individuals ingesting more than 3–4 mg·kg<sup>-1</sup> of caffeine.

ing products before sports competition. Table 1 represents the co-utilization of caffeine with other substances, but only in samples with urinary caffeine concentrations higher than 4 µg·mL<sup>-1</sup>. We set this threshold to select individuals ingesting more than 3–4 mg·kg<sup>-1</sup> of caffeine, as suggested by previous data (Pasman et al. 1995; Cox et al. 2002), since this urinary cut-off may be representative of the use of caffeine to increase performance (rather than social consumption). Interestingly, 81% of individuals consumed caffeine alone, while ~7% combined caffeine with other stimulants (pseudoephedrine and cathine). The remaining individuals co-ingested caffeine with analgesics and (or) anti-inflammatory medications. Thus, there is an elevated proportion of competitors ingesting moderate to high doses of caffeine without combining it with other substances.

In summary, the removal of caffeine from the WADA prohibited list has not changed the utilization of this substance by athletes. Currently, 74% of athletes consume caffeine before or during competition, judging from the content of caffeine in urine samples obtained from 2004 to 2008. Participants in endurance sports have higher levels of caffeine in urine than their counterparts from shorter lasting or intermittent sports. Finally, urine caffeine concentration increases with age, suggesting an increased use of this substance by older competitors.

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