Dirasat, Educational Sciences, Volume **35**, Supplement, 2008

Caffeine Content in Turkish Coffee: A Question of Concern in Sport Community

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ABSTRACT

Caffeine (trimethylxanthine) is an alkaloid present in many beverages and food sources. It is widely consumed and it is known for its stimulating properties and potential performance-enhancing effects. World Anti-Doping Agency (WADA) has placed caffeine on the 2006 monitoring program for stimulants in-competition only substance if its presence in the urine exceeding 12.0 µg/ml.

Turkish coffee is the most commonly used type of coffee in the Middle-East region. However, athletes who are Turkish coffee consumers may not easily appreciate how much caffeine they can ingest before being accused of breaching the new WADA regulation of caffeine monitoring program for stimulants in-competition. Such problem could be avoided by establishing caffeine content in each cup of Turkish coffee, based on the understanding of the cultural practice in drinking and preparing Turkish coffee, and estimating the dose of caffeine present in each cup of Turkish coffee.

Twenty-four volunteers participated in the study as group A and B and consumed Turkish coffee cups (40ml each) with caffeine mean dose of 8.56 (4 cups) and 12.84 (6 cups) mg/kg respectively over a two-hour interval. Volunteers of Group A who had 4 cups showed urinary maximum caffeine concentration of 8.86±2.36 µg/ml three hours post ingestion. However, Group B who consumed 6 cups of coffee under the same experiment conditions showed a maximum urinary concentration of 12.36±2.02 µg/ml, such caffeine concentration is above the permitted level of 12.0 µg/ml. In Conclusion, athletes who are Turkish coffee drinkers are advised to lower their intake of Turkish caffeine to less than two cup of regular coffee during or before sport competition especially if the athlete ingests soft or hot drinks containing caffeine or taking permitted drugs containing caffeine. The small volume of Turkish coffee cup of 40ml could be misleading to athletes, and a cross over with the new WADA regulations of caffeine misuse will be reported if the athlete consumed 6 regular cups of Turkish coffee only.

KEYWORDS: Turkish coffee, Doping, Sport, Cut-off, Jordan.

1. INTRODUCTION

Coffee is consumed world–wide, with other beverages such as tea and chocolate, which are natural sources of caffeine (Bispo et al., 2002). In addition, caffeine is also appreciated for its stimulating properties and performance–enhancing capabilities among the athletes and as an ergogenic substance at a dose of 3-6mg/kg (Chambaz et al., 2001; Merwe and Muller, 1988; Stuart et al., 2005). Caffeine is used in sport events by athletes due to its presence in an inexpensive way, readily available, medically quite safe and legal (Graham, 2001). For that reason, athletes usually abstain from caffeine ingestion 48-72 hours prior to competition to suppress tolerance phenomenon and they consume caffeine on the day of the game 2-3 hours prior to the play. (Spriet, 1995; Nehling et al., 1992).

Caffeine was classified according to the International Olympic Committee (I.O.C) as a doping agent in 1962. It was removed from the list in 1972, but was reclassified once more as a restricted drug in 1984 if its concentration in urine exceeds 12.0 µg/ml (Silver, 2001; Dekhuijzen, 1999; Thomas, 1988; International Olympic Committee, 2003). Recently, the world anti-doping code issued by the World Anti-Doping Agency (WADA) has placed caffeine on the 2006 monitoring program for stimulants in-competition only substances (WADA Website, 2006).

Caffeine is rapidly absorbed with a 100%

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bioavailability; the peak plasma concentration is reached 15-20 minutes after ingestion. Caffeine is metabolized by cytochrome P450 in the liver to it's predominant metabolite (84%) dimethylxanthines, 10-15% into theobromine and the ophylline, with less than 0.5-3% of caffeine excreted unchanged in urine (Paluska, 2003; Holland et al., 1998; Mcleane and Graham, 2002; Mandel, 2002; Chambaz et al., 2001; Graham, 2001) and eliminated by first order kinetics, and mainly excreted in urine. The peak urinary caffeine concentration occurs within 2-5 hours, but its clearance is nonlinear and significantly lower in higher doses (Paluska, 2003; Mcleane and Graham, 2002).

In the literature, there are few studies related to caffeine intake and consumption among athletes and doping. Merwe and Muller (Merwe and Muller, 1988) showed the intake of doses from 1.52 mg/kg to 17.53 mg/kg of caffeine in the form of tea, coffee and coca cola (which is equivalent to 8 cups [1cup = 210 ml]), within 15 minutes, their caffeine concentration in urine will exceeds 14.0 µg/ml after 3 hours of ingestion. Another study conducted by Lelo et al. (Lelo et al., 1986) showed that caffeine concentration in the urine could exceed the acceptable limit if the athlete consumed 4-11 drinks per day (cup mean volume 177ml). Moreover, when caffeine tablets were administered to cyclists' athletes at a dose of 13 mg/kg, urine caffeine concentration was above the allowable limit of 12.0µg/ml (Pasman et al., 1995)

Turkish coffee is the most commonly used type of coffee in West Asia especially in the Middle-East region. Based on our social habits in preparing and drinking Turkish coffee, Jordanian athletes are at a high risk of being accused of caffeine misuse in sport since there is no study addressing caffeine content of each cup of Turkish coffee and as a result consumers may not easily appreciate how much caffeine they can ingest. It is known that the content of caffeine could vary according to brew strength and volume (Bell et al., 1996). In the western countries, data are available regarding caffeine content per cup of coffee and it was found to be in the range from 40 to more than 200 mg (Chambaz et al., 2001). In our region, which is known for being a big consumer of Turkish coffee, no data were available for athletes, sport doctors, trainers, coaches and sport authorities regarding the dose of caffeine present in each cup of Turkish coffee in respect to our method of preparation, types of coffee and method of coffee roasting.

The aim of this study was to establish the caffeine content in each cup of Turkish coffee, which enable us to keep the athletes in compliance with the new WADA regulation regarding caffeine monitoring program for stimulants in competition, and to elaborate the effects of other factors which might contribute to increasing the level of caffeine in urine with a normal life style of eating, drinking, drugs intake and other environmental and health factors.

Study Design and Protocol

1. Coffee Solution Preparation

In order to have proper data regarding Turkish coffee preparation method and consumption, a forty-four questionnaire was distributed to the public; each person was required to answer questions regarding their coffee intake from food and beverage with emphasis on Turkish coffee source, type, roasting degree, mode of preparation and how many spoons of coffee the person used in preparing a cup of coffee to enable us to determine the quantity in grams of coffee and to estimate the dose of caffeine present in each cup.

2. Volunteers

The study sample were 24 healthy university student athletes, 12 non-smoker females, 9 non-smokers males and 3 male smokers. Mean (±SD) age of the volunteers was 24.91±3.09 years (range from 19-27 years) and mean body weight and height was 77.83±13.10 Kg and 171.87±11.72 centimeter respectively. Their medical history, physical activity and history of caffeine intake were recorded. Moreover, they were asked to show their caffeine sources from food and beverages such as chocolate intake, or drugs that contain caffeine in their prescription and or OTC medications. Kidney function tests and urine analysis were conducted for all volunteers. Volunteers were prohibited from food or beverages containing caffeine for 5 days before and during the study day. On the study day, they were asked to empty their bladder before the ingestion of coffee cups. These urine samples were collected to check for the abstinence of caffeine containing food and drinks and were considered as a baseline. The volunteers were divided into two groups; group A and B consumed 4 and 6 cups of Turkish coffee respectively in a two-hour interval. Each cup contained 6.66 g of coffee and it was prepared from the same coffee batch. Urine samples collected from

volunteers at 1,2,3,4,5,6,9,12,24,30 and 36 hours post ingestion were stored at –20C° until analysis time.

3. Method of caffeine analysis

Urine samples were prepared by adding 100µl urine to 800µl distilled water, 120 mg of ammonium sulphate and 100µl paracetamol (30 µg/ml prepared in distilled water) as an internal standard and they were extracted with 8 ml of chloroform: isopropanol (85: 15 v/v) respectively. After vortex mixing for 1 minute, the tubes were centrifuged at 2500 rpm for 5 minutes and the aqueous (upper) phase was aspirated. The organic phase was allowed to evaporate using nitrogen gas with a hot plate at 40°C. The dried residue was reconstituted with 250µl of the mobile phase, mixed and 100µl was injected directly into the HPLC system.

Table 1. shows the sources of caffeine in different products; Prescribed and non-prescribed medication, Cold and hot Beverages containing caffeine

Prescription drugs containing caffeine	**Content per tablet mg**	**Uses**
Anolor300®	40	Pain relieve and sedative
Belcomp-BP®	40	Headache
Cafergot®	100	Migraine
Darvon®	32.4	Narcotic analgesic
Fembutal®	30	Pain reliever and sedative
Norgesic®	30	Skeletal muscle relaxant
Wigrame®	100	Migraine headache
Non-Prescription drugs containing caffeine	**Content per tablet mg**	**Uses**
Excedrin®	65	Pain Relievers
NoDoz, Vivarin®	200	Stimulants
Vanquish®	33	Pain Relievers
Aqua Ban®	200	Diuretics
Coryban-D®, Triaminicin®	30	Cold remedies
Caffeine – Containing Beverages	**12 OZ Serving (mg)**	
Coca Cola®	34	
Diet Coca Cola®	45	
Nestea Lemon Tea®	16	
Pepsi Cola®	38	
Diet Pepsi Cola®	36	
Caffeine in Hot Beverages	**Caffeine Per Cup(mg)**	
Brewed Coffee	100-200	
Instant Coffee	85-100	
Decaffeinated coffee	2-5	
Tea	60-75	
Cocoa	5-40	

Adopted from: (Durrant, 2002 and Tyler et al., 1988).

Caffeine in different types of coffee was determined by weighing 0.1 gram of coffee; boiled water was added to extract caffeine. The solution was left in a boiling water bath to boil for further 10 minutes in order to have the same parameter conditions of the coffee cup preparation. After cooling, the solution was filtered using filter paper. To the filtrate, 0.5 gram of sodium carbonate was added to adjust the pH into an alkaline condition then extracted by organic solvent prior to its analysis. Caffeine in urine and coffee samples was measured by HPLC-Diode array detector, according to a modification of Bendriss (Silver, 2001) and Ventura (Dekhuijzen, 1999) methods.

Table 2. Drugs containing caffeine in Jordan market

Drug	Content Per Tablet(mg)	Uses
Rodavan®	50	Anti-emetic anti-nausea
Somadril®	32	Muscle relaxant
Algesic®	50	Narcotics
Migril®	100	Anti-migraine
Coldex®	65	Anti-asthma
Triatussic®	25	Anti-cough
Neo-synephrine®	15	Anti-tussive
Cafergot®	100	Anti-migraine
Panadrin®	65	Analgesic
Saridon®	50	Analgesic
Dolocet®	65	Analgesic
Excedrin®	65	Analgesic
Panadol -Extra®	65	Analgesic

Adopted from: (Jordan National Drug Formula, 2002, Ministry of Health, Amman-Jordan).

Table 3. Drugs which inhibit or potentiate metabolism of caffeine

A. Drugs which inhibit or potentiate metabolism of caffeine
Clozapine, Disulfiram
Theophyline, Warfarin
B. Drugs increase caffeine metabolism
Barbiturates, Nicotine, Smoking
Acetaminophen, Phenytoin, Flavenoids in food
C. Drugs inhibit caffeine metabolism
Cimitidine, Ciprofloxacin, Erthromycin
Isoniazid, Oral contraceptives
D. Caffeine increases the urinary excretion of
Lithium
E. Caffeine impairs absorption of minerals of
Calcium, Iron

Adopted from: (Durrant, 2002 and Nehlin et al., 1992)

Table 4. Factors affecting cytochrome P450 1A2 (CYP1A2) activity

Factors	Effect
Lean individuals	↑
Obese individuals	↓
Coffee	↑
Exercise	No effect
Alcohol	↓
Grapefruit	↓
Grilled meat	↑
Liver disease	↓
Male	↑
Female	↓
Pregnancy	↓
Smoking	↑

Adopted from: (Carrillo, J. and Bentiz, J., 2000).

Table 5. Public habits practice in preparing coffee

Question	Answer	Percentage
Time for coffee intake	- All the day - Only morning - Only morning and night - No difference	52% 23% 18% 7.0%
Coffee Brewing	- Without foam - With foam - No difference	48% 32% 20%
Drinking coffee while smoking	- No - Yes	70% 30%
Degree of coffee roasting*	- 1/2 Dark + 1/2medium - 3/4 medium + 1/4 Dark - 1/4 medium+ 3/4 Dark - Irrelevant	57% 23% 14% 6.0%
Coffee sources	- Brazilian - Yamani - No difference	90% 5.0% 5.0%
Coffee concentration(Type)	- Regular - Strong	52% 48%

* Roasting of coffee beans is of two different types according to intensity of roasting. Mainly the coffee beans are either lightly roasted [golden yellow] medium roasted [yellowish brown color] or dark roasted [blackish brown in color]. According to personal tastes, the two types are mixed in different ratios.

Table 6. Caffeine concentration in each gram of coffee and caffeine dose in each cup of coffee

Type of Coffee cup	Mean Coffee weight in grams± SD	Mean Caffeine concentration(mg) per cup of Turkish coffee(40ml)	% of users
Regular	6.84±3.65	165	80%
Strong	12.41±6.58	313	20%

4. Caffeine Sources and interaction with drugs

Caffeine is present in many food sources and natural products of black and green tea, coffee, cacao beans, cola nuts, mate leaves, over the counter (OTC) drugs including analgesics which are used for their central nervous system stimulation. Also it can be used as a flavoring agent in sport drinks (Mandel, 2002) (Ratanayake et al., 1993; Chen and Mou, 1998; Bispo, 2002). (Table 1) shows the caffeine content from beverages, food, prescription and non prescription drugs or (OTC) (Bocci, 2002; Durrant, 2002). Local Jordanian drugs that contain caffeine are shown in (Table 2) (Tylor et al., 1998). There are many factors that could affect the metabolism of caffeine, the interaction between caffeine and a variety of drugs are shown in (Table 3) (Nehling et al., 1992; Bocci, 2002), while factors affecting cytochome P450 1A2 (CYP1A2) metabolism is presented in (Table 4) (Jordan National Drug Formula, 2002).

2. RESULTS AND DISCUSSION

1. Coffee solution preparations

The different cultural and habitual preparation methods of Turkish coffee within the Jordanian community are given in (Table 5). It shows the amount of coffee in grams consumed in each cup of coffee according to the public preparation form, which shows the variation and different caffeine resources and consumption quantities. Most Jordanians 90% consume Brazilian coffee and 57% of Jordanians prefers their coffee to be a mixture of ½ medium and ½ dark roasted coffee beans. In term of coffee preparation, 80% of Jordanians prefer what we call the regular cup of coffee; in which around 6.86±3.65 grams of ground coffee is

used to prepare one cup of Turkish coffee of 40ml, while 20% like strong coffee, in which 12.41±6.58 gram of coffee were used. The expected caffeine concentration presents in each cup of Turkish coffee according to the method of preparation is presented in (Table 6). Based on our findings from the public questionnaire, we prepared the coffee for volunteers as regular caffeine level in which a mixture of ½ medium and ½ dark roasted ground coffees is used. To produce what is known as a regular cup of coffee, accurately weighed amount of 35grams of finely ground Brazilian coffee was added to 210 ml of boiling water and they were allowed to boil for a further 1 minute until coffee foam disappeared from coffee solution (Ratanayake et al., 1993). Each cup (40 ml) of Turkish coffee contained about 6.66 grams coffee.

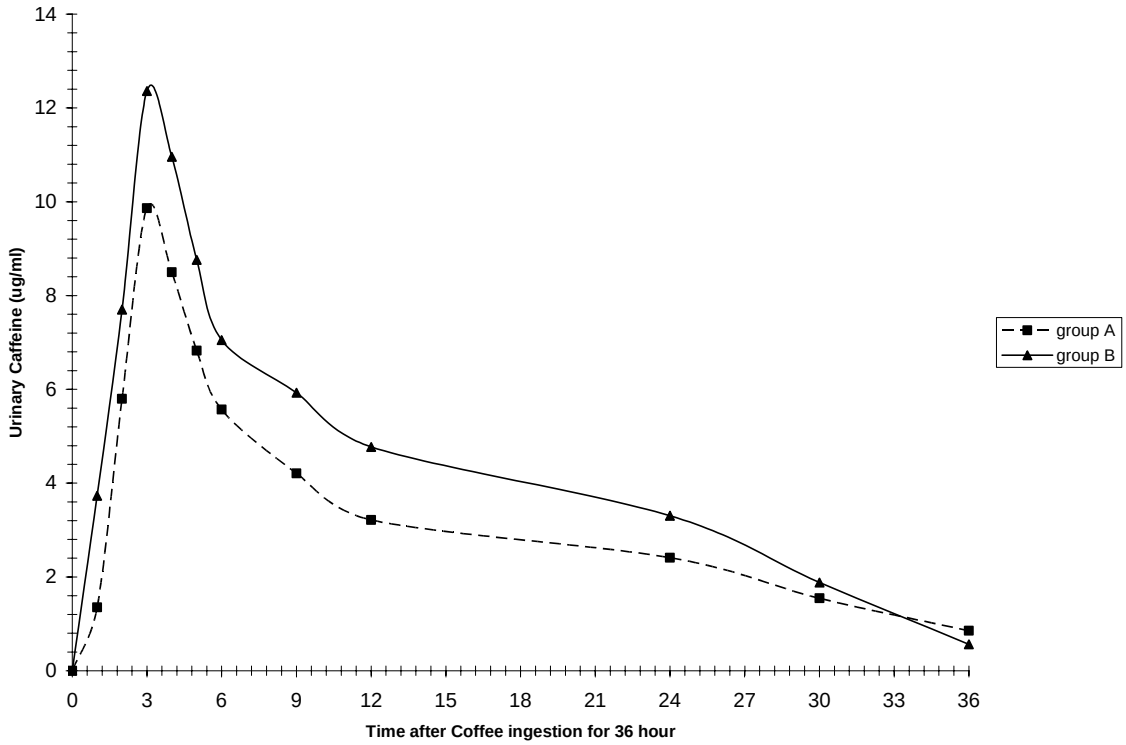

Figure 1: Caffeine excretion in urine for group A and B

No significant difference in the concentration of caffeine present in medium or dark roasted coffee was noticed, (Table 7) shows the caffeine concentration of 24.9 and 25.6 mg/gram in medium and dark roasted Brazilian coffee respectively. In calculating the concentration of caffeine in each cup of coffee a concentration of 25.2 mg/g was taken as an average of 24.9 and 25.6 mg/gram in medium and dark. In our study we used the regular coffee for volunteers, the caffeine dose in mg/kg per cup (40ml) was calculated and the total caffeine dose given to volunteers in each group was estimated.

2. Volunteers

All volunteers had acceptable baseline urine caffeine of less than 1µg/ml. The results of urine specific gravity, pH and creatinine for all volunteers were within the normal range. Caffeine concentration was measured in the urine for group A and B. The time at which maximum concentration occurred was 3 hours post ingestion. Figure 1 reflects caffeine urinary excretion with respect to the maximum concentration in the urine over a 36 hours period for group A and B. The highest concentration of caffeine in the urine of group A which consumed 4 cups over 2 hours with a mean caffeine dose of 8.56 mg/kg was observed at 3 hours post ingestion with a peak urine caffeine concentration of 8.86 µg/ml. In group B the highest caffeine concentration in urine of 12.36 µg/ml was noticed when 6 cups of coffee were consumed in 2 hours with a mean caffeine dose of 12.84 mg/kg. The maximum urinary caffeine excretion was at 3 hours for both groups A and B respectively. Our findings are in agreement with those noticed in the literature regarding the caffeine maximum excretion in urine 2-5 hours after

oral administration (Mandel, 2002). However, the current limit of 12.0 µg/ml of caffeine in the urine was not attained in any of the volunteers in group B. Part of the inter-individual variation in caffeine excretion may be assigned to the metabolic clearance by the liver (Carrillo and Benitez, 2000). Among other causes of variability is the ingestion of certain drugs or food that would inhibit or delay the metabolism and excretion of caffeine.

Table 7. Caffeine concentration in mg/ gram of different types of coffee

No.	Coffee Type Non Roasted	Caffeine Concentration in mg/gram of Coffee (n=5)
1	Yamani	4.3896 ± 0.3438
2	Brazilian	4.2062 ± 0.5708
3	Columbian	3.7074 ± 0.2195
4	Habashi	2.8628 ± 0.2219
	Roasted	
5	Light Brazilian	22.297 ± 0.9372
6	Medium Brazilian	24.9508 ± 0.5952
7	Dark Brazilian	25.6046 ± 0.3344
8	Medium Columbian	24.786 ± 0.8705
9	Dark Columbian	26.5876 ± 0.9002
10	Medium Yamani	24.5716 ± 0.3503
11	Dark Yamani	25.8782 ± 0.3236
12	Yamani Bean Cover	3.2566 ± 0.1993
13	Medium Habashi	24.803 ± 0.3184
14	Dark Habashi	25.5044 ± 0.2210
15	Light Kenian	20.3782 ± 0.5181
16	Dark Kenian	25.4644 ± 0.4512
17	Light Costarican	24.7138 ± 0.4467
18	Dark Costarican	26.3952 ± 0.1835
	Roasted Decaffeinated	
19	Brazilian (Coffee City)	0.5666 ± 0.0344
20	Brazilian (Brazilian Coffee House)	4.4892 ± 0.0827

3. Caffeine estimation in coffee samples

A total of 20 samples of different types of coffee were taken. The samples included roasted, non-roasted and decaffeinated Turkish coffee. The concentrations of caffeine in different types of coffee available in Jordan market are presented in (Table 7). Data on caffeine content per cup of coffee is in agreement with previous studies in which caffeine was directly measured in beverages (Lelo et al., 1986).

The caffeine concentration in coffee solution is influenced by various coffee preparation methods. These variables include the coffee grounds to water volume ratio, which influence the caffeine content being extracted. The grinding process varies for each coffee store. Larger extent of grinding leads to significantly higher caffeine concentration. This is due to the high surface area of ground coffee to the boiling water molecules, which leads to more interaction between caffeine and boiled water and thus increasing the amount of caffeine being extracted. Longer brewing time implies a longer contact time between water and coffee leading to more complete caffeine extraction, the time of extraction is also an important factor in estimating caffeine in coffee drinks. A study conducted by Bell et al (Bell et al., 1996) showed that boiling coffee for 3 minutes will extract 40% more of caffeine than the coffee solution being boiled for one minute. The time of extraction implemented in our study was 10 minutes which was an adequate time for the total caffeine extraction. Caffeine concentration per gram of coffee ranged from 2.8 – 4.3 mg/g for non-roasted, while roasted types contained 20.3–26.5 mg/g (equivalent to 2.03% to 2.65%) with the exception of Yamani coffee,

which showed the lowest caffeine concentration of 3.25 mg/g. Our finding is in agreement with a study conducted by Bell et al (Bell et al., 1996) in which caffeine present at 2.0% of the dry weight of roasted mature beans of coffee. The roasted types of coffee showed an increase in the caffeine concentration from the non-roasted types in the range of 5.30-8.90 folds for all types. The fold increase in caffeine concentration in roasted coffee is due to the moisture level being decreased within the coffee beans. The decaffeinated coffee showed a variation in caffeine concentration from one shop to another, depending on the decaffeination process being implemented by each shop.

3. CONCLUSION

It is clear that athlete who purposely took shortly before competition 6 cups of regular or 3 cups of strong Turkish coffee will attain the stated limit of 12.0 µg /ml urine. Emphasis on educating athletes regarding the dose of caffeine in every cup of coffee he drinks, sources of caffeine containing drinks and medications, factors which might interfere with the metabolism of caffeine like; permitted medications, natural juices, smoking is of paramount value in protecting their health and reduce the number of positive cases in WADA report regarding caffeine monitoring program in competition especially in athletes from west Asia and athlete's who play in west Asia region.

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كميات الكافيين في القهوة التركية: سؤال ذو أهمية في المجتمع الرياضي

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ملخص

هدفت هذه الدراسة إلى التعرف إلى كمية الكافيين الموجودة في القهوة التركية وتأثيرها على الرياضيين ومقارنة عدد الفناجين المعطاة لدى عينة الدراسة حيث أن الكافيين هو مادة قلوية توجد في العديد من المشروبات والمأكولات تستخدم لتأثيرها المنشط المعروف وقدرتها على زيادة وتحسين الأداء. قامت الوكالة العالمية لمكافحة المنشطات بوضع الكافيين ضمن قائمة المواد المنشطة عام 2006 والتي يجب مراقبتها خلال المسابقات فقط إذا كان تركيزها أكثر من 12 ميكروغرام –ملليتربول.

ولتحقيق هدف الدراسة تم إشراك أربع وعشرين متطوعاً من الشباب الرياضي الأردني حيث قسموا إلى مجموعتين مجموعة (أ) مجموعة (ب) ومن ثم تم إعطاء المجموعة (أ) أربعة فناجين قهوة تركية، والمجموعة (ب) ستة فناجين، كل فنجان يحتوى على (40) ملليتراً من القهوة بحيث كان معدل جرعة الكافيين هو (8.56) ملغم و(12.48) ملغم لكل كيلو غرام من وزن الجسم الرياضي خلال ساعتين للمجموعتين (أ) و(ب) على التوالي .

وتم إجراء التحاليل المخبرية حيث تبين أن المتطوعين الرياضيين في المجموعة (أ) الذين تناولوا أربعة فنجاين قهوة تركية كان مستوى الكافيين في البول لديهم (9.86 ±2,36) ملغم – ملليتر –بول بعد (3) ساعات من تناولهم القهوة التركية، أما المجموعة (ب) الذين تناولوا (6) فنجاين قهوة تركية كان مستوى الكافين في البول لديهم (12.36 ±2,02) ملغم –ملليتر-بول بعد (3) ساعات وهو اعلى من الحد المسموح به ضمن نظام المراقبة الصادرة عن الوكالة العالمية .

من هنا يوصي الباحثون بعدم تناول الرياضي القهوة التركية باستمرار وينصح بتقليل عدد فناجين القهوة التي يتناولها إلى أقل من (2) خلال المسابقات الرياضية أو قبلها وخصوصاً إذا كان تناول مشروبات مرطبة أو ساخنة أو أدوية تحتوى على مادة الكافيين .

أن حجم فنجان القهوة البالغ أربعين ملليتراً قد تبدو قليلة وبالتالي لاتحدث مخالفة لنظام الوكالة الدولية لمكافحة المنشطات، وإن تناول الرياضي لـ(6) فناجين قهوة تركية سوف يوقع الرياضي ضمن قائمة المخالفين لنظام الوكالة العالمية .

الكلمات الدالة: القهوة التركية، المنشطات، الرياضة، الحد الفاصل، الاردن.

* كلية الدراسات العليا، كلية التربية الرياضية، كلية الطب، الجامعة الأردنية. تاريخ استلام البحث 2007/2/6، وتاريخ قبوله 2007/6/28.