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Effect of the chronic administration of caffeine on adipose mass and lipid profile of Wistar rats

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ABSTRACT. This work aimed at verifying the effect of the chronic ingestion of caffeine on body weight and adiposity of Wistar rats. Sixteen male Wistar rats weighting on average 240 g were divided into two groups, one control and the other caffeine-treated (5 mg kg^{-1}, orally) for five weeks. At the end, the groups were evaluated for their differences in body weight; weight of the periepididymal, retroperitoneal, subcutaneous and mesenteric fat pads; size of the retroperitoneal adipocytes; liver and heart weight; glycemia and plasma lipids. Statistically significant differences were observed in adipocyte size and total serum cholesterol, while the results for the other parameters were not statistically different. Therefore, this study showed that, using an oral dose of caffeine within acceptable (non toxic) limits, it is possible to reduce the size of adipocytes of non-obese Wistar rats, as well as to reduce the serum cholesterol levels, even in the absence of physical activity or other active compounds.

Keywords: adipocytes, fat, lipolysis, cholesterol.

Efeito da administração crônica de cafeína na massa adiposa e no perfil lipídico de ratos Wistar

RESUMO. Este trabalho teve como objetivo verificar o efeito da ingestão crônica de cafeína no peso corporal e na adiposidade de ratos Wistar. Foram utilizados 16 ratos Wistar machos com peso em torno de 240 g divididos em dois grupos, um controle e outro tratado com cafeína (5 mg kg^{-1}, via oral) por cinco semanas. Ao final, foi avaliada a diferença entre os dois grupos em relação ao peso corporal; ao peso dos depósitos de gordura periepididimal, retroperitoneal, subcutânea e mesentérica; ao tamanho dos adipócitos retroperitoneais; ao peso do coração e do fígado; e aos valores de glicemia e lipídeos plasmáticos. Foram observadas diferenças estatisticamente significativas no tamanho dos adipócitos e na quantidade plasmática de colesterol total, sendo que o resultado das demais variáveis não foi significativo. Portanto, este estudo mostrou que, usando uma dose oral de cafeína dentro dos limites considerados aceitáveis (não-tóxicos), é possível reduzir o tamanho dos adipócitos de ratos Wistar não-obesos, bem como reduzir seus níveis séricos de colesterol, mesmo na ausência de atividade física ou de outros compostos ativos.

Palavras-chave: adipócitos, gordura, lipólise, colesterol.

Introduction

In order to assure their survival even in conditions of shortage of nutrients in the environment, mammals are capable of storing the excess of calories ingested and not immediately required for their metabolic needs especially as lipids (triacylglycerols) and carbohydrates (glycogen). Lipids, being hydrophobic, can be stored in large amounts without solvent water (FONSECA-ALANIZ et al., 2006).

The adipose tissue is the major energy reservoir of the organism; the adipocytes are cells specialized in the storage of lipids as triacylglycerols (TAGs) in their cytosol (FONSECA-ALANIZ et al., 2006). These cells have all the enzymes and regulatory proteins necessary for the synthesis of fatty acids (lipogenesis) and storage of TAGs when the supply of energy is large, and for the mobilization through lipolysis when there is a caloric deficit in food intake.

The adipose tissue has many functions, such as: thermal insulation, barrier against mechanical trauma, energy storage and secretion of signaling proteins and peptides. Its capacity of energy storage is virtually unlimited and results both from the increase of the volume of each adipocyte and the replication and differentiation of preadipocytes (COSTA; DUARTE, 2006).

Curi et al. (2002) describe the adipose tissue as a special type of connective tissue, a loose association of fat-storing cells – the adipose cells or adipocytes –

with cells of the vascularized stroma, linked to a matrix of collagen and reticular fibers; other cells found in this tissue are cells of the vascular bed, fibroblasts, leukocytes and macrophages. In mammals there are two types of adipose tissue: 1) white, yellow or unilocular adipose tissue, and 2) brown or multilocular adipose tissue; they differ in color, amount, vascularization, metabolic activity, number of organelles and distribution in the body.

In humans, about 15% of the body weight of an adult male and 22% of the adult female are adipose tissue. Contrary to other tissues, it has a high capacity of changing its size: it can increase in weight more than 100% or decrease it to 3% normal (in pathological conditions such as obesity or anorexia nervosa, respectively) (CURI et al., 2002).

The autonomic nervous system has a direct control over the adipose tissue through its sympathetic and parasympathetic divisions; the sympathetic innervation exerts mainly catabolic (i.e. lipolytic) actions, mediated by beta-adrenergic receptors and dependent of the activity of the hormone-sensitive lipase. On the other hand, the parasympathetic nervous system is involved with anabolic (i.e. lipogenic) effects on the adipose tissue, including insulin-induced glucose and fatty acid uptake (DIEPVENS et al., 2007; FONSECA-ALANIZ et al., 2006).

Caffeine is an alkaloid, identified as 1,3,7-methylxanthine, containing a purine skeleton and found in large amounts in coffee seeds and green tea leaves, as well as in other plant products, particularly cocoa, guarana and South American holly (MARIA-CARLOS; MOREIRA, 2007).

Dall'Agnol and Ciero (2002) report that caffeine is one of the major xanthines, which has stimulant effects, antagonizes adenosine receptors and inhibits phosphodiesterase with resulting buildup of cyclic adenosine monophosphate (cAMP). These actions are also reported by other authors (DIEPVENS et al., 2007; MELLO et al., 2007; WALD et al., 1976). In addition, it was proposed that caffeine could also have an inhibitory action over the lipoprotein lipase of mature adipocytes (COUTURIER et al., 1998).

Studies about the behavioral effects of caffeine detected an increase in alertness, fast and clear thoughts, and increased mental awareness; stimulation of the cardiac muscle, secretion of gastric acid, increased urine volume, decreased fatigue and delayed need of sleep were also reported (DALL'AGNOL, 2001).

Curi et al. (2002) state that caffeine, in addition to stimulating the central nervous system through the increase in the plasma concentration of adrenaline, directly stimulates lipolysis; during physical exercise, the effect on the nervous system is not relevant, but the direct influence on lipolysis is significant in the supply of fatty acids to the blood flow. These authors state that the dose that causes these effects is 3-6 mg kg^{-1} body weight (3-4 coffees); a dose of 8 mg kg^{-1} is considered doping and a dose higher than 10-15 mg kg^{-1} is toxic, causing gastrointestinal disturbances, arrhythmia, anxiety and hallucinations.

Altimari et al. (2005) and Mendes and Brito (2007) report that the metabolism of caffeine occurs mainly in the liver, where there is a greater concentration of cytochrome P450 1A2, an essential enzyme for the metabolism of this substance. The brain and the kidneys play an important role in caffeine metabolism because they also synthesize the cytochrome.

The adipose tissue is a dynamic organ and is related to processes that contribute to some diseases such as atherosclerosis, arterial hypertension and other cardiovascular diseases, insulin resistance, and type 2 diabetes (DRAY et al., 2007; HIGDON; FREI, 2006); these observations justify the importance of studying different methods for losing weight and adiposity, such as caffeine, to improve life quality. Caffeine, among other compounds, can increase the energy expenditure and antagonize the decrease of the metabolic rate that takes place during weight loss, a decrease that obviously is not interesting in this situation (DIEPVENS et al., 2007).

We hypothesized that the chronic use of caffeine could have a fat-reducing effect, even when obesity or exercise are not present. Our objective in this study was to test whether the chronic administration of caffeine to Wistar rats reduce body weight and fat mass.

Material and methods

The rats were supplied by the Central Animal House of the State University of Maringá, kept in plastic cages in groups of four animals at the animal house of the Department of Physiological Sciences under controlled conditions of light (12 hours light 12 hours $dark^{-1}$) and temperature (22 ± 2°C). Water and rodent chow (Nuvital, Curitiba, Paraná, Brazil) were provided freely during the experimental period. All the procedures were approved and certified by the Ethical Committee on Animal Experimentation (Report 056/2009-CEAE).

Sixteen male Wistar rats, weighting about 240 g and aging 60 days, were randomly assigned to two groups:

Caffeine Group – these animals were given caffeine (Farmácia São Paulo Manipulação, Maringá, Paraná, Brazil) once a day, in the morning, through gavage. Caffeine concentration was 5 mg kg^{-1} body weight; caffeine was dissolved in distilled water immediately before administration.

Control Group – these animals were given only distilled water at the same time of the day and in equal volume as the Caffeine Group.

There are many ways of administering caffeine to both humans and animals subjected to experimental protocols: intraperitoneal, subcutaneous, or intramuscular injections; suppositories; orally. The latter is the easiest and most used (MENDES; BRITO, 2007). In rats, the oral administration of caffeine as gavage, in addition to causing little stress to the animal when carried out by a handy experimenter, assures the administration of the desired dose, and that is why it was chosen for the treatment of the animals in this protocol.

The experimental period lasted five weeks. The amount of food ingested was recorded at the beginning and at the end of each week. Body weight was recorded on the first and each seven days. Food ingestion and body weight were used to calculate food conversion, that is, the weekly body weight gain as a function of ingested metabolizable energy. The energy content employed for this calculation was that provided by the manufacturer (12.26 kJ g^{-1} chow).

At the end of the fifth week, the rats were fasted overnight, anesthetized with thiopental (40 mg kg^{-1} body weight) and euthanatized by exsanguination. The periepididymal, retroperitoneal, subcutaneous and mesenteric fat pads were completely removed and weighted in a digital scale. Heart and liver were also weighted and blood was collected for biochemical measurements.

Plasma or serum were obtained by centrifuging the blood samples and were used to determine the plasma concentrations of glucose and triglycerides and the serum concentrations of total cholesterol and HDL cholesterol through enzymatic-colorimetric methods (GoldAnalisa, Belo Horizonte, Minas Gerais, Brazil). The samples were read in a Bio2000 spectrophotometer (BioPlus, São Paulo, São Paulo, Brazil) and the values were expressed as mg dL^{-1}.

Adipocytes from six rats of each group were collected for further analysis. The retroperitoneal adipose tissue was mechanically fragmented with fine-tipped scissors and placed in digestive buffer (DMEM/HEPES 24 mmol L^{-1}, 4% bovine serum albumin (BSA) fraction V, collagenase II 1.25 mg mL^{-1}, pH 7.4 at 37°C) for 20 minutes under constant stirring. Next, the digested tissue was filtered and washed three times with 25 ml of EARLE/HEPES buffer (EHB) 20 mmol L^{-1} containing 1% BSA, glucose-free sodium pyruvate 1 mmol L^{-1}, pH 7.4 at 37°C (RODBELL, 1964). The isolated adipocytes were photographed under optical microscope for morphometric evaluation immediately after isolation. One-hundred cells of each rat had their diameters measured using the computerized image analyzer Image-Pro Plus 4.5 (Media Cybernetics, Bethesda, USA).

The data were statistically treated using a non-paired Student t test. A p value of 0.05 was considered significant. The statistical analyses were carried out with Prism version 4 (GraphPad, San Diego, USA). Data are shown as mean ± standard deviation.

Results

The relative amount of ingested food was not different between the Control Group and the Caffeine Group (Figure 1). Also, both groups gradually decreased their relative ingestion of food during the five weeks of observation and treatment ($p < 0.05$).

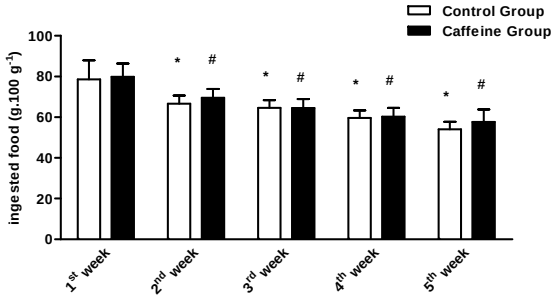

Figure 1. Relative ingested food of the Control Group and Caffeine Group during five weeks. Caffeine was administered orally at the dose of 5 mg kg^{-1} every morning. Data are shown as mean ± standard deviation; n=8 for each group. ⋆ $p < 0.05$ compared with the first week of the Control Group, # $p < 0.05$ compared with the first week of the Caffeine Group, non-paired Student t test.

Food conversion was not different between the Control Group and the Caffeine Group, and progressively decreased during the five-week period in both groups (Figure 2). In other words, caffeine did not influence the gain of body weight of the animals per amount of metabolizable energy ingested, which gradually decreased as the animals became older.

The weight gain per week was calculated as the difference between the body weight at the end and at the beginning of the week. The differences between the groups were not significant, but the weight gain gradually decreased along the five

weeks, as shown in Figure 3. At the end of the experimental period, both groups had similar body weight nasoanal length ratio^{-1} (Table 1).

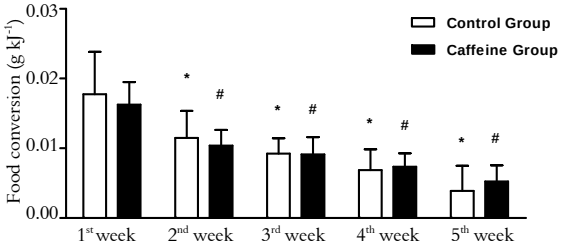

Figure 2. Food conversion of the Control Group and Caffeine Group during five weeks. Caffeine was administered orally at the dose of 5 mg kg^{-1} every morning. Data are shown as mean ± standard deviation; n=8 for each group. ⋆ $p < 0.05$ compared with the first week of the Control Group, # $p < 0.05$ compared with the first week of the Caffeine Group, non-paired Student t test.

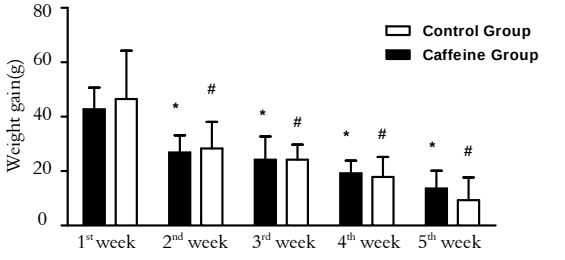

Figure 3. Body weight gain of the Control Group and Caffeine Group during five weeks. Caffeine was administered orally at the dose of 5 mg kg^{-1} every morning. Data are shown as mean ± standard deviation; n=8 for each group. ⋆ $p < 0.05$ compared with the first week of the Control Group, # $p < 0.05$ compared with the first week of the Caffeine Group, non-paired Student t test.

Table 1. Biometric and biochemical parameters of the Control Group and Caffeine Group after five weeks of oral administration of caffeine (5 mg kg^{-1}).

Parameter	Control Group (n=8)	Caffeine Group (n=8)
Body weight nasoanal length ratio^{-1}	14.79±0.90	14.78±1.22
Heart (g 100 g^{-1})	0.36±0.02	0.38±0.07
Liver (g 100 g^{-1})	3.19±0.21	3.16±0.23
Retroperitoneal fat pad (g 100 g^{-1})	1.16±0.30	1.24±0.51
Periepididymal fat pad (g 100 g^{-1})	1.09±0.28	1.13±0.26
Subcutaneous fat pad (g 100 g^{-1})	1.05±0.37	1.06±0.18
Mesenteric fat pad (g 100 g^{-1})	0.95±0.17	0.92±0.21
Retroperitoneal adipocytes diameter (μm)	91.50±7.39⋆	77.58±6.85⋆
Plasma glucose (mg dL^{-1})	103.11±14.98	107.55±11.79
Serum triglycerides (mg dL^{-1})	55.44±17.26	51.87±8.49
Serum total cholesterol (mg dL^{-1})	148.17±14.49⋆	108.78±11.53⋆
Serum HDL cholesterol (mg dL^{-1})	93.75±14.45	94.86±10.76

Data are shown as mean±standard deviation. ⋆$p < 0.05$ between the groups, non-paired Student t test.

Heart and liver, which were weighted as important representatives of the metabolically and physiologically active mass of an animal, did not differ between the groups (Table 1).

The weight of the retroperitoneal, periepididymal, subcutaneous and mesenteric fat pads, corrected for body weight (i.e., per 100 g body weight), was not statistically different between the groups, either compared separately (Table 1) or together (not shown). However, the measurements of the retroperitoneal adipocytes revealed that the adipose cells of the caffeine-treated animals had a diameter significantly smaller than those of the control animals (Table 1; $p < 0.05$).

Total cholesterol was markedly lower in the Caffeine Group (Table 1; $p < 0.05$), while HDL cholesterol was not different between the groups.

Discussion

By the end of 2003, caffeine was one of the forbidden substances of the World Anti-Doping Agency (WADA), in the class of stimulants, but later it was withdrawn. Caffeine has pharmacological actions on the Central Nervous System, with the ability of exciting or restoring cerebral and bulbar functions without being considered a therapeutic drug. It is commonly used and freely commercialized because of its low power of addiction (ALTIMARI et al., 2005).

In this investigation caffeine was administered through gavage to rats for five weeks to assess some effects of this substance, particularly on body weight and adiposity, because about 80% of the general population uses this substance on a daily basis (SILVA, 2003).

According to Altimari et al. (2005) and Mendes and Brito (2007), almost 100% of the caffeine ingested is absorbed by the gastrointestinal tract, reaching a peak plasma concentration after 15 to 120 minutes. The half-life of caffeine in the circulation ranges from 4 to 6 hours (MELLO et al., 2007).

The dose used was 5 mg kg^{-1} body weight; Silva (2003) reports that low doses of caffeine (2-10 mg kg^{-1} body weight) cause enhanced alert, decreased sleepiness, fatigue relief, and increased breath and heart rates, catecholamine release, metabolism and diuresis.

To Curi et al. (2002), besides its stimulant effect on the Central Nervous System by increasing the plasma concentration of noradrenaline, caffeine also directly stimulates lipolysis, at doses ranging from 3 to 6 mg kg^{-1} body weight (3-4 coffees). To adult humans, doses of up to 6 mg kg^{-1} body weight have not been linked to toxic, cardiovascular, or other adverse effects (NAWROT et al., 2003).

A literature review by Greenway (2001) concludes that caffeine is efficient as an agent of weight loss. In this study, the dose of 5 mg kg^{-1} body weight of caffeine, administered daily for five weeks, did not have any detectable effect on the total body weight or the weight of any of the major store of

white adipose tissue. However, the treatment did significantly reduce the diameter of the retroperitoneal adipocytes, as well as the serum concentration of cholesterol. The dermatologic use of caffeine in rats also caused a 17% decrease in the diameter of adipocytes (VELASCO et al., 2008). In a study in humans, Phillips et al. (1981) observed a positive correlation between coffee and/or tea ingestion and LDL cholesterol reduction. Consuming decaffeinated coffee also decreased the LDL cholesterol in other study in humans (SUPERKO et al., 1991), indicating that components of coffee other than caffeine can also have a biological effect.

It is possible that caffeine exerts a significant effect on body weight loss a) when body weight is above the normal range, such as in overweight or obese individuals; b) when it is associated with other synergistic compounds, such as ephedrine; c) when it is associated to physical activity (GREENWAY, 2001; HALLER et al., 2004; HINO et al., 2007; MAGKOS; KAVOURAS, 2004; MELLO et al., 2007).

Conclusion

The chronic oral administration of caffeine to adult, non-obese Wistar rats at the dose of 5 mg kg^{-1} did not decrease their body weight nor their fat mass. However, it did decrease the size of their adipocytes and reduced the serum levels of cholesterol, even in the absence of physical activity or other active compounds.

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