



ORIGINAL ARTICLE

## Lipid metabolism in trained rats: Effect of guarana (*Paullinia cupana* Mart.) supplementation

Waldecir P. Lima<sup>a</sup>, Luiz C. Carnevali Jr<sup>a</sup>, Robson Eder<sup>a</sup>, Luis Fernando B.P. Costa Rosa<sup>a</sup>, Elfriede M. Bacchi<sup>b</sup>, Marília C.L. Seelaender<sup>a,\*</sup>

<sup>a</sup>Department of Cell and Developmental Biology, Institute of Biomedical Sciences, Av. Prof. Lineu Prestes 1524, São Paulo, SP, CEP 05508-900, Brazil

<sup>b</sup>Department of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

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### KEYWORDS

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### Summary

**Background and aims:** Guarana is widely consumed by athletes, either in supplements or in soft drinks, under the belief that it presents ergogenic and “fat burning” effects. We examined the effect of guarana supplementation (14 days) upon aspects of lipid metabolism in sedentary (C) and trained rats (T).

**Methods:** To isolate the effect of caffeine from that of other components of guarana, we adopted two different doses of whole extract (G1—0.130 g/kg; G2—0.325 g/kg) or decaffeinated extract (DG1, DG2). Body weight, food and water intake; muscle fat content, oleate incorporation, glycogen content, and carnitine palmitoyltransferase I (CPT I) activity and mRNA expression; along with plasma lactate concentration, were assessed.

**Results:** Muscle oleate incorporation was decreased in rats receiving decaffeinated guarana in relation to G1 and G2; as was CPT I mRNA expression in the gastrocnemius. Whole extract supplementation, but not DG induced reduced plasma lactate concentration in trained rats. G1 showed higher muscle glycogen content compared with all other groups. The results show an effect of guarana on aspects of lipid metabolism, which is abolished by decaffeination.

**Conclusion:** The changes in lipid metabolism of supplemented rats herein reported are associated with the methylxanthine content of guarana.

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### Introduction

Guarana (*Paullinia cupana* Mart. var. *sorbilis*), a sprawling shrub or woody vine, found in northern Brazil,<sup>1</sup> is widely used as a flavouring for soft

\*Corresponding author. Tel.: +5511 30918039;  
fax: +5511 30917402.

E-mail address: seelaend@icb.usp.br (M.C.L. Seelaender).

drinks, and in the composition of a variety of dietary supplements throughout the world. It is claimed to have stimulant and ergogenic properties and to be of therapeutic value for a variety of conditions, showing alleged antidiarrheic, diuretic, and antineuralgenic properties.<sup>2,3</sup> Guarana has been shown to have an antioxidant effect,<sup>3</sup> to inhibit platelet aggregation,<sup>4,5</sup> and to have a gastroprotective action.<sup>6</sup> In Brazil, the use of guarana by athletes is widespread, with claims of performance improvement, and it has also been adopted as a supplement in the diet of racing horses.<sup>7</sup> Guarana and Ma Huang mixtures are commercialized as weight reducing supplements, and seem to effectively promote fat loss in overweight subjects,<sup>8</sup> despite considerable adverse effects.<sup>9</sup>

The role of changes in lipid metabolism in the anecdotal weight loss induced by guarana consumption has never, to our knowledge, however, been previously investigated. The existing studies assume that the effects of guarana equal those of caffeine, what is not necessarily true.

Guarana seeds contain caffeine (2.5–5%), theobromine and theophylline (small amounts), and tannins (up to 16%).<sup>10</sup> Although the methylxanthine content of guarana may explain many of the effects attributed to the plant, some studies demonstrated that treatment of rats with caffeine in similar doses to those found in guarana fails to induce many of the responses observed after guarana supplementation.<sup>2</sup> It was thus suggested<sup>2,3</sup> that tannins could play a part in these responses to guarana supplementation.

The aim of this study was to examine the effects of guarana supplementation upon tissue lipid metabolism in rats receiving different doses of aqueous extract of guarana (GE), and compare the results with those presented by animals whose diet was supplemented with decaffeinated guarana extract (DG), hence isolating the influence of the methylxanthine content from that of other components present in the extract. Trained rats were included in the study as to allow the comparison of the effects of exercise and supplementation upon lipid metabolism. An intermittent exercise training protocol was chosen, since anecdotal evidence shows frequent consumption of guarana is common (67%) among male teenager (14–18 years-old) volleyball and other court sports players in Brazil.

## Materials and methods

### Materials

Solvents, buffer reagents, and Tween 20 were purchased from LABSYNTH (Brazil); palmitoyl CoA,

carnitine, albumin, lipid standards, and solvents for HPLC and mass spectrometry from the Sigma Chemical Co., USA. <sup>14</sup>C-oleate and <sup>3</sup>H-carnitine were purchased from Amersham, UK.

Guarana powder (batch GUAR04/01, *P. cupana* HBK, *Sapindaceae*) was the kind gift from Santos Flora Ervas Mediciniais Ltd. (Brazil).

### Obtainment and analysis of guarana extracts

One litre of an ethanol:water solution (6.6:3.4 v/v) was added to each 2 kg of guarana crude powder and then, percolated with 6 l of the same solution, as in Prista et al.<sup>11</sup> After 7 days the percolate was evaporated and the resulting extract lyophilized, yielding 327 g of dry extract. The extract was submitted to HPLC analysis (Shimadzu), following the method of Salvadori et al.<sup>7</sup> that determined a caffeine content of 0.153 g/g of extract.

The decaffeinated extract was obtained after chloroform extraction,<sup>12</sup> which eliminated all the methylxanthines present in the extract. Tannins and catechins were not removed with this process being therefore present in the DG extract. Each 5 g of dry GE was mixed with 40.0 ml of chloroform and 5 ml 10% ammonium hydroxide. After vigorous agitation, followed by a decanting period of 15 min, the mixture was filtered with cotton pads. The final filtrate was mixed with 15 ml distilled water and 0.5 ml of a 10% aqueous solution of sulphuric acid. The mixture was heated for 2 min, filtered and brought to alkaline pH with 10% ammonium sulphate. Caffeine was extracted three times with chloroform. The obtained decaffeinated extract was submitted to HPLC analysis to ensure the effectiveness of the process.

### Animals and supplementation

Male adult Wistar rats (160–250 g), obtained from the Institute of Biomedical Sciences, University of São Paulo, were maintained under a 12 h light/12 h dark cycle (lights on at 7:00 a.m.), and controlled temperature conditions (23 ± 1 °C), receiving water and food (commercial chow, Nuvilab<sup>®</sup>, Nuvital, Brazil) ad libitum. Weight gain and food intake were assessed daily by measuring the amount of chow and the volume of water consumed by each animal, kept in a metabolic cage. The animals were killed by decapitation, between the interval of 8:00 and 11:00 a.m. The Biomedical Sciences Institute/USP Ethical Committee for Animal Research approved all the adopted procedures, which were carried out in accordance with the ethical principles stated by the Brazilian College of Animal Experimentation.

The doses of supplemented guarana were calculated based on the caffeine content of the extract. Guarana supplementation was carried out for 14 days. The following scheme was adopted (Table 1).

A lower dose was also adopted (0.065 g/kg of guarana, corresponding to 0.010 g/kg of caffeine), but found in preliminary studies (data not shown) to be insufficient to promote any significant changes in the studied parameters. The trained rats were supplemented with 0.130 g/kg body weight, after the results with sedentary rats were obtained, showing that this dose would be enough to promote changes in the investigated parameters, without presenting deleterious side effects (diarrhoea).

### Training protocol

All non-sedentary animals were submitted to an adaptation period of 6 days prior to the beginning of the training protocol. Training consisted of swimming in individual PVC tanks (100 × 60 × 60 cm) filled with circulating water at  $31.0 \pm 1.0^\circ\text{C}$ .<sup>13</sup> The training program was the following (Table 2).

During the training period exercise intensity was equivalent to 100%  $\text{VO}_2$  max, as the lactate threshold is attained with an extra load of 5–6% of body weight.<sup>14</sup> Body weight was assessed daily as to ensure proper calculation of the extra load. During the resting times the rats were kept outside the water.

### Measurement of fat content and $^{14}\text{C}$ -oleate incorporation into the skeletal muscle

Animals received 0.5 ml of [ $^{14}\text{C}$ ]-triolein (approximately 2.5  $\mu\text{Ci}$ ) intragastrically, as in Oller do Nascimento and Williamson<sup>15</sup> After 5 h the soleus, the gastrocnemius and the digestive tract were removed and submitted to the method described in Stansbie et al.<sup>16</sup>: duplicate samples were digested with 30% KOH (w/v) for 15 min, and then, after absolute ethanol was added, incubated for 2 h at  $70^\circ\text{C}$ . The free fatty acids from the saponifiable lipid fraction were extracted three times with petroleum ether and, after evaporation; the mass of the lipid present in the sample was assessed. Scintillation fluid was added to samples, whose radioactivity was determined in a scintillation

**Table 1** Experimental design.

Group	GE (g/kg body weight)	Caffeine content (g/kg body weight)	DG (g/kg body weight)
<i>Sedentary</i>			
C	0	0	0
G <sub>1</sub>	0.130	0.020	0
G <sub>2</sub>	0.325	0.050	0
DG <sub>1</sub>	0	0	0.130
DG <sub>2</sub>	0	0	0.325
<i>Trained</i>			
T	0	0	0
TG <sub>1</sub>	0.130	0.020	0
TDG <sub>1</sub>	0	0	0.130

**Table 2** Training program.

Period	Number of bouts	Activity duration (min)	Rest duration (min)	Load (% body weight)	Total time (min)
1st day (adaptation)	5	1	1	3	10
2nd day (adaptation)	5	1	1	5	10
3rd day (adaptation)	5	1	1	7	10
4th day (adaptation)	8	1	1	7	16
5th day (adaptation)	8	1	1	10	16
6th day (adaptation)	8	1	1	10	16
2nd–5th week (training)	10	1	1	10	20

counter (Packard, TRICARB 2100). To assess the total radioactivity in the tissue, 0.5 ml of NaOH (1N) was added to 300 mg of either liver or digestive tract, and the samples incubated for 30 min at 70 °C. An aliquot of 100 µl was transferred to a vial containing scintillation fluid, 100 µl of HCl and some drops of hydrogen peroxide (130V). The total radioactivity present in the tissue was measured to provide a control value for comparison with the amount of radiolabelled oleate incorporated in the form of lipid.

### Measurements of carnitine palmitoyltransferases I and II activities

To isolate the mitochondria<sup>17</sup> the muscles were minced with scissors and homogenized manually in isolation buffer (mannitol 220 mM, sucrose 70 mM, Hepes 2 mM, EDTA 0.1 mM, pH 7.4). The homogenate was filtered and centrifuged twice at 1000 rpm (12 min). The supernatant was then centrifuged twice at 8500 rpm for 15 min. The isolated mitochondria were suspended in a buffer consisting of 0.15 mM KCl and 5 mM Tris-HCl (pH 7.5), centrifuged (10,000g, 15 min), resuspended in 10 mM phosphate buffer (pH 7.5), frozen in liquid nitrogen and thawed. Samples were then ultracentrifuged (100,000g, 1 h—Hitachi). The resulting pellet was suspended in phosphate buffer to which Tween 20 (1% w/v) had been added, and stirred on ice for 30 min, in order to separate CPT I (membrane bound) from CPT II.<sup>18</sup> Another ultracentrifugation followed, after which the fractions containing CPT I (pellet) and CPT II (supernatant) were obtained.

CPT activity was measured with the method of Bremer,<sup>19</sup> which was modified by Seelaender et al.<sup>20</sup> Assay medium consisted of 60 mM KCl, 40 mM mannitol, 20 mM Hepes, 0.15 mM EGTA, 1.5 mM KCN, defatted bovine serum albumin (0.5%), 42 µM palmitoyl CoA, 0.35 mM carnitine (0.6 Ci <sup>3</sup>H-methylcarnitine) and approximately 0.03 mg of the isolated enzyme fraction or distilled water (blanks). The final volume of the assay mixture was 0.5 ml, and the pH, 7.3. The assay was stopped by addition of 1.5 ml of 7% perchloric acid, and the acylcarnitine formed was extracted with *n*-butanol, as described previously.<sup>21</sup> CPT activity is expressed as nmol/min per mg of protein in the isolated enzyme fraction.

### Analysis of gene expression

Total RNA was obtained from aliquots of 100 mg of the muscle of the animals after TRIZOL<sup>®</sup> reagent

extraction, accordingly to Chomzynski and Sacchi.<sup>22</sup> RNA concentration was determined spectrophotometrically (Beckman DU 640, Fullerton, CA, USA).

A semi-quantitative reverse transcriptase-polymerase chain reaction method was used for the estimation of the concentration of CPT I and CPT II mRNA. A 33 µl assay mix containing 3 µg RNA, 10 units of placental RNase inhibitor, 2 µl oligo(dt), 2 µl dNTP (10 nmol), 2 µl DTT, 10 units of Moloney-murine leukaemia virus reverse transcriptase (Invitrogen, USA), and 4 µl 10× reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 150 mM MgCl<sub>2</sub> in nuclease free water) was used to produce cDNA. The RT-mixture was incubated at 80 °C for 3 min, followed by 21 °C for 10 min, 42 °C for 30 min and then 99 °C for 10 min. 2 µl of the product obtained were fractionated in 1% agarose and ethidium bromide gel to assess the quality of the reaction. The primers were designed after the published Genebank sequences: CPT I [sense: CAAGGCCCTGGCTGATGATGTG; antisense: AGTCTCTGTCCGCCCTCTCG]<sup>23</sup>; CPT II [sense: GATAAGCA-GAATAAGCACACC; antisense: GGAGGAACAAAGCGAATGAGT].<sup>24</sup>

Amplification of cDNA was carried out in 30 cycles of 35 s at 94 °C, 60 s at 70 °C, and 60 s at 72 °C. The RPL19 gene was used as the internal control. Each 3 µl PCR mixture contained 40, 8 or 4 ng (three different cDNA dilutions were used) of cDNA, 0.5 units AmpliTaq Gold Polymerase (Perkin Elmer, Foster City, CA, USA), 2.5 nmol each dNTP, and 1.0 µM of the primers in reaction buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl<sub>2</sub>). Five microlitres of the PCR mixture were fractionated in polyacrilamide gel (agarose 1.2% and ethidium bromide). Semi-quantitative analysis was performed after image obtainment with the Typhoon<sup>®</sup> (Molecular Dynamics). Image analysis was carried out with the program ImageQuaNT TM.<sup>25,26</sup>

Sample protein content was assessed with the method of Lowry et al.<sup>27</sup>

Lactate concentration was measured (lactate analyzer 1500 YSI Sport) in the plasma obtained on the last day of training (at rest, and after the 5th and the last bouts of exercise).<sup>14</sup>

Glycogen content of the gastrocnemius was evaluated by a histological method: After sample fixation (4% paraphormaldehyde) for 18 h, the specimens were kept (24 h) in 70% ethanol, and then immersed into 96% and 100% ethanol solutions, xylol and Paraplast<sup>®</sup>. The samples were kept under 60 °C for 5 h. After rehydration they were incubated with a solution containing perchloric acid, Schiff reagent, and sodium sulphide. Sections

were examined with a Nikon Eclipse E600 microscope. Images were captured using a digital camera (cool Snap—PROcf color), and image Pro Plus software (Media Cybernetics, Silver Spring, MD) was applied for the morphometric analysis.

Results are expressed as mean±standard error of mean. Statistical analysis was performed with one-way ANOVA, followed by Tukey's post-test. Significance level was set at, at least,  $P < 0.05$ .

## Results

Guarana supplementation with the higher dose studied caused, after 14 days, the decrease of the total intake of food (Table 3), which was not found when the decaffeinated extract was supplemented. Although the total weight gain in the same period was not significantly changed by treatment with whole GE, both doses of DG induced lower weight gain in the period as compared with controls, despite the lack of difference in food consumption. Training reduced the weight gain rate of all groups. Water consumption was unaffected by the supplementation and training protocols.

The relative weight (percentage of total body weight represented by the tissue) and the neutral lipid content of both soleus and gastrocnemius muscles was not different among the studied groups (Table 4). Animals receiving the higher dose of guarana showed, nevertheless, decreased carcass fat content ( $9.14 \pm 0.40\%$  of total tissue weight,  $n = 7$ ), as compared with controls ( $10.77 \pm 0.2\%$ ,  $n = 7$ ,  $P < 0.05$ ) and with  $G_1$  ( $12.5 \pm 0.36\%$ ,  $n = 6$ ,  $P < 0.05$ ). Exogenous oleate incorporation

was decreased in the soleus and gastrocnemius muscles of  $DG_1$  and  $DG_2$  in relation to  $G_1$  and  $G_2$ . This parameter was not affected by the training protocol adopted.

Maximal long-chain fatty acid mitochondrial transport capacity, measured as the maximal activity of the CPT I system was not changed due to supplementation or training with the intermittent exercise protocol (Table 5), nor was the mRNA expression for CPT I in the muscles of rats receiving the different guarana treatments (Table 6). However, chronic consumption of DG led to decreased CPTI mRNA expression in the gastrocnemius of trained rats (Table 7).

When plasma lactate was measured (Table 8) at rest, after 5 or after 10 bouts, a significantly lower concentration was found for rats receiving guarana supplementation as compared with controls, after the 5th bout. This difference did not persist until the 10th bout, as the result for  $TG_1$  was not different from T. The decaffeinated extract did not induce a different response compared with controls. Figure 1A–C show the Periodic Acid of Schiff (PAS) reaction in the gastrocnemius of C,  $G_1$  and  $DG_1$ . A more intense reaction was obtained for  $G_1$ , suggesting higher glycogen content in this group.

## Discussion

Guarana (*Paullinia cupana*) consumption is increasing in the world, as it takes part in the composition of many commercial dietary supplements,<sup>28</sup> as a weight loss-promoting adjuvant.<sup>8</sup> In Brazil, GE has been also used as a stimulant and in the therapeutics of depression, fatigue, and migraine.<sup>29</sup>

**Table 3** Weight, total food and water variations after the 14 days of supplementation.

Group	Weight variation after 14 days (g)	Food consumption (g/14days)	Water consumption (ml/14days)
C	42.08 ± 3.47	360.00 ± 4.39	583.00 ± 31.37
$G_1$	35.00 ± 3.52	381.00 ± 13.29	476.00 ± 22.67
$G_2$	37.08 ± 2.98	329.50 ± 13.30**	473.00 ± 24.32
$DG_1$	29.10 ± 3.29	361.66 ± 15.91	505.00 ± 19.09
$DG_2$	27.22 ± 3.61**	357.00 ± 12.72	497.00 ± 26.87
T	20.13 ± 1.66*,a,d	391.66 ± 18.42	542.00 ± 32.60
$TG_1$	17.13 ± 1.71*,b,e	418.00 ± 20.69 <sup>e</sup>	528.00 ± 38.96
$TDG_1$	11.50 ± 1.55*,c,f,g,h	376.50 ± 14.75	507.00 ± 32.54

C, control sedentary,  $G_1$ , sedentary rats supplemented with 0.130 g/kg body weight of guarana extract; or ( $DG_1$ ) decaffeinated guarana extract;  $G_2$ , sedentary rats supplemented with 0.325 g/kg body weight of guarana extract; or ( $DG_2$ ) decaffeinated guarana extract; T, trained control rats;  $TG_1$ , trained rats supplemented with 0.130 g/kg body weight of guarana extract; or ( $TDG_1$ ) decaffeinated guarana extract.

Results are mean ± s.e.m. of 12 (sedentary) or 8 (trained) animals. \* $P < 0.001$  in relation to C; \*\* $P < 0.05$  in comparison with C; <sup>a</sup> $P < 0.05$  in relation to  $G_1$ ; <sup>b</sup> $P < 0.01$  in relation to  $G_1$ ; <sup>c</sup> $P < 0.001$  in relation to  $G_1$ ; <sup>d</sup> $P < 0.05$  in relation to  $G_2$ ; <sup>e</sup> $P < 0.01$  in relation to  $G_2$ ; <sup>f</sup> $P < 0.001$  in relation to  $G_2$ ; <sup>g</sup> $P < 0.01$  in relation to  $DG_1$ ; <sup>h</sup> $P < 0.01$  in relation to  $DG_1$ .

**Table 4** Relative weight, fat content and oleate incorporation of gastrocnemius (GAS) and soleus (SOL) muscles.

	Weight (% body weight)		Fat content (mg fat/g tissue)		Incorporation (%)	
	GAS	SOL	GAS	SOL	GAS	SOL
C	0.585 ± 0.072	0.040 ± 0.004	16.13 ± 0.53	28.12 ± 2.41	0.232 ± 0.032	0.181 ± 0.020
G <sub>1</sub>	0.564 ± 0.042	0.039 ± 0.004	15.19 ± 0.71	27.12 ± 2.98	0.391 ± 0.042	0.373 ± 0.070
G <sub>2</sub>	0.609 ± 0.068	0.041 ± 0.005	15.69 ± 1.06	29.12 ± 1.75	0.296 ± 0.030	0.289 ± 0.041
DG <sub>1</sub>	0.640 ± 0.077	0.041 ± 0.005	17.22 ± 0.75	30.12 ± 3.48	0.205 ± 0.020*	0.176 ± 0.044*
DG <sub>2</sub>	0.593 ± 0.082	0.039 ± 0.006	18.59 ± 1.22	29.12 ± 1.75	0.153 ± 0.029**	0.142 ± 0.029**
T	0.713 ± 0.098	0.044 ± 0.006	15.15 ± 0.80	30.12 ± 2.93	0.295 ± 0.046	0.278 ± 0.064
TG <sub>1</sub>	0.692 ± 0.083	0.042 ± 0.004	17.10 ± 0.48	31.45 ± 3.02	0.345 ± 0.067	0.293 ± 0.037
TDG <sub>1</sub>	0.663 ± 0.079	0.041 ± 0.005	16.58 ± 1.08	30.23 ± 4.13	0.302 ± 0.062	0.269 ± 0.058

C, control sedentary, G<sub>1</sub>, sedentary rats supplemented with 0.130 g/kg body weight of guarana extract; or (DG<sub>1</sub>) decaffeinated guarana extract; G<sub>2</sub>, sedentary rats supplemented with 0.325 g/kg body weight of guarana extract; or (DG<sub>2</sub>) decaffeinated guarana extract; T, trained control rats; TG<sub>1</sub>, trained rats supplemented with 0.130 g/kg body weight of guarana extract; or (TDG<sub>1</sub>) decaffeinated guarana extract.

Results are mean ± s.e.m. of 12 (body weight), 15 (fat content) sedentary animals; or 8 (body weight and fat content) for trained rats. For the oleate incorporation experiments 6 animals from each group were used. \**P* < 0.05 in relation to G<sub>1</sub>; \*\**P* < 0.01 in relation to G<sub>2</sub>.

**Table 5** Maximal activity of carnitine palmitoyltransferase I (CPT I) and II (CPT II).

	CPT I		CPT II	
	GAS	SOL	GAS	SOL
C	3.06 ± 0.29	3.39 ± 0.30	2.14 ± 0.20	1.33 ± 0.12
G <sub>1</sub>	3.34 ± 0.35	2.91 ± 0.36	2.01 ± 0.39	1.48 ± 0.20
G <sub>2</sub>	3.10 ± 0.23	4.41 ± 0.51 <sup>a</sup>	2.43 ± 0.30	1.46 ± 0.18
DG <sub>1</sub>	3.03 ± 0.29	3.45 ± 0.38	2.17 ± 0.45	1.19 ± 0.17
DG <sub>2</sub>	2.68 ± 0.33	2.63 ± 0.15*	1.89 ± 0.10	0.93 ± 0.05
T	3.21 ± 0.21	3.24 ± 0.23	1.97 ± 0.32	1.09 ± 0.14
TG <sub>1</sub>	3.31 ± 0.44	2.95 ± 0.45	2.48 ± 0.37	0.98 ± 0.09
TDG <sub>1</sub>	2.39 ± 0.41	3.03 ± 0.39	2.03 ± 0.10	1.27 ± 0.19

nmol/min/mg of protein in the isolated enzyme fraction.

C, control sedentary, G<sub>1</sub>, sedentary rats supplemented with 0.130 g/kg body weight of guarana extract; or (DG<sub>1</sub>) decaffeinated guarana extract; G<sub>2</sub>, sedentary rats supplemented with 0.325 g/kg body weight of guarana extract; or (DG<sub>2</sub>) decaffeinated guarana extract; T, trained control rats; TG<sub>1</sub>, trained rats supplemented with 0.130 g/kg body weight of guarana extract; or (TDG<sub>1</sub>) decaffeinated guarana extract.

Results are mean ± s.e.m. of 12 (sedentary) or 8 (trained) animals. <sup>a</sup>*P* < 0.05 in relation to G<sub>1</sub>; \**P* < 0.01 in comparison with G<sub>2</sub>.

**Table 6** mRNA expression for CPT I in the gastrocnemius (gastro) and soleus muscles of sedentary groups.

	C	G <sub>1</sub>	G <sub>2</sub>	DG <sub>1</sub>	DG <sub>2</sub>
Gastro	0.905 ± 0.116	0.929 ± 0.245	1.192 ± 0.276	0.970 ± 0.116	1.092 ± 0.381
Soleus	1.252 ± 0.136	1.338 ± 0.300	1.019 ± 0.103	1.505 ± 0.389	1.473 ± 0.156

Results are mean ± s.e.m. of the ratio CPT I/RPL 19 of expression for 7 (gastrocnemius) or 3 (soleus) animals.

C, control sedentary, G<sub>1</sub>, sedentary rats supplemented with 0.130 g/kg body weight of guarana extract; or (DG<sub>1</sub>) decaffeinated guarana extract; G<sub>2</sub>, sedentary rats supplemented with 0.325 g/kg body weight of guarana extract; or (DG<sub>2</sub>) decaffeinated guarana extract.

Although caffeine is considered to be the active component of guarana, there is evidence that its other components may also be involved in the

response to supplementation.<sup>2,3</sup> Although there is extensive knowledge on the effects of caffeine upon lipid metabolism, to our knowledge no study

**Table 7** mRNA expression for CPT I in the gastrocnemius and soleus muscles of trained groups.

	TG <sub>1</sub>	TDG <sub>1</sub>
Gastrocnemius	0.767 ± 0.076	0.590 ± 0.041*
Soleus	1.120 ± 0.093	1.068 ± 0.235

Results are mean ± s.e.m. of the ratio CPT I/RPL 19 of expression for 7 (gastrocnemius) or 4 (soleus) animals. TG<sub>1</sub>, trained rats supplemented with 0.130 g/kg body weight of guarana; TDG<sub>1</sub>, trained rats supplemented with 0.130 g/kg body weight of decaffeinated guarana. \**P* < 0.005 for comparison with TG<sub>1</sub>.

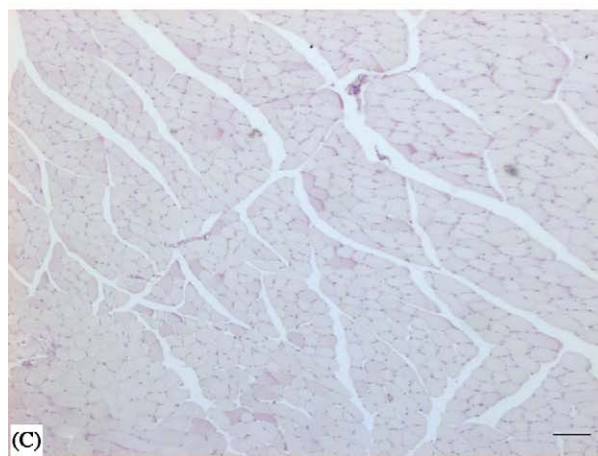
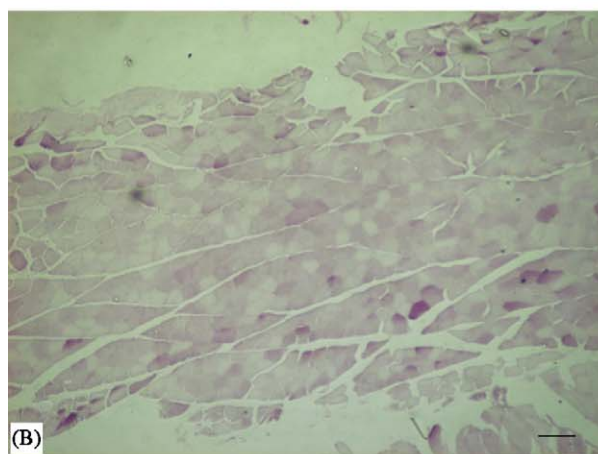
**Table 8** Plasma lactate of trained rats (mmol/l), supplemented with 0.130 g/kg body weight of guarana (TG<sub>1</sub>) or decaffeinated guarana (TDG<sub>1</sub>), and control (T).

	Rest	5th bout	10th bout
T	1.84 ± 0.26	6.14 ± 0.48*	9.84 ± 0.68***
TG <sub>1</sub>	1.79 ± 0.23	3.68 ± 0.59 <sup>a</sup>	9.02 ± 0.89***
TDG <sub>1</sub>	1.52 ± 0.24	7.84 ± 0.54 <sup>a,b</sup>	11.87 ± 0.60***

Results are mean ± s.e.m. of the plasma samples obtained from 9 (T) or 8 (TG<sub>1</sub>) and 6 (TDG<sub>1</sub>) rats. \**P* < 0.001 in relation to rest; \*\*\**P* < 0.001 in relation to the 5th bout; <sup>a</sup>*P* < 0.01 in relation to T; <sup>b</sup>*P* < 0.001 in relation to TG<sub>1</sub>.

has addressed so far the effect of guarana supplementation, except in relation to lipid peroxidation.<sup>3</sup> We have thus examined aspects of lipid metabolism in sedentary and trained rats, submitted to guarana or DG supplementation, in order to isolate the effects of caffeine from those of the other components.

Food intake was found to be decreased in the group receiving the higher dose of guarana/caffeine (0.325/0.05 g/kg, respectively). Racotta et al.<sup>30</sup> also reported diminished food consumption after caffeine injection in rats, although others found no differences in this parameter<sup>31</sup> between caffeine treated animals and controls, or still, an increase.<sup>32</sup> The discrepancies among studies may be related to the different doses of caffeine and models adopted. Andersen and Fogh<sup>33</sup> showed that in overweight human subjects gastric emptying was delayed by the consumption of a herbal preparation consisting of a mixture of guarana, yerbe maté and damiana, consequently modifying food energy intake and inducing weight loss. Guarana, when combined with Ma Huang, has been shown to induce weight loss in overweight men, women and

**Figure 1** PAS reaction in the gastrocnemius of animal treated with guarana extract (G<sub>1</sub>—A), decaffeinated guarana extract (DG<sub>1</sub>—B) and controls (C—C).

adolescents, as reviewed by Carlini.<sup>10</sup> In the same review the author stresses the anorectic properties of guarana, and emphasizes the role of constituents of the extract, other than caffeine, upon many of the reported physiological alterations induced by supplementation. Indeed, the final weight gain of the animals consuming 0.130 g/kg DG was presently

found to be decreased, as compared with controls and animals submitted to whole guarana supplementation. Theophylline, known to be present in GE, a potent adenosine receptor antagonist, has been linked to increased lipolysis.<sup>34,35</sup> In the present study carcass fat content was also reduced as a response to guarana supplementation, an effect which may be attributed to both caffeine and theophylline. However, the fact that the final weight gain of animals consuming the decaffeinated extract was lower than that of the group receiving whole guarana suggests that tannins and catechins may also contribute to the result, especially after the removal of methylxanthines. The tannin content of GE may reach 8.5%,<sup>33</sup> and the consumption of tannins has been associated<sup>36</sup> with decreased body weight in various animal species.

As the use of mixtures containing guarana is increasing among athletes it was also our aim to examine the effect of the supplementation upon muscle lipid metabolism in sedentary and trained rats. Training caused the weight of the animals to be reduced in all groups compared with the sedentary counterparts, but guarana supplementation (TG<sub>1</sub> and TDG<sub>1</sub>) did not allow further significant decreases of this parameter. Interestingly, nevertheless, a trend towards reduced weight gain in TDG<sub>1</sub> was detected, similarly to what was observed for sedentary animals. Although the fat content of the soleus and gastrocnemius muscles was unchanged by either guarana consumption or training, oleate incorporation was decreased in both muscles from the animals receiving the decaffeinated extract. This method measures the amount of exogenous oleate that is found in tissues 5 h after the administration of an intragastric *bolus* of long-chain fatty acid-rich triacylglycerol.<sup>15</sup> Therefore, the results with the sedentary, DG consuming groups reflect either a decreased capacity of fatty acid uptake by the muscle or still, enhanced oxidation of this substrate associated with reduced reesterification, in comparison with G1 and G2, which showed a trend towards increased oleate incorporation. Mittal et al.<sup>37</sup> reported decreased tissue fat level in mice fed proanthocyanidins, attributing this effect to possible changes in lipolysis or lipogenesis. Our results suggest that a diminished capacity for fatty acid uptake from the plasma may be involved in one such effect. The fact that trained rats did not show the same decrease in oleate incorporation is very possibly related to the increase in intrafibrillar triacylglycerol content promoted by training reported by our group in a previous study,<sup>38</sup> when we found an important contribution of very low density lipoproteins

(VLDL)-derived triacylglycerol to the replenishment of these stores.

The major regulatory step in the oxidation of long-chain fatty acids is the entry of this substrate into mitochondria, whose rate is a reflex of the activity of CPT I.<sup>39</sup> This enzyme catalyzes the formation of long acyl-carnitine complexes, which, according to the most recent models<sup>40</sup> enter the intermembrane space through a porine, being then transported by carnitine-acylcarnitine translocase (CACT) to the matrix compartment in which they are exposed to the catalytic action of carnitine palmitoyltransferase II (CPT II), regenerating carnitine and acyl CoA. Whole GE supplementation (G<sub>2</sub>) induced an increase of the activity of CPT I in the soleus, but not in DG<sub>2</sub>, suggesting caffeine takes part in the observed response. The literature provides no evidence of a direct effect of caffeine on the activity of this enzyme. However, the effects of caffeine upon lipolysis, and consequently, on substrate availability, could account, at least partially, for the increased activity observed, as it is also associated with an increase in resting oxygen consumption in humans.<sup>9</sup> The other methylxanthines in the extract could also interfere with CPT activity, as Alhomida<sup>41</sup> demonstrated an increase in rat heart carnitine palmitoyltransferase activity after theophylline administration; while Greer et al.<sup>42</sup> reported improved performance in the cycle ergometer after theophylline supplementation.

Measuring CPT activity in an isolated system presents the advantage of abolishing the influence of alterations in malonyl CoA concentration and in the sensitivity to malonyl CoA-induced inhibition. It is thus possible to discard the influence of these aspects in the present results of activity, which regard the catalytic capacity of CPT I, but not its concentration. On the other hand, CPT I mRNA expression was not modified by supplementation in sedentary rats, a parameter that, if different among the studied groups, could point out to eventual differences in the concentration of the enzyme (although a semi-quantitative analysis was carried out). These results suggest that the increase in the catalytic capacity herein reported could be linked to post-translational modification of CPT I.

Although CPT I activity was not altered by intermittent exercise training, nor by the combination of training and the supplementation protocols, CPT I mRNA expression was reduced in the gastrocnemius of trained rats receiving DG in comparison with TG<sub>1</sub>. These results suggest that, while in the soleus caffeine is able to induce changes in the catalytic capacity of CPT I, in the gastrocnemius the regulation would rest upon the control of

enzyme expression. This latter effect would be only apparent in trained animals. A possibility which cannot be discarded, nevertheless, is that the other components of guarana play a part in the discrepancy of CPT I mRNA expression found between TDG<sub>1</sub> and TG<sub>1</sub>. We have not been able to find studies in the literature concerning the effect of tannins upon CPT activity.

In order to investigate the eventual occurrence of a glycogen-sparing effect in the gastrocnemius induced by the treatment with guarana, we examined histological sections after PAS reaction. The stronger positivity of the reaction obtained for G<sub>1</sub> suggests an influence of the caffeine content of guarana upon this parameter. This hypothesis is corroborated by the fact that plasma lactate was reduced in TG<sub>1</sub> as compared with C and TDG<sub>1</sub>, after the 5th bout of exercise. Greer et al.<sup>42</sup> found no effect of either caffeine or theophylline ingestion in exercising subjects regarding muscle glycogen content and plasma lactate concentration. The present muscle glycogen results were obtained 24 h after the last exercise bout, while Greer et al.<sup>42</sup> collected the biopsies immediately after the exercise session.

Taken together the results show that guarana consumption is able to induce changes in lipid metabolism, but the predominant element inducing the alterations reported seems to be the methylxanthine content of the extract.

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