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Tea decoctions prevent body weight gain in rats fed high-fat diet; black tea being more efficient than green tea

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ABSTRACT

Background/Aims: In contrast to the usual tea infusion, the anti-obesity effect of tea decoction (TD) is poorly documented. Here, we compared and contrasted the chronic effect of short-time decoction (15-min) of green versus black tea (GTD, BTD) prepared at a dose of 5% on lipid digestion and weight gain in rats fed high-fat diet (HFD) for 10 weeks.

Methods: The rats were assigned into three groups (n = 10–12 each) and given ad libitum the HFD + water (CTRL) or GTD (GTGr) or BTD (BTGr). The food and fluid intake were measured daily and weight gains once/week. The fecal matters were collected twice/week for TPC, caffeine, total lipids and triglycerides (TG) analysis. In addition, the liver, perirenal and epididymal adipose tissues (AT) were removed and blood was collected for the same analysis and leptine level.

Results: 10-weeks TD consumption increased fecal TG excretion (+170 in GTGr and +230% in BTGr; $P < 0.001$ vs CTRL). It reduced liver TG by 25 and 35% ($P < 0.001$ vs CTRL) and plasma TG by 36.6 and 48% ($P < 0.01$ vs CTRL) in GTGr and BTGr, respectively. The AT gains were reduced by 26.5 and 56.4% in GTGr and 60% in BTGr ($P < 0.001$ vs CTRL). The reduced AT was consistent with a reduction of 27 and 59% of leptin levels ($P < 0.001$ vs CTRL) and 21 and 55% of weight gains in GTGr and BTGr ($P < 0.01$ vs CTRL), respectively.

Conclusion: Chronic GTD and BTD prevent fat storage in the liver, lowering blood lipids and glucose, increasing fecal excretion of TG, decreasing AT and weight gains in rats fed HFD, with a strong effect of BTD compared to GTD. Therefore, these beverages containing high amounts of TPC and caffeine could constitute a natural alternative in the prevention of obesity.

1. Introduction

Obesity and its co-morbidities remain a public health problem worldwide [1]. Epidemiological studies from North African countries reported that obesity and overweight affect about 15% of men in Tunisia population [2]. Expansion of visceral obesity was reported to be associated with the progression of multiple metabolic alterations, including exacerbation of insulin resistance, which led to diabetes and increased risks of cardiovascular diseases [3,4].

Many dietary regimens, pharmacological therapies and popular beverages have been advocated to combat obesity. More attention was focused on tea consumption because both green and black tea leaves contain numerous polyphenolic compounds and caffeine that have been involved in the control of abdominal obesity [5,6]. It has been reported that chronic administration of decaffeinated polyphenol extracts from green, Oolong or black tea decreases body weight, total visceral fat volume, liver lipid content and

Abbreviations: GTD, green tea decoction; BTD, black tea decoction; 15-min GTD, green dried tea leaves are cooked in boiling water for 15 min; 15-min BTD, black dried tea leaves are cooked in boiling water for 15 min; HFD, high-fat diet; TPC, total phenolic compounds; EGCG, epigallocatechingallate; CTRL, control group; EGC, epigallocatechin; ECG, epicatechingallate; RP-HPLC-MS, reverse phase-HPLC-mass spectrometry; TG, triglycerides; TF, theaflavins; TF_1, a mixture of theaflavin; TF_2A, theaflavin-3-gallate; TF_2B, theaflavin-3′-gallate; TF_3, theaflavin-3, 3′-digallate.

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inflammation in mice fed high-fat or high-sucrose diets [7]. In addition, a supplementation for 4 months of a high-fat diet (HFD) with dietary EGCG, one of the major green tea polyphenols, reduces body weight gain; body fat mass, liver triglycerides in mice, these effects are associated with increased fecal lipids [8]. These data are consistent with those showing that EGCG purified from green tea, supplemented in the diet, attenuated diet-induced body fat deposit because of a reduced diet digestibility in mice [9]. Moreover, highly purified theaflavins, a major polyphenols in black tea, significantly decreased the body weight, food intake, adiposity index and serum levels of total cholesterol, triglycerides and LDL-cholesterol in high-fat diet fed rats [10]. Furthermore, administration of black tea polyphenols reduced body weight gain, adipose tissue mass, and liver lipid content in mice fed a high-fat diet [6]. However, most of the studies on tea-polyphenols were performed with pure catechin, catechin-extract, purified theaflavins, tea infusion, but not with tea leaves prepared as decoction although this method is popular through large areas of North African countries, especially in Tunisia. In these countries, both green and black tea decoction (GTD, BTD) are prepared by cooking dried tea leaves with sugar in boiling water for a variable period of time [11]. On the other hand, in most Western and Asiatic countries, tea is consumed as an infusion and is prepared by adding dried tea leaves in hot water and let them brewed for a few minutes. Tea decoctions are different from tea infusions because long decoction process in boiling water could change the polyphenolic profile, and thus, their bioactive function. We have previously reported that a short time decoction of green tea (15-min GTD) contained higher amount of polyphenolic compounds than tea prepared as an infusion or cooked for a longer period of times (30-min) [12]. The most predominant peaks in 15-min GTD extract were EGCG, epigallocatechin (EGC), catechin, EGC-3-methyl gallate, epicatechingallate ECG, vanillic acid ester, kaempferol 3-glycoside and caffeine, whereas peaks in equivalent 15-min BTD remain unidentified.

In this study, we identified the major polyphenolic compounds of a short time (15-min) decoction of BTD as theaflavins, caffeine, gentissic acid esters, gallic acid esters, catechin, EGCG, kaempferols, and quercetin. Since, these compounds were in mostly different from the one identified in GTD, we compared and contrasted the effects of 10-week oral consumption of GTD and BTD without sugar on lipid digestion of HFD-fed rats by measuring fat excreted in feces, liver fat content, weight of abdominal fat tissues, food intake and body weight gain. The data show that chronic consumption of GTD, BTD increased fecal excretion of fats together with total polyphenolic compounds (TPC), reduced adipose tissue mass and liver triglyceride content along prevention of body weight gain with BTD being more efficient than GTD.

2. Materials and methods

2.1. Preparation of green tea decoction (GTD) and black tea decoction (BTD)

The decoctions were freshly prepared throughout the experimental period. Fifty grams (50 g) of green or black tea leaves (*Camellia sinensis*) purchased from local market (Tunis area center, Tunisia) were soaked in hot water and cooked in 1 L of distilled water for 15 min, then cooled to room temperature before distribution. All studies were performed with same batches of tea to avoid possible variations in the properties of the green or black tea sample.

2.2. Preparation of high-fat diet (HFD)

The HFD given to rats during the experimental period was prepared in our laboratory as previously reported [13]. All ingredients and chemical compounds of HFD including vegetable oil and butter, as a major source of lipids, are presented in Table 1. After melting at 100 °C, the butter was mixed with all ingredients and chemical compounds in a stainless blender. The homogeneous diet was transformed into a piece of cake, then dried at 45 °C and stored at +4 °C for short periods. The HFD provides 3400 kcal/kg with fat accounting for 24%, carbohydrate for 52% and protein for 24% of calories. Energy from fat was about 4-fold higher in HFD than that of a normal diet for rats. Detailed of fatty acid composition of HFD is presented in legend of Table 1.

2.3. Experimental protocol

Male Wistar rats aged 6 weeks ago and weighing between 120 and 140 g were purchased from Siphat company-Tunisia and housed in stainless steel cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C and light 12-h light/dark cycle, lights on 07.00 to 19.00) with tap water and regular standard food for rat [13] provided ad libitum. The animals were treated in accordance with the European Community guidelines based on declaration of Helsinki concerning the care and use of laboratory animals. After 1-week acclimation, the rats were weighed and randomly assigned into three groups (n = 10–12 rats each) with comparable body weight, and given ad libitum HFD and free access to water (CTRL), GTD (GTGr) or BTD (BTGr) for 10 weeks. The HFD, water, GTD and BTD were distributed to rats every morning at 08.00 h. However, to standardize the fluid intake between tea groups and the CTRL group, each rat of GTGr and BTGr was given about 5 ml of distilled water before tea distribution (Table 3). In addition, the body weight gains were determined once/week and feces were individually collected twice a week, dried and stored for total polyphenolic compounds (TPC), triglycerides and caffeine analysis. At the end of the experimental period, the rats were weighed, and then killed by decapitation. Fasting blood was

Table 1
Ingredients of the high-fat diet.

Ingredients	Amounts, g/kg diet
Powder skim milk[a]	380
Soya oil[f]	60
Butter[f b]	30
Maize starch	300
Sucrose	155
$CaCO_3$	20
Na_2PO_4	20
KCl	5
NaCl	5
Egg yolk[c]	17
Mineral mixture[d]	2.7
Vitamin mixture[e]	5.5

[a] As source of protein (Inesfood- Tunisia).
[b] As source of supplemented fat.
[c] As source of choline and L-cysteine.
[d] Mineral mixture (grams per kilogram dry weight of diet): $MgSO_4 \cdot 7H_2O$ = 1.85; $ZnSO_4 \cdot 7H_2O$ = 0.50; $MnSO_4 \cdot 4H_2O$ = 0.15; $CuSO_4 \cdot 5H_2O$ = 0.020; KIO_3 = 0.0015; $FeSO_4 \cdot 7H_2O$ = 0.200.
[e] Vitamin mixture (per kilogram dry weight of diet): synthetic vitamin A concentrate = 6.500 IU; cholecalciferol = 1.300 IU; α-tocopherol acetate = 2.6 mg; pyridoxine hydrochloride = 2.6 mg; thiamin hydrochloride = 2.6 mg; riboflavin sodium phosphate = 1.95 mg; nicotinamide = 13 mg; ascorbic acid = 65 mg; dexpanthenol = 5.2 mg and finely powdered sucrose to make 5 g.
[f] Total lipids composition of HFD (%) 8.85 ± 0.6. Fatty acids from total lipid content were 30.4% saturated fatty acids, mainly palmitic acid (C16: 0) and myristic acid (C14:0), 36.4% monounsaturated fatty acids, mainly oleic acid (C18:1 n-9), 33.3% polyunsaturated fatty acids, mainly linoleic acid (C18:2 n-6), linolenic acid (C18:3) and *Docosahexaenoic acid* (C22:6 n-3).

drawn in vacutainer tubes, centrifuged at 3000 rpm for 10 min, and then, the plasma was collected for the analysis of TPC, caffeine, glucose, triglycerides, cholesterol and leptin parameters. In addition, the liver, perirenal and epididymal white adipose tissues from abdominal cavity were removed, weighed and stored at − 20 °C until use.

2.4. Extraction and evaluation of TPC and caffeine

The extraction of TPC was performed according to the method used by Dhaouadi et al. [14]. Samples of 1 ml of plasma or 1 g of dried fecal matter were mixed with 3 ml of water, vigorously vortexes and sonicated for 20 min. Then, 7 ml of cold acetone (−20 °C) was added to the mixture. After centrifugation 10,000 × g for 15 min, the residue was re-extracted twice with 5 ml of acetone (−20 °C). The supernatants were collected, pooled and concentrated at 60 °C using a rotary evaporator and recovered to a final volume of 3 ml. To prevent oxidation of the polyphenols, extraction was rapidly achieved and extracts were immediately used or stored in darkness at −20 °C until further use. The TPC was estimated spectrometrically by the Folin-Ciocalteu assay described by Singleton et al. [15], using catechol as standard. The profile of phenolic and caffeine compounds in GTD, BTD, plasma and fecal matter was analyzed from the extract by RP-HPLC-MS technique [14].

2.5. Determination of liver and fecal lipids and plasma parameters

The total lipids from liver homogenates and fecal matters were extracted according to Folch et al. method [16] modified by Bligh and Dyer [17]. The liver and fecal triglycerides as well as plasma glucose, triglycerides, total cholesterol and HDL-cholesterol were analyzed by colorimetric methods using kits supplied by Biomaghreb Company (Tunis, Tunisia). Plasma leptin concentration was determined using Rat leptin RIA Kit (Cat RL-83K) supplied by Linco Research, Inc. USA.

2.6. Statistical analysis

All data are presented as the mean ± SEM. Comparisons between groups were performed with one ANOVA followed by Turkey multiple comparison to compare three groups and 2-ways ANOVA followed by Bonferroni post-test for kinetic studies. Statistical evaluation was carried out using Graph Pad Prism version 5.00 for Windows, (Graph pad software Inc., San Diego, CA). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Comparison of polyphenol contents between green and black tea decoctions

We compared the phenol contents of 15-min decoction of green tea versus black tea (Table 2). The sum of phenolic compounds was 3 times more important in GTD versus BTD ($p < 0.0001$vs BTD). However, the caffeine content was higher in BTD than in GTD ($p < 0.001$vs GTD). In addition, some of phenolic compounds were different. Both decoctions display free catechins and EGCG although GTD presenting 28-time more EGCG than BTD ($p < 2.10^{-7}$ vs BTD). More interestingly, in black tea decoction the most predominant peaks were gallic acid esters, gentissic acid esters, theaflavins and thearubigins that are poorly detectable at GTD. Conversely, GTD displayed EGC, ECG and gallic acids that are not detected in BTD. This difference urges us to compare their respective effect as chronic consumption in HFD fed rats.

Table 2
Comparison of polyphenolic compounds and caffeine content between green and black tea decoction.

Phenolic compounds	15-min GTD	15-min BTD
Gallic acid	6.0 ± 0.17	–
Gallic acid esters	–	185.3 ± 15.7
Gentissic acid esters	–	138.9 ± 11.8
Free catechins	6.26 ± 0.21	10 ± 0.7[a]
EGC	416 ± 2.3	–
EGCG	789.8 ± 9.6[b]	28.4 ± 0.6
ECG	18.1 ± 0.5	–
Theaflavins	–	38.3 ± 2.6
Kaempferols	–	14.2 ± 13.2
Thearubigins	–	20 ± 7
Sum of phenolic compounds	1236 ± 11.3[c]	435.1 ± 43.3
Caffeine	261.3 ± 8.7	319 ± 7.6[d]

Results are expressed as mean ± S.E.M in mg/100 ml of decoction (n = 4 measurements/sample).Values followed by different letters are significantly different between 15-min GTD and 15-min BTD: [a]: $p < 0,04$; [b]: $p < 2.10^{-7}$; [c]: $p < 0.0001$; [d]: $p < 0.001$.

3.2. Amounts of HFD and fluid intake during the experimental period

The food and fluid intake measured throughout the experimental period were presented in Table 3. The food intake daily was significantly higher in CTRL (18.02 ± 0.4 g) and GTGr (17 ± 0.6 g) than in BTGr (14.4 ± 0.4 g; $P < 0.01$vs CTRL). The volumes of water, GTD and BTD consumed were 30.2 ± 0.3; 28.3 ± 0.2 and 27.9 ± 0.3 ml/rat/day for the CTRL, GTGr and BTGr, respectively. Therefore, at equal volume consumption, the GTD provides 346 mg TPC or 221 EGCG mg and 73 mg caffeine whereas the BTD provides 121.4 mg TPC and 89 mg caffeine.

3.3. Enhanced plasma and fecal levels of TPC and caffeine in HFD rats consuming GTD or BTD

In Fig. 1A, HFD rats consuming daily GTD and BTD for 10 weeks, have a significant 3-fold ($P < 0.001$ vs CTRL) and 2.5-fold ($P < 0.001$ vs CTRL) increase in plasma TPC concentrations, respectively. Plasma caffeine concentrations were also enhanced reaching a 6-fold ($P < 0.01$ vs. CTRL) for GTD and 10-fold ($P < 0.01$ vs. CTRL) for BTD in HFD rats (Fig. 1B). Moreover, the TPC excretion in feces was high in both group of HFD rats consuming tea decoction as compared to CTRL ($P < 0.001$) (Fig. 2A). The appearance of TPC in feces was rapid-as early as 5 weeks-and dramatic. The amount of TPC excreted in feces varied from 500 μg at week 5th to 700 μg EC/g feces at week 10th for GTD and BTD. This high excretion of TPC in feces would suggest an important presence of tea-polyphenols within the intestinal lumen able to influence the processes involved in lipid digestion and absorption. By contrast, tea-caffeine is rapidly absorbed from the intestine, since plasma caffeine levels were high reaching values of 4–6 μg caffeine per ml (Fig. 1B) and excretion of caffeine in feces was detected in small amounts varying from 0.6 to 0.8 μg per g feces in HFD rats consuming GTD and BTD, respectively (Fig. 2B).

Table 3
Food intake and water, GTD and BTD consumption during the experimental period in different groups.

	CTRL	GTGr	BTGr
Food intake, g/day	18.02 ± 0.42	17 ± 0.81	14.40 ± 0.39**
Tea consumption, ml/day	–	28.3 ± 0.2	27.9 ± 0.3
Water consumption, ml/day	30.2 ± 0.3	5	5

Results are expressed as mean ± SEM. ** Significant reduction of food intake by 20% ($P < 0.001$ vs CTRL) in BTGr.

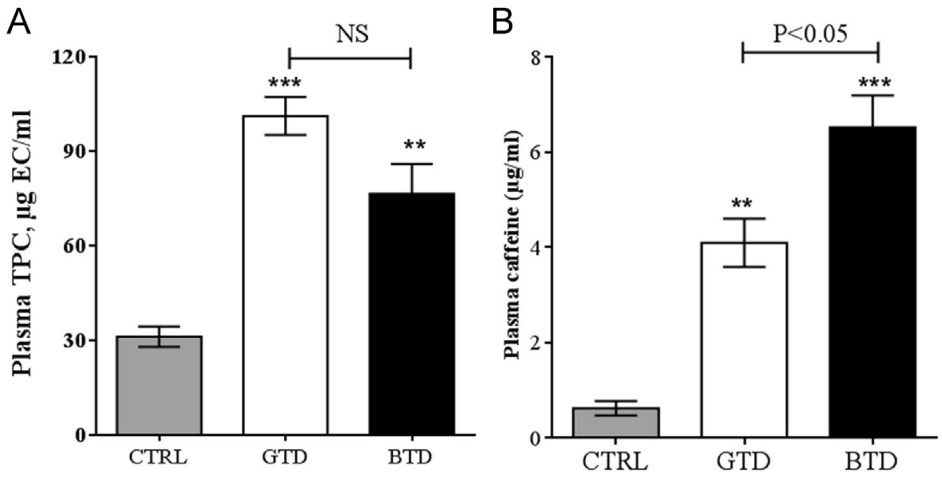

Fig. 1. Increased plasma TPC (A) and Caffeine (B) levels by chronic consumption of GTD or BTD in rats feeding HFD. (**A**) Plasma levels of TPC and (**B**) caffeine in rats fed HFD. Results are expressed as mean ± SEM (n = 12 rats for each group).***P < 0.001 *vs.* CTRL.

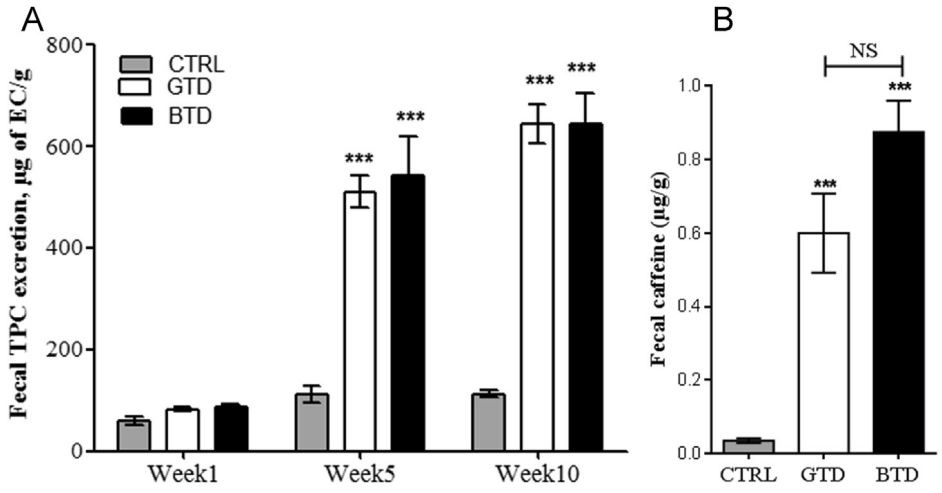

Fig. 2. Enhanced fecal TPC (**A**) **and Caffeine** (**B**) **by chronic consumption ofGTD or BTD in rats feeding HFD**. (**A**) Fecal excretion of TPC and (**B**) caffeine in feces of rats fed HFD. Results are expressed as mean ± SEM (n = 12 rats for each group). ***P < 0.001 *vs.* CTRL. Note the rapid and dramatic increases in fecal TPC from 5 to 10 weeks of GTD and BTD consumption.

3.4. Decreased liver content of total lipids and triglycerides in HFD rats consuming GTD and BTD

The comparative effects of GTD and BTD consumption on the liver content of lipids and liver triglycerides were shown in Fig. 3. Chronic10-weeks consumption GTD and BTD led to a significant reduction of liver content of total lipids by 20% and 30% (P < 0.001 vs CTRL), respectively. This was associated with significant reduction of liver TG content (25% and 35%; P < 0.001 vs CTRL) for GTD and BTD respectively. It could be noted that the relative liver weights did not significantly differ among groups (2.96± 0.31% for GTD and 2.8 ± 0.29% for BTD vs. 2.77± 0.41% for CTRL) indicating that GTD or BTD did not have a toxic effect on the liver.

Except for plasma HDL-cholesterol, chronic GTD and BTD-HFD fed rats had reduced total cholesterol (32.4% and 49%, P < 0.001 vs CTRL, respectively), plasma triglycerides (36.6% and 48%; P < 0.01 vs CTRL respectively) and blood glucose by 25 and 27.5%, respectively (P < 0.01 vs CTRL) Table 4.

3.5. Dramatic enhancement of fecal triglycerides excretion in HFD rats consuming GTD and BTD

As shown in Fig. 4, fecal triglycerides excretion in HFD rats consuming GTD and BTD significantly increased as a function of time. This increase of fecal triglycerides excretion was rapid-as early as two weeks and was dramatic from 3rd to 10th week of tea consumption. At 10th week fecal triglycerides excretion has reached +170% (P < 0.001 vs CTRL) in HFD rats consuming GTD and, those consuming BTD have excreted +230% (P < 0.001 vs CTRL) of TG in feces. Note that all time-period, TG excretion in feces was significantly higher in HFD rats consuming BTD than in those consuming GTD suggesting a great efficacy of BTD to reduce intestinal absorption of TG.

3.6. Potent reduction of abdominal fat mass in HFD rats consuming GTD and BTD

We next investigated the impact of this higher fecal excretion TG

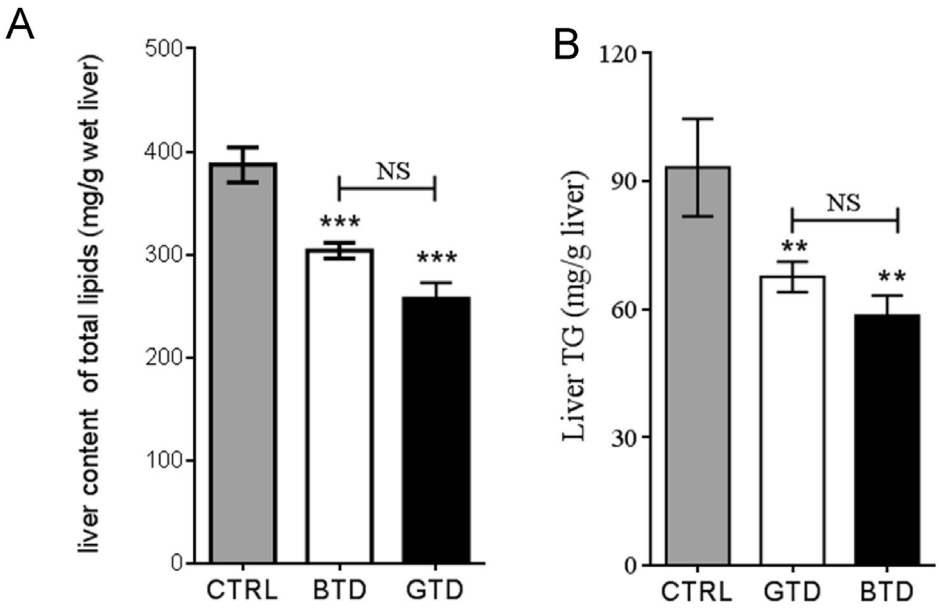

Fig. 3. Reduced liver content of total lipids (A) and triglycerides (B) in HFD fed rats treated with GTD and BTD for 10 weeks. Effect of chronic GTD and BTD consumption on total lipids and triglycerides amounts in the liver of HFD rats treated with GTD and BTD. Results are expressed as mean ± SEM of n = 10–12 rats for each group.**P < 0.01: ***P < 0.001 vs CTRL.

Table 4
Effect of GTD and BTD consumption on plasma lipids, glucose and circulating leptin levels in HFD rats.

Parameters	CTRL	GTGr	BTGr
Plasma glucose, mmol/L	5.60 ± 0.20	4.20 ± 0.10*	4.06 ± 0.10**
Plasma Triglycerides, mmol/L	1.23 ± 0.10	0.78 ± 0.04**	0.64 ± 0.06 ***
Blood cholesterol, mmol/L	2.10 ± 0.09	1.42 ± 0.10**	1.07 ± 0.10**
HDL cholesterol, mmol/L	1.07 ± 0.04	1.03 ± 0.04	0.97 ± 0.03
Plasma leptin, ng/ml	7.50 ± 1.10	5.50 ± 1.10**	3.10 ± 0.80**

Results are expressed as mean ± SEM. The consumed volume of GTD/BTD (28 ml/day) decreased plasma glucose, triglycerides, cholesterol and leptin. *P < 0.05, **P < 0.001, ***P < 0.0001vs CTRL: Values are significantly different from the control group.

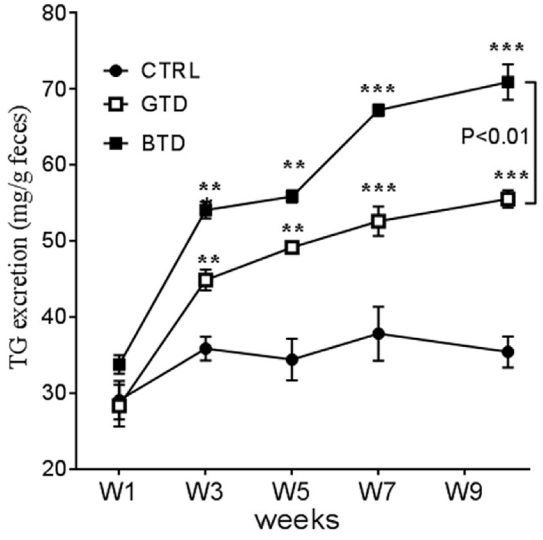

Fig. 4. Time dependent increase of triglyceride excretion in feces. Effects of GTD and BTD consumption on excretion of triglycerides in feces as a function of time during HFD feeding. Results are expressed as mean ± SEM of n = 10–12 rats for each group.*P < 0.05,**P < 0.01,***P < 0.001 vs CTRL.

abdominal adipose tissue mass, as markers of adiposity. In Fig. 5A, the weights of epididymal and perirenal adipose tissues were reduced by 26.5% and 56.4% (P < 0.001 vs CTRL) in HFD rats consuming GTD. These reductions were 60% (P < 0.001 vs CTRL) for both epididymal and perirenal adipose tissue in HFD rats consuming BTD. This reduction of epididymal and perirenal adipose tissue weights was consistent with a reduction of 27% and 59% (P < 0.001 vs CTRL) of circulating leptin levels in HFD rats consuming GTD and BTD, respectively (Table 4). These results suggest that the abdominal adipose tissue mass are direct targets of both tea beverages with a particular strong effect of BTD.

3.7. Reduced body weight gains in HFD rats consuming GTD and BTD

Finally, we examined the consequences of the changes above on body weight and food intake. In Fig. 5B, body weight gain during HFD feeding was significantly reduced in rats consuming GTD and BTD. The initial body weights did not differ among groups: 129.8 ± 5.9; 130.3 ± and 130.5 ± 4.9 g for CTRL, GTGr and BTGr respectively. However, there was a significant time dependent reduction of body weight gain in HFD rats consuming GTD and BTD. At 10 week, this decrease of weight gain was 21% (P < 0.001 vs CTRL) and 55%, (P < 0.01 vs CTRL) for HFD rats treated with GTD and BTD, respectively (Fig. 5B) arguing for higher efficiency of BTD over GTD. The amount food intake did not change between GTD and CTRL, but was reduced by 20% (P < 0.001 vs CTRL) in BTD -HFD rats (Table 3) suggesting that it could contribute to the greater reduction of weight gain.

4. Discussion

The prepared dose of 50 g tea leaves/L water is somewhat similar to those used throughout consumer habits in Tunisia and other North African areas. Then, the consumed GTD and BTD (Table 3) provides comparable doses of TPC, EGCG and caffeine with those used in other studies in both animals [18,19] and humans [20,21]. Here, we demonstrate that this chronic consumption of GTD and

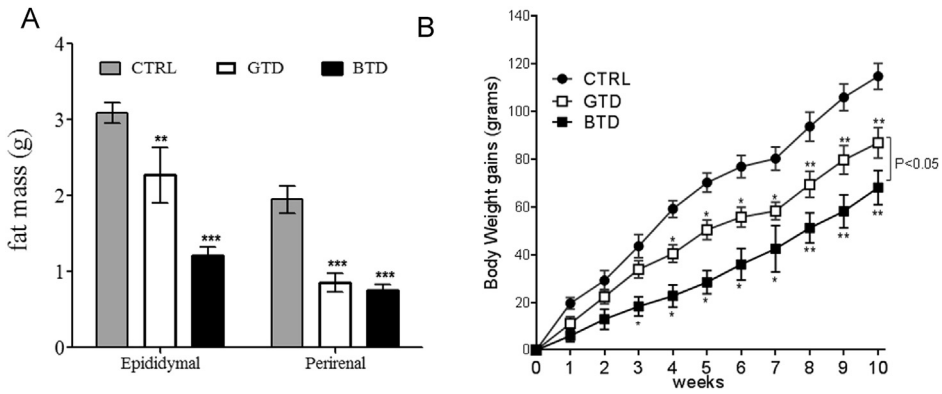

Fig. 5. Chronic consumption of GTD or BTD reduces fat deposits and prevents weight gain. Effect of chronic 15-min GTD and 15-min BTD consumption on weight of perirenal, and epididymal adipose tissue and body-weight gain curves of HFD rats. Results are expressed as mean ± SEM of n = 10–12 rats for each group.*P < 0.05, **P < 0.01, ***P < 0.001vs CTRL.

BTD during a HFD can have beneficial health effects, preventing storage of fats in the liver, increasing fecal excretion of triglycerides, decreasing abdominal fat tissue weight and reduces body weight gain in rats feeding HFD. These findings are in agreement with those using purified EGCG (major green tea polyphenols) or purified theaflavins (major black tea polyphenols) in rats or mice fed HFD [22]. They are also in accordance with data showing that green tea EGCG effectively reduced adipose tissue mass and ameliorated plasma lipid profiles in high-fat diet-induced obese mice [23]. In normal conditions, the lipid excretion in feces did not exceed 3–5% of ingested lipids. In this study, we found that consumption of GTD and BTD results a dramatic excretion of lipids, mostly triglycerides, which was associated with a high TPC excretion in feces. These findings may suggest that black and green tea-containing TPC could interfere with dietary lipids, mostly triglycerides, making them indigestible and therefore increased their excretion [5,24]. Previous studies have reported that green tea catechins (EGCG, EGC and EC) or black tea theaflavins, thearubigins and gallic acids strongly affect the hydrolysis process of triglycerides degradation by inhibiting pancreatic lipase activities and interfering with lipids emulsification and micellar solubility in the intestinal lumen [6,25–27]. However, although the major polyphenolic compounds exert their effects across luminal intestine, the absorbed amount of catechins, theaflavins and their derivative compounds are thought to modulate actively the fat deposition in white adipose tissues and liver [9]. Therefore, the final circulating levels of TPC, in particular EGCG and theaflavins may be involved in the reduction of abdominal adipose tissue gain and lowering blood and liver lipids. The EGCG acts through the regulation of the expression of multiple genes involved in adipogenesis, lipolysis, beta-oxidation, thermogenesis, fatty acid and triglycerides synthesis in white adipose tissue and liver [5,9,28]. As results, the EGCG induced the inhibition of lipogenic enzymes activity and/or expression, such as acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, glucose-6-phosphate dehydrogenase that may explain the hypolipidic liver, fat cells, and blood [28,29]. Accordingly, a short-term supplementation of dietary EGCG showed to increase energy excretion, decreased post-prandial triglyceride and glycogen content in liver, increased oxidation of dietary lipids and decreased incorporation of dietary enriched lipids into fat tissues, liver and skeletal muscle [29]. Moreover, it has been shown that EGCG reversed HFD-induced effects on intestinal substrate transporters (CD36, FATP4 and SGLT1) and down regulated lipogenesis-related genes (ACC, FAS and SCD1) in liver in the post-prandial state [29].

Although the amount of TPC provided by GTD was 3-fold higher than that provided by BTD, the effect of TPC from BTD on all studied parameters was much higher. This pronounced BTD effect could be attributed to the nature of polyphenolic compounds. Indeed, GTD contained predominantly: EGCG, EGC, catechin, EGC-3-methyl gallate, ECG and other identified compounds, including phenolic acid ester, kaempferol 3-glycoside and caffeine. However, BTD contained mainly theaflavins (TF), a mixture of theaflavin (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B), theaflavin-3,3′-digallate (TF_3), thearubigin and gallic acid esters as well as caffeine and gentissic acid esters. Therefore, BTD provided much more gallic compounds as a polymerized form than GTD. Consequently, TFs and TF1, which are rich in gallic acid, appeared to exhibit the strongest beneficial effect on the reduction of abdominal adipose tissue gains, lowering of blood and liver lipids than other phenolic compounds of tea [10]. Our results are consistent with those showing that black tea extract or highly purified solution of TFs and TF1 suppressed body weight gain and adipose tissue formation, reduce the serum levels of cholesterol, triglycerides and attenuate hepato-cellular damage in rats fed HFD [10,22]. They are also in accordance with those showing that black tea extract and gallic acid suppressed body weight gain in a mouse model of diet-induced obesity [25]. Specifically; these findings demonstrated that gallic acid, abundant in black tea extract, strongly inhibited pancreatic lipase activities and reduced post-prandial blood triglycerides [6,25]. Additionally, we found that food intake was affected by BTD (−20%), but not GTD. This supplementary effect of TPC from BTD may contribute to the reduction of adipose tissue mass and related lipid parameters. Therefore, our data indicate that GTD and BTD containing TPC may act via the modulation of lipid digestion and fat deposition in white adipose tissues, but in addition, BTD-TPC also affects food intake that could explain the marked effect of black tea. Moreover, both GTD and BTD treated rats exhibited high-reduced circulating glucose levels with a more marked effect of BTD. Several mechanisms have been involved in the reduction of blood glucose by tea-polyphenols including the modulation of the intestinal transporter of glucose. Accordingly, we previously demonstrated that a chronic administration of GTD reduced SGLT-1/GLUT-2 ratio in the luminal intestine in rats fed HFD [12]. Such effect may reduce glucose absorption and therefore, the metabolic conversion of carbohydrates to fat in adipose tissue.

On the other hand, caffeine is an important bioactive compounds in both green and black tea. Our results showed that 15-min cooking tea released a high amount of caffeine in GTD and more in BTD, but the caffeine of BTD is better absorbed than that of GTD. This difference could be related to the manufacturing process of the two teas which is different. While green tea arises from natural fresh tea leaves without fermentation, the black tea was obtained

after withering, crushing, rolling, fermenting and drying of fresh tea leaves [30]. Because during this process, the tea leaves have been shredded, so there are more edges and more surface area as well as a breakdown of leaf proteins, which increases the availability of freed caffeine and therefore more caffeine can be extracted in water [31]. As results, the increase of plasma caffeine was concomitant with the reduction of adipose tissue gain and related blood and liver triglycerides whereas fecal caffeine was not. Consistent with other studies [22,32,33], we believe that the high absorption of caffeine from GTD and more from BTD may be efficient in the decrease of adipose tissue mass in rats fed HFD. Therefore, caffeine from tea decoction, especially from BTD could be considered as an additional potential factor for stimulating thermogenesis and promotes fat oxidation. However, the caffeine content of GTD and BTD cannot completely explain the reduction of adipose tissue mass because it was demonstrated that thermogenic effect of tea extract containing both caffeine and catechin polyphenols is greater than that of an equivalent amount of caffeine [34]. Thus, the caffeine may act independently or synergistically with catechins and theaflavins of GTD and BTD [23]. The anti-obesity effect of caffeine from tea, especially in animals, has been observed in different experimental conditions [35]. Hence, the intake of caffeine doses (0.025–0.1%) for 21 days reduced body fat mass gain in rats fed a high-fat diet in a dose dependant manner [35]. This reduction was concomitant with an increase of serum epinephrine, norepinephrine, dopamine and free fatty acid levels, suggesting an active catecholamine synthesis and lipolysis [35]. In addition, the caffeine consumption showed to inhibit the enzyme phosphodiesterase and leading to increase intracellular cAMP as well as sympathetic nervous system and lipase activities, which promotes lipolysis [36]. Accordingly, and being richer in TFs and caffeine than the GTD, BTD was found more efficient in term of reduction of abdominal adipose tissue gain and lowering blood and liver lipids.

Some other phenolic compounds, including vanillic acid ester, kaempferol 3-glycoside and gentissic acids were found in GTD and BTD although as little amounts. It has been reported that D-glucosyl-kaempferol isolated from Sauropus at a dose of 60 mg/kg significantly reduced food intake in rats by 15%, resulting in decreases in body weight and free triglyceride without obvious histopathological changes [37]. Unlike to synthetic or isolated glucosyl-kaempferol compounds, it is difficult to highlight the distinctive effect of kaempferol from GTD and BTD on the decrease of adiposity or body weight gains because of the interference of several polyphenolic compounds present in tea decoctions. However, we can consider that the anti-adiposity effect of these simple phenolics present in tea is far lower than that of gallic acid, known for its powerful action on the modulation of dietary lipids digestion and adiposity.

In summary, chronic administration of short-time tea decoctions (15-min GTD or 15-min BTD) during 10-weeks, dramatically reduced lipid digestion, lowering liver and blood lipids, adipose tissue mass and body weight gains in rats fed HFD. The data showed a higher efficiency of BTD compared to GTD, which confer to these traditional cooking beverages, rich in TPC mostly EGCG and TFs as well as caffeine, a natural alternative in the prevention of obesity.

Conflicts of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

Authors' contribution

MHH and AB designed the project, data analysis and wrote the paper. CS carried out the animal work and contributed to data analysis. KD and SF performed polyphenolic and caffeine compounds analysis. RD and MLG contributed to the preparation of manuscript drafting. All authors approved the final manuscript.

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