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Basic nutritional investigation

Comparative effect of black, green, oolong, and white tea intake on weight gain and bile acid metabolism

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ABSTRACT

Objective: The beneficial effects of tea on health, including obesity, are well known. However, the comparative effects of black, green, white, and oolong teas, which are prepared from the same fresh leaves, on weight gain and the potential mechanisms involved are not yet fully understood. Bile acids (BAs) are shown to be powerful regulators of metabolism; however, to our knowledge, no studies have investigated the effect of tea on BA metabolism. The aim of this study was to investigate the modulatory effects that green, black, white, and oolong teas that were prepared from the same raw tea leaves have on the plasma BA profile.
Methods: Female rats were dosed with the aforementioned tea types as their sole source of drinking fluid for 28 d. We then investigated their weight and effect on BA metabolic profile using advanced ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS)-based metabolomics.
Results: The UPLC-MS/MS analysis of the plasma show that the levels of murocholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurochenodeoxycholic acid, tauroursodeoxycholic acid, taurodeoxycholic acid, tauromuricholic acid, and taurocholic acid were increased; whereas levels of taurolithocholic acid and isolithocholic acid were decreased after drinking green, oolong, and white tea types compared with control. Surprisingly, oolong tea significantly influenced reduction in relative weight compared with control, black, and green tea; whereas black, green, and white teas had no effects on weight compared with control.
Conclusions: Green, black, oolong, and white teas altered the BA metabolism. This change in BA metabolism could be associated with the health benefit effects of tea. Oolong tea was most effective in reducing weight.

Introduction

Tea, an infusion of the leaves of *Camellia sinensis*, includes green tea, black tea, white tea, and oolong tea, which are very popular drinks because of their unique aroma and characteristics that are the result of their different levels of fermentation and manufacturing process. An increasing number of studies have shown that tea lowers fasting serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [1–5], may have preventive effects against cancer [6–9], exerts antiobesity effects, and is capable of lowering the risk for obesity [10–15]. Although there are many mechanistic investigations in to the beneficial effects of tea, the exact mechanisms are yet to be worked out [16,17].

In humans, the major portion of the physiologic cholesterol is converted to bile acids (BAs) by cascade of metabolic processes in hepatocytes leading to removal of physiologically active cholesterol in the system [18]. In fact, it is estimated that about half of the 800 mg of cholesterol synthesized daily is used for BA synthesis, amounting to about 200 to 600 mg daily in humans [19], thus BA synthesis is a regulator of body cholesterol. The classical cholesterol metabolic pathway produces the primary BAs, cholic acid (CA), and chenodeoxycholic acid (CDCA) in roughly equal amounts, whereas the alternative pathway produces mainly CDCA [20] (Figure 1). Most BAs are further conjugated with either glycine or taurine, with a 3:1 predominance of glycine over taurine [18,21]. In the gut, BAs undergo further metabolism, mainly deconjugation and dihydroxylation, which generate unconjugated free BAs and secondary BAs [22] (Figure 1). Recent studies have shown that BAs are potent signaling molecules that interact with farnesoid X receptor, vitamin D receptor, and TGR5 receptor to trigger cellular

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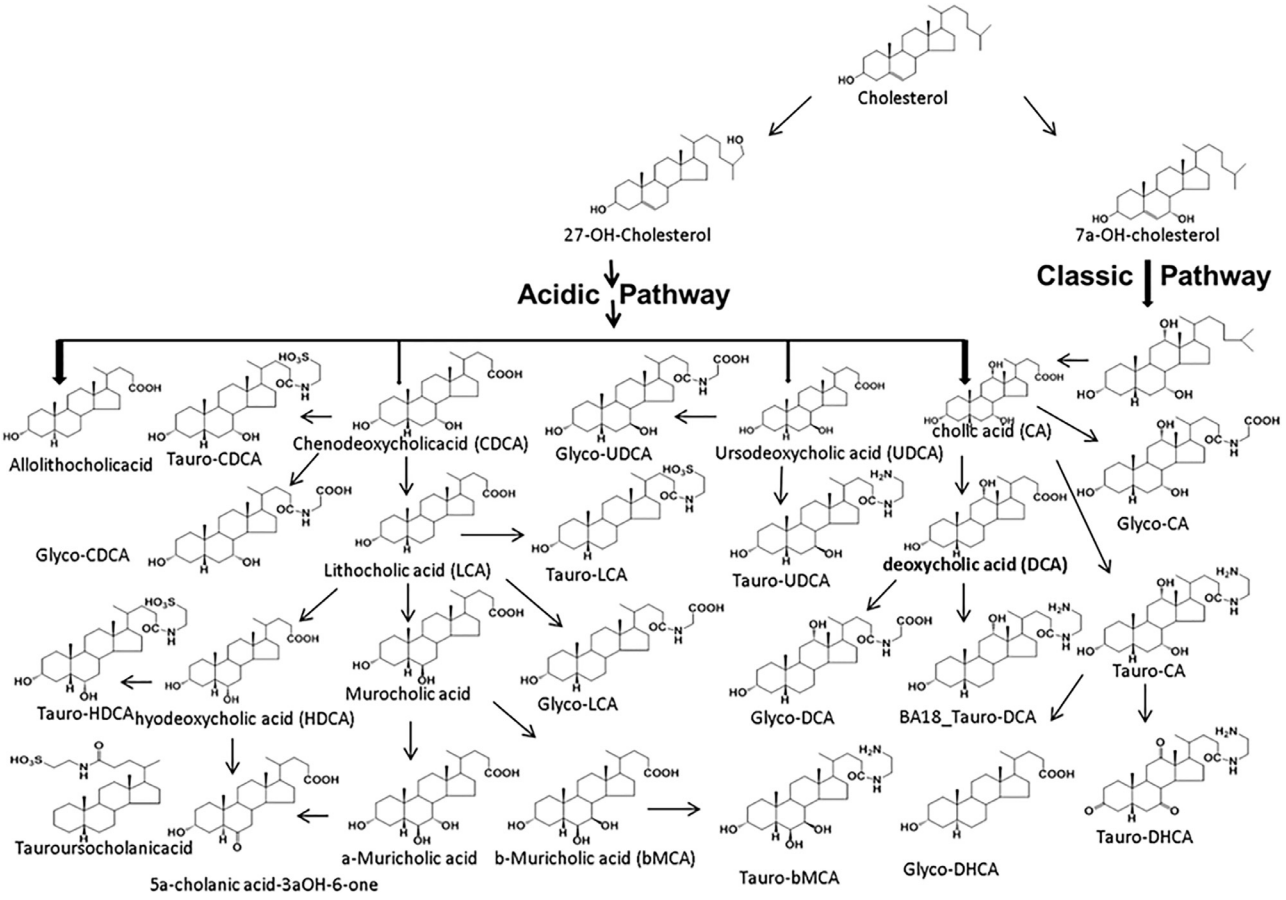

Fig. 1. Bile acid metabolic pathways.

responses that play essential roles in host lipid metabolism, electrolyte transport, and immune regulation [23–25]. Hence, disturbance of gut microbial flora can change the balance of BAs, which in turn effect the digestive and signaling processes. A number of studies have demonstrated that perturbations in microbial flora are associated with changes in BA profiles that are seen in many diseases. For example, irritable bowel syndrome [26], inflammatory bowel disease [27], short bowel syndrome [28], and *Clostridium difficile* infection [29] all exhibit simultaneous alterations in the composition of the gut microbiota and changes to host BA profiles. Furthermore, it is also suggested that asthma and obesity could be linked to changes in BA profiles in the host [30–32]. BAs are powerful regulators of metabolism. Moreover, a modulation of plasma BA levels has been found that could affect weight [18]. It has been found that increased levels of plasma BAs are associated with a significant reduction in diet-induced obesity and resulted in increased whole body energy expenditure and dissipation of energy in the form of heat, and that lean mice exhibited higher blood BA concentrations relative to the obese mice [31,33,34].

The comparative study on the effects of green, black, white, and oolong teas that are prepared from the same fresh tea leaves, on weight has not, to our knowledge, been reported. Moreover, effect of these tea types on BA metabolism has not been investigated. To address this, in the present study, we dosed rats with green, black, white, and oolong teas as their sole source of drinking fluid for 28 d and investigated their weight and the effect on BA metabolic profile using an advanced ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS)-based metabolomics platform. To our knowledge, this is the first study to show the modulatory effects of green, black, white, and oolong teas on plasma BA profile and their possible link to weight loss.

Materials and methods

Chemicals and reagents

(-)-epigallocatechin 3-gallate (EGCG), (-)-epicatechin-3-O-gallate, (-)-gallocatechin, and (-)-epigallocatechin (EGC) were purchased from Cayman Chemicals (Ann Arbor, MI, USA), whereas (-)-epicatechin (EC) and (−)-catechin gallate (CG) were from Sigma-Aldrich Co. (St. Louis, MO, USA). (-)-Gallocatechin gallate was from AdooQ BioScience (Irvine, CA, USA). The purity of these chemical standards was >98%. Reference standards of BAs (Table 1) were purchased from Steraloids (Newport, RI, USA). All solvents were high-performance liquid chromatography grade and all other chemicals used were of the highest grade available.

Tea samples and preparation

The fresh tea (*Camellia sinensis cv. Fuding-dabaicha*) leaves were plucked from Chengdu, Sichuan, China, at the same time of same species and made into green, black, white, and oolong teas. Tea extracts were prepared similarly to human consumption by the addition of freshly boiled deionized (DI) water to tea and brewing for 5 min (2%). The extracts were prepared daily, filtered, and cooled to room temperature before being given to the rats.

Measurements of catechins using UPLC-MS/MS

From tea

Tea sample preparation for UPLC-MS/MS analysis was done following the method by Zhang [35]. In brief, a 30-mg tea sample was weighed into a 5-mL glass tube and extracted by 1.5-mL extraction solvent, 75% (v/v) methanol aqueous solution, in a water bath for 30 min at 70°C. The tube was cooled to room temperature and content was transferred to Eppendorf tube and centrifuged for 10 min at 100 000*g*. Supernatant was transferred to 1.5-mL vial and stored at −20°C until UPLC-MS/MS analysis.

Table 1
Levels of bile acids measured in plasma of rats treated with various teas

		Control	Black tea group	Green tea group	Oolong tea group	White tea group
No.	Bile acid	Median (min–max) pg/ mL	Median (min–max) pg/ mL	Median (min–max) pg/ mL	Median (min–max) pg/ mL	Median (min–max) pg/ mL
1	Chenodeoxycholic acid	67603 (1004 – 501397)	54637 (0 – 179926)	143868 (2933 – 830420)	130172 (2828 – 2951822)	41479 (4940 – 1357430)
2	Ursodeoxycholic acid	162502 (0 – 661659)	90097 (0 – 207125)	200166 (0 – 855519)	313628 (0 – 1567037)	63348 (44588 – 1494638)
3	Murocholic acid*	186945 (0 – 1036040)	626425 (0 – 976325)	895780 (35204 – 1319203)	584976 (136285 – 984100)	723742 (511503 – 1663018)
4	Hyodeoxycholic acid	115715 (8450 – 457269)	51104 (0 – 128765)	131613 (0 – 728236)	161987 (0 – 969078)	46939 (6718 – 1013601)
5	Allolithocholic acid	3705 (0 – 52536)	1606 (737 – 86938)	1938 (637 – 60727)	2302 (665 – 232796)	815 (317 – 10688)
6	Lithocholic acid	73716 (4483 – 499987)	11375 (3675 – 895829)	19083 (5900 – 287257)	17473 (9615 – 2724244)	8371 (2884 – 65537)
7	Taurohyodeoxycholic acid	69048 (26118 – 169734)	66394 (0 – 304991)	179126 (17705 – 363075)	103791 (0 – 1133054)	148704 (34888 – 318959)
8	Cholic acid	253945 (0 – 1568309)	238282 (4004 – 432791)	596483 (0 – 1699946)	364368 (9023 – 4916393)	110065 (43803 – 3726034)
9	Taurochenodeoxycholic acid*	78938 (11674 – 428132)	259746 (29662 – 1145078)	347364 (23469 – 1077125)	327928 (25455 – 2320939)	237858 (77544 – 1342859)
10	Desoxycholic acid	3854 (0 – 19375)	5446 (0 – 7852)	11885 (0 – 40546)	16239 (0 – 56286)	9069 (5388 – 78015)
11	Taurolithocholic acid*	20222 (1863 – 138564)	3979 (850 – 61185)	5593 (1831 – 39898)	7159 (2909 – 128096)	3494 (1759 – 15798)
12	Glycolithocholic acid	0 (0 – 0)	0 (0 – 345)	0 (0 – 936)	0 (0 – 197)	0 (0 – 1234)
13	6-Keto-allolithocholic acid	22918 (3519 – 2337506)	8305 (0 – 2095858)	8117 (1715 – 1772190)	11447 (0 – 300149)	3004 (405 – 174852)
14	Glycodeoxycholic acid*	5407 (0 – 15396)	4452 (0 – 17173)	21468 (2262 – 49310)	13891 (0 – 40359)	17145 (1570 – 84232)
15	Glycochenodeoxycholic acid*	1415 (0 – 24955)	135 (0 – 9729)	16932 (0 – 384211)	10340 (0 – 219449)	15485 (2507 – 92451)
16	Tauroursodeoxycholic acid*	100462 (34074 – 250285)	98756 (161 – 459061)	267614 (16216 – 550914)	138324 (0 – 1732865)	209074 (59369 – 486019)
17	Taurodeoxycholic acid*	77462 (2791 – 547348)	299661 (8060 – 1401678)	427385 (21416 – 1279500)	286428 (17374 – 2777272)	299629 (60667 – 1537053)
18	Glycodeoxycholic acid	244 (0 – 507)	176 (75 – 2419)	416 (0 – 6821)	488 (53 – 3017)	208 (30 – 7281)
19	a–Muricholic acid	19380 (0 – 245945)	40451 (0 – 127317)	113736 (0 – 369702)	55472 (0 – 1144753)	15574 (6382 – 888061)
20	b-Muricholic acid	8641 (0 – 173887)	28973 (0 – 149160)	86429 (9668 – 295034)	80798 (1866 – 874408)	19897 (3115 – 585965)
21	Tauro-b-Muricholic acid*	100727 (0 – 516265)	266025 (7254 – 1291970)	501917 (61389 – 1017350)	442527 (43868 – 3377082)	445336 (210631 – 1370383)
22	Taurocholic acid*	170153 (67742 – 686264)	259429 (75767 – 1547374)	450128 (98289 – 1408857)	473471 (59076 – 5016448)	426433 (209674 – 1450222)
23	Isolithocholic acid*	6537702 (230355 – 32574885)	291677 (238480 – 8020976)	315365 (242688 – 10650364)	362515 (250328 – 12823690)	300807 (207186 – 663512)
24	Ursocholic acid	306815 (2639 – 1707673)	248857 (6777 – 661804)	735193 (3227 – 1811138)	426277 (33670 – 5289613)	121786 (45336 – 3948965)
25	Glycohyodeoxycholic acid	1615 (0 – 6810)	3156 (0 – 7880)	13288 (1535 – 25482)	6398 (0 – 19441)	9321 (659 – 44671)
26	Glycocholic acid*	15485 (1759 – 59101)	53456 (483 – 191333)	115533 (20144 – 190940)	82261 (11852 – 234016)	114990 (25951 – 355837)

*Bile acids that are significantly altered after exposure to tea.

From plasma

Urine (500 μL) or plasma (500 μL) was extracted twice with 1 mL chloroform. Each time the mixture was vortexed for 30s and centrifuged for 5 min; chloroform layer was transferred to a 2-mL tube and dried. The aqueous layer from the chloroform extract was freeze dried and the resulting residue was extracted with 1 mL MeOH. The MeOH layer mixture after 5 min centrifugation was added to above chloroform extract. This mixture was dried and resuspended in 125 μL MeOH and filtered using 5 kD membrane filters. Filtrates were transferred to vials for UPLC-MS/MS analysis.

Animal care and experimental design

All experiments involving rats were approved by the Institutional Animal Care and Use Committee. Forty 6- to 8-wk-old female Wistar rats weighing 168 to 228 g were ordered from the Charles River and kept in vivarium under controlled conditions of temperatures at 22.5°C ± 2.5°C with 50% ± 15% relative humidity during a photoperiod of 12 h. Rodent Diet (2018 Teklad Global 18% protein rodent diet, Madison WI, USA) were available ad libitum. After 4 d of acclimatization, rats were treated for 4 wk. Rats were randomly and equally divided (eight rats per group) into control (C), black tea (B), green tea (G), oolong tea (O), and white tea (W) groups. The tea group rats were exposed to black, green, oolong, and white tea extracts. The animals had free access to the tea as their sole source of drinking fluid, whereas the control group received DI water. Body weight and fluid intake of all groups were monitored daily throughout the experimental period. In addition, feces excretion and food intake were measured on the day before tea treatment as baseline and on days 1, 14, and 28. During these measurements, rats were kept in metabolic cages separately for these 4 d. At the end of the experiment on day 28, blood plasma was collected by heart puncture and kept in heparinized tubes. After centrifugation (3000*g* at 4°C for 15 min), plasma was collected and stored at −80°C.

Metabolomic profiling by UPLC-MS/MS

Sample preparation

Sample preparation was performed using slight modification of reported method [36]. Briefly, the plasma (500 μL) was extracted twice with 1 mL chloroform. Each time the mixture was vortexed for 30s and centrifuged for 5 min; chloroform layer was transferred to 2-mL tubes and dried. The aqueous layer from the chloroform extract was freeze dried, and the resulting residue was extracted with 1 mL MeOH. The MeOH layer mixture after 5 min centrifugation was added to above chloroform extract. This mixture was dried and resuspended in 125 μL MeOH and filtered using 5 kD membrane filters. Filtrates were transferred to vials for UPLC-MS/MS analysis.

UPLC-MS/MS analysis

UPLC/MS-MS analyses of all the samples were conducted using a Waters Acquity UPLC system connected with Xevo-TQ triple quadruple mass spectrometer (Waters, Milford, MA, USA). The MS/MS analysis for both BAs and catechins was done using Electro Spray Ionization (ESI) in either positive (PI) or negative ion (NI) mode, capillary voltage of 3.0 kV, extractor cone voltage of 3 V, sample cone voltage of 32 V, and detector voltage of 500 V. Cone gas flow was set at 50 L/h and desolvation gas flow was maintained at 600 L/h. Source temperature and desolvation temperatures were set at 150°C and 350°C, respectively. Analytical separations for BAs on the UPLC system were conducted using an Acquity UPLC C18 1.6 μm column (2 × 150 mm) at a flow rate of 0.15 mL/min. The gradient started with 100% A (0.1% formic acid in water) and 0% B (0.1% formic acid in CH_3 CN) for 2 min, then changed to 80% A over 2 min, followed by 45% A over 5 min and 20% A for 2 min. Finally, over 1 min it was changed to original 100% A, resulting in a total separation time of 12 min. The elutions from the UPLC column were introduced to the mass spectrometer. Analytical separations for catechins on the UPLC system were conducted using an Acquity UPLC C18 1.6 μm column (2 × 150 mm) at a flow rate of 0.15 mL/min. The gradient started with 100% A (0.1% formic acid in water) and 0% B (0.1% formic acid in CH_3 CN) for 0.1 min, changed to 80% A over 6 min and then 45% A over 4 min, followed by 20% A in 1 min and then to 0% A over 1 min. Finally, over 2 min it was changed to original 100% A, resulting in a total separation time of 14 min. The elutions from the UPLC column were introduced to the mass spectrometer. Resulting data were analyzed and processed using MassLynx 4.1 software.

Statistical analysis

Statistical analyses were done with SPSS version 23 (IBM, Armonk, NY, USA). A repeated measure analysis of variance (ANOVA) with a Greenhouse-Geisser correction and post hoc tests using the Bonferroni correction was used to compare the drinking fluid consumption, relative body increase, feces excretion, and food intake between groups over time. $P < 0.05$ was defined as statistically significant (two-sided). Data sets with a non-normal distribution were analyzed using the non-parametric Kruskal–Wallis test. Differences with $P < 0.05$ were considered significant. To evaluate the effect of tea on BA metabolism *t* test was used and $P < 0.05$ was considered significant. Differences in plasma EGCG among groups were analyzed the Kruskal–Wallis non-parametric ANOVA followed by Dunn's pairwise comparison. Significant differences of the composition between different types of tea were tested using a one-way ANOVA. Data are presented as mean ± SEM.

Results

Food intake and feces excretion

Fluid consumption in any tea group was not significantly affected compared with the control group (data not presented). A repeated measures ANOVA with a Greenhouse-Geisser correction was used to determine the effect of tea types on the weight of food intake and feces. The Greenhouse-Geisser test suggests that time effect on both food intake [$F(2.457, 85.99) = 125.624$, $P < 0.05$] and weight of feces [$F(1.877, 65.699) = 49.326$, $P < 0.05$] were significant. However the time × tea roup interaction of food intake [$F(9.827, 85.99) = 1.087$, $P = 0.381$] and weight of feces [$F(7.509, 65.699) = 1.933$, $P = 0.074$] was not significant (not showed), therefore, the trend for each group is not significantly different, which means the food intake and feces excretion were not influenced by drinking tea. Figure 2 shows that, in general, the feces weight of green tea group was lowest at every time point, followed by white tea group, whereas the food intake of oolong tea group was found to be considerably higher than the other tea groups and control.

Effect of various tea types on weight

Results from repeated measures ANOVA with a Greenhouse-Geisser correction clearly show that tea types have a significantly

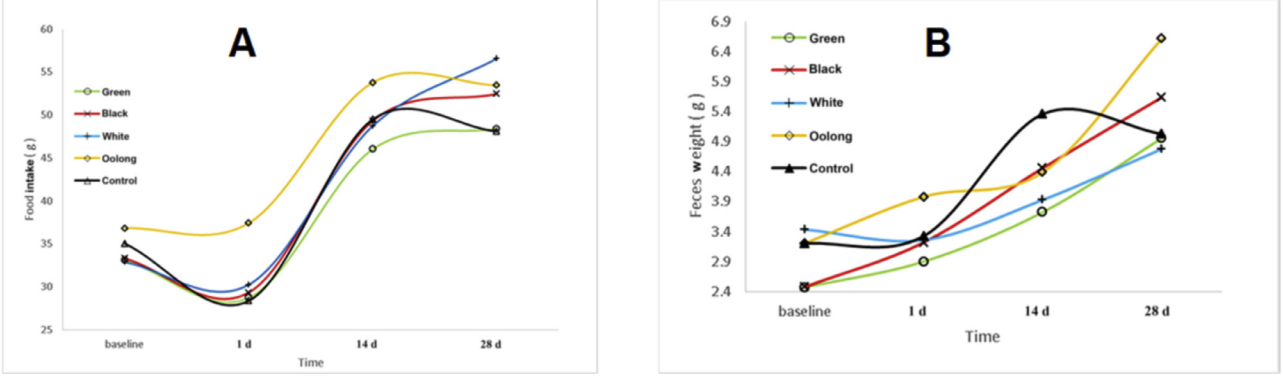

Fig. 2. Effect of various types of tea on food intake (**A**) and feces weight (**B**) in Wistar rats during the period of 4 wk.

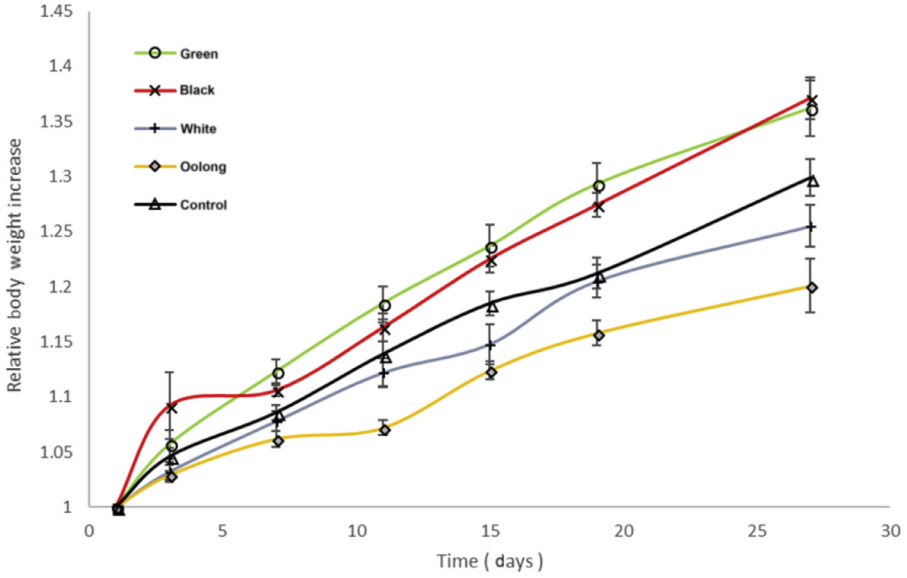

Fig. 3. Effect of various types of tea on weight development in Wistar rats during the period of 4 wk. Groups had free access to green, black, white, and oolong teas and deionized water, respectively. Each point represents mean + SE (n = 8).

different ($P < 0.05$) effect on the weight increase. Post hoc tests using the Bonferroni correction revealed that, at the end of study, oolong tea can significantly influence the relative weight compared with control ($P = 0.023$), black ($P = 1.927\ e^{-5}$) and green ($P = 4.516\ e^{-5}$) tea groups, whereas no significant effects were found between the oolong and white tea groups. Furthermore, white tea had significant effects on weight compared with black ($P = 0.004$) and green ($P = 0.009$) tea. Oolong tea had the most significant effect on slowing a weight increase during the 28 d (Fig. 3).

Metabolomic analysis of BAs after tea consumption

Twenty-six plasma BAsids were detected and measured by UPLC-MS/MS (Table 1). A *t* test revealed that ≥11 BA were significantly affected after drinking tea. The levels of murocholic acid (Fig. 4A), glycochenodeoxycholic acid (Fig. 4B), glycocholic acid (Fig. 4C), glycodeoxycholic acid (Fig. 4D), taurochenodeoxycholic acid (Fig. 4E), tauroursodeoxycholic acid (Fig. 4F), taurodeoxycholic acid (Fig. 4G), tauromuricholic acid (Fig. 4H), and taurocholic acid (Fig. 4I) were increased, whereas levels of taurolithocholic acid (Fig. 4J) and isolithocholic acid (Fig. 4K) were decreased after exposure to tea. *P*-values are presented in Figure 4.

Analysis of flavan-3-ols and xanthines present in tea and plasma

Catechins and xanthine content of the various teas, namely, black, green, oolong, and white, prepared from same freshly harvested tea leaves was determined by UPLC-MS/MS. Table 2 shows the concentration of various catechins, caffeine, theobromine, and paraxanthine. The concentration of catechins and xanthines were highest in green tea, whereas levels of these compounds were lowest in black tea. No significant difference in levels of these compounds was found between white and oolong teas. Interestingly, the EGC concentration of all four types of tea is higher than the EGCG concentration, which supports data from Kuo et al. [37], except oolong tea. In this study, the concentration of caffeine was found highest in green tea, followed by black tea, whereas oolong and white tea were much lower. Theobromine was mostly found in green tea. Figure 5 shows levels of polyphenol compounds in the plasma of different tea groups of rats. The mean concentration of EGCG in control rats was negligible—37.5 pg/mL—whereas, the levels of EGCG in green, black, white, and oolong teas were 4506, 2622, 4691, and 4688 pg/mL, respectively.

Discussion

In this study, for the first time, we report the comparative effects of green, black, white, and oolong teas on weight gain and bile acid metabolism. We prepared green, black, white, and oolong tea from the same fresh tea leaves and exposed Wistar rats to them as their sole source of drinking fluid for 28 d. We then investigated their effect on weight and BA metabolism. Our results, for the first time, show modulatory effects of green, black, white, and oolong tea on plasma BA profile as well as on weight loss.

Weight in rats was significantly influenced by oolong tea intake (Fig. 3), whereas no significant differences were found in green, white, and black tea consumption compared with controls. Moreover, there was no significant difference between white and oolong teas. Additionally, food intakes were not significantly influenced by drinking any of the types of tea (Fig. 2). This clearly suggests that oolong tea attenuated weight gain through mechanisms other than reduction in food intake. Based on this observation, it is logical to assume that oolong tea affects physiologic pathways that are involved in weight gain (Fig. 6). Broadly, the weight gain could be altered through either direct effect of bioactive tea components on physiologic pathways that are involved in weight gain process or through gut microflora. It is known from recent advances that obesity or weight can be significantly influenced by gut microflora through metabolic processes that involve BAs or through other signaling mechanisms (Figs. 4 and 6) [38,39]. BAs are known to affect metabolism [40], moreover, they have been shown to be excellent indicators of perturbed metabolism by gut microbiome [41,42]. BA synthesis is carried out in the liver. Tea bioactive components can either directly affect the BA synthesis in liver or through disturbance of gut microflora [43]. BAs are powerful regulators of metabolism and because of their specific role in fat metabolism, they could facilitate the removal of fat and thereby lower the diet-induced obesity [1,2,44–46]. Increased levels of plasma BAs were shown to be associated with a significant reduction in diet-induced obesity and resulted in increased whole body energy expenditure and dissipation of energy in the form of heat [31]. In addition, they have been implicated in electrolyte transport and immune

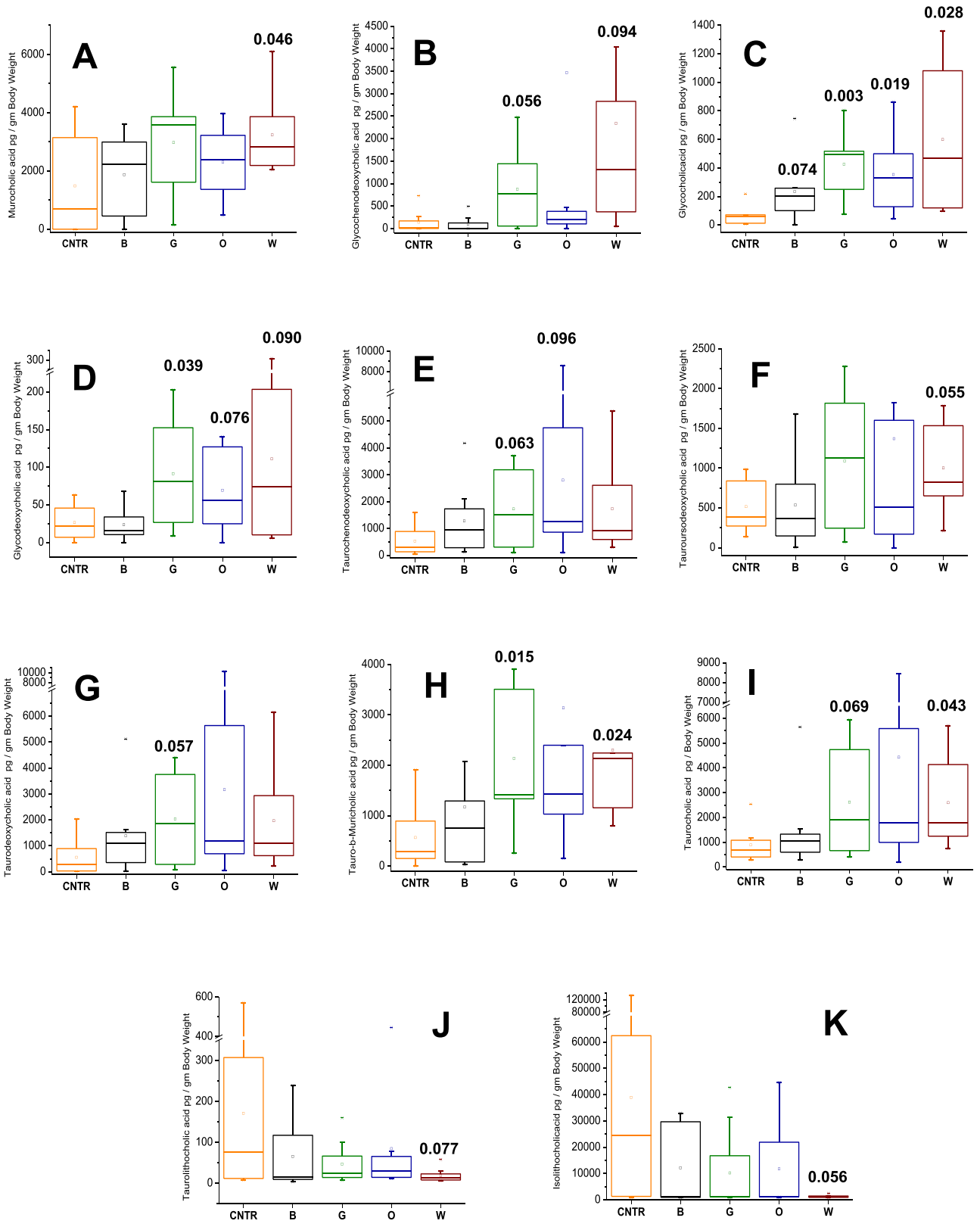

Fig. 4. Box and whisker plots of bile acids that were significantly changed by exposure to various types of tea (pg/mL). *P*-values were calculated using *t* test and are shown at the top of bar.

regulation through signaling of farnesoid X receptor [23], vitamin D receptor [25], and TGR5 receptor [24] to trigger cellular responses that play essential roles in host [47]. In this metabolomics investigation, the plasma levels of nine BAs were found to be differentially elevated (Fig. 4A–I) or suppressed (Fig. 4J, K) by specific tea treated rats groups (Table 1 and Fig. 4). In general, all the tea types

Table 2
Flavan-3-ols and xanthines present in the methanolic extract of various teas

No.	Tea Component	Black tea μg/mg	Green tea μg/mg	Oolong tea μg/mg	White tea μg/mg
1	Catechin (C)	0.33 + 0.13	3.53 + 0.73	0.85 + 0.03	1.02 + 0.08
2	Catechin gallate (CG)	0.08 + 0.01	0.56 + 0.13	0.38 + 0.01	0.36 + 0.01
3	Epicatechin (EC)	1.12 + 0.37	6.81 + 0.52	3.22+0.15	3.42 + 0.35
4	Epicatechin-3-O-gallate (ECG)	0.36 + 0.08	2.1 + 0.2	1.09 + 0.03	1.08 + 0.06
5	Epigallocatechin (EGC)	0.91 + 0.34	12.03 + 2.07	7.36 + 0.31	7.84 + 0.67
6	Epigallocatechin-3-gallate (EGCG)	0.67 + 0.18	7.42 + 1.22	2.68 + 0.13	2.88 + 0.38
7	Gallocatechin (GC)	0.33 + 0.26	6.74 + 1.44	4.01 + 0.19	3.46 + 1.01
8	Gallocatechin gallate (GCG)	0.44 + 0.25	2.81 + 0.35	1.76 + 0.18	1.38 + 0.14
9	Caffeine	7.36 ± 1.94	11.05 ± 0.63	3.19 ± 0.12	3.46 ± 0.22
10	Theobromine	0.97 ± 0.43	4.72 ± 1.79	0.28 ± 0.02	0.4 ± 0.07
11	Theophylline	0.05 ± 0.02	0.02 ± 0	0 ± 0	0.01 ± 0.01
12	Paraxanthine	0.11 ± 0.03	0.13 ± 0.02	0.02 ± 0.01	0.05 ± 0.02

Each value is expressed as mean ± SE (n = 3).
BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; EGCG, (-)-epigallocatechin 3-gallate; ECG, (-)-epicatechin-3-O-gallate; GC, (-)-Gallocatechin; and EGC, (-)-epigallocatechin; EC, (-)-epicatechin; CG, (−)-Catechin gallate; GCG, (-)-Gallocatechin gallate.

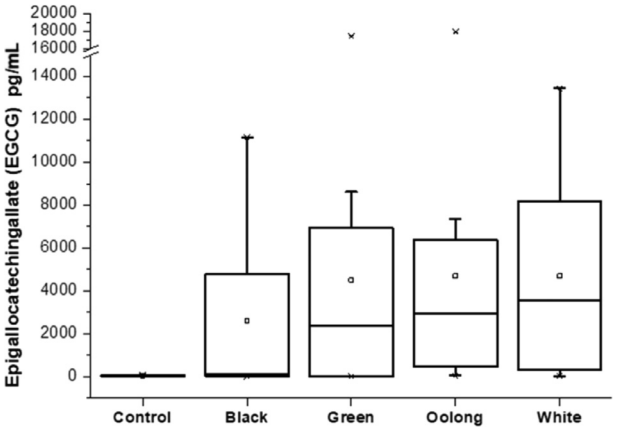

Fig. 5. Box and whisker plots of the plasma EGCG concentration of different tea groups. EGCG, (-)-epigallocatechin 3-gallate.

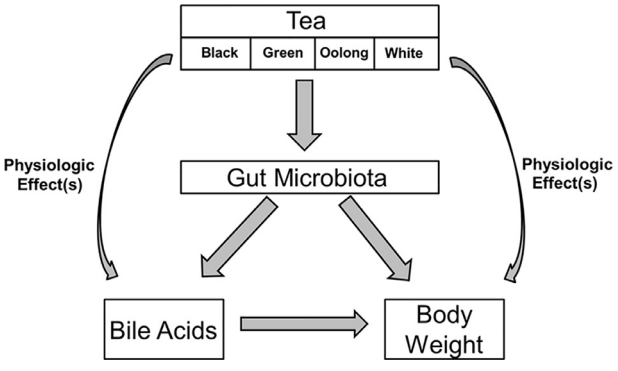

Fig. 6. Proposed mechanisms of tea induced reduction in weight gain .

affected increase in the levels of murocholic acid (Fig. 4A), glycochenodeoxycholic acid (Fig. 4B), glycocholic acid (Fig. 4C), glycodeoxycholic acid (Fig. 4D), taurochenodeoxycholic acid (Fig. 4E), tauroursodeoxycholic acid (Fig. 4F), taurodeoxycholic acid (Fig. 4G), tauromuricholic acid (Fig. 4H), and taurocholic acid (Fig. 4I), whereas levels of taurolithocholic acid (Fig. 4J) and isolithocholic acid (Fig. 4K) were decreased. Specifically, the levels of glycocholic acid (Fig. 4C) in the black tea treatment group were significantly increased compared with the control group. Treatment of green tea significantly increased the levels of glycochenodeoxycholic acid (Fig. 4B), glycocholic acid (Fig. 4C), glycodeoxycholic acid (Fig. 4D), taurochenodeoxycholic acid (Fig. 4E), taurodeoxycholic acid (Fig. 4G), tauromuricholic acid (Fig. 4H), and taurocholic acid (Fig. 4I). It is important to note that oolong tea, which caused the blockage of weight gain, significantly increased the levels of only glycocholic acid (Fig. 4C), glycodeoxycholic acid (Fig. 4D), and taurochenodeoxycholic acid (Fig. 4E), indicating that these three BAs may have important role in the weight gain mechanism. Finally, among all four tea types, white tea caused significant increases in the levels of all nine BAs except taurochenodeoxycholic acid (Fig. 4E) and taurodeoxycholic acid (Fig. 4G); moreover, white tea also significantly reduced levels of taurolithocholic acid (Fig. 4J) and isolithocholic acid (Fig. 4K). Although at this stage the exact molecular mechanisms about how changes in BA could affect weight gain processes are not known, it could be assumed that the cumulative effect of changes in the BA metabolic profile could be responsible for reduction of weight gain in oolong tea-treated rats. In summary, oolong tea can induce weight loss by directly affecting physiologic changes, disturbing gut microflora, and through changes in the BA metabolic profile. However, follow-up validation study is needed to confirm these findings.

The concentration of catechins and xanthines were highest in green tea, whereas levels of these compounds were lowest in black tea. No significant difference in levels of these compounds was found between white and oolong tea. Interestingly, the EGC concentration of the teas is higher than the EGCG concentration, which corroborates an earlier report [37], except oolong tea. In this study, the concentration of caffeine was found highest in green tea, followed by black tea, whereas in oolong and white tea the caffeine levels were much lower. Theobromine was largely found in green tea. UPLC-MS/MS analysis showed presence of EGCG in plasma. The mean concentration of EGCG in plasma of control rats was negligible, and in the black tea group was much less, whereas the concentration was much higher in green-, white-, and oolong tea-treated rats. Statistical analysis found no correlation between plasma EGCG and the weight of the rats. EGCG absorption is extremely low and moreover its half-life is short; perhaps this is the reason we did not find correlation. Furthermore, it may also be possible that catechin profiles measured from the intestinal tract could correlate well with other parameters including weight. However, these results indirectly support our observation that tea may have elicited its effect through inducing changes in gut microflora.

In the present study, we used rats that were maintained on a normal diet and were treated with tea extracts. Here our aim was to perform comparative investigation of the effects of black, green, oolong, and white teas on BA metabolism and other parameters. Commonly, others have used rats that were treated with high-fat diet in their antiobesity studies with tea. Moreover, these

investigations also report use of test substance at higher dosage. As a result, owing to differences in the methods, the conclusion obtained in this study may differ from previous reports. We think that our experimental design very closely resembles to normal human tea consumption. Hence, the data obtained through such a study is more relevant to humans.

Conclusion

The effects of black, green, oolong, and white teas that were prepared from the same fresh tea leaves on weight and BA metabolism was evaluated in rats in a comparative study. The results demonstrate that plasma levels of nine BAs were elevated and two BAs were suppressed in rats after the consumption of specific black, green, oolong, and white teas. Furthermore, it was found that oolong tea significantly blocked weight gain, whereas black, green, and white tea dosed rats had no effect on weight compared with control rats. Finally, results from this study clearly show that oolong tea most effectively blocked weight gain.

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