



HEALTH SCIENCES

Effects of green tea extract combined with brisk walking on lipid profiles and the liver function in overweight and obese men: A randomized, double-blinded, placebo-control trial

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Abstract: This study was aimed to investigate the effect of green tea extract (GTE) combined with brisk walking on lipid profiles and the liver function in overweight and obese men. Twenty-four participants were randomized to either the GTE group or the placebo group for 12 weeks with a 4-week follow-up. The walking program consisted of four 60-min-sessions/week and all participants were asked to consume two GTE (150mg) or placebo tablets daily. After 12-week intervention, GTE group resulted in a significant difference in the low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels when compared to placebo group ($P < 0.01$). There was also a significant reduction in the aspartate aminotransferase levels ($P < 0.01$) in the GTE group, but no change in the placebo group ($P > 0.05$). There was no change in the triglyceride or high-density lipoprotein cholesterol (HDL-C) levels in the placebo group, but a significant reduction was noted in the HDL-C levels in the GTE group ($P < 0.05$). GTE combined with brisk walking resulted in a significant change in the LDL-C and TC levels, however, a significant reduce in HDL-C in the GTE group. The study has a more positive effect on the liver function than brisk walking alone.

Key words: green tea extract, brisk walking, overweight and obese men, lipid profiles, liver function.

INTRODUCTION

The prevalence of overweight and obesity has increased dramatically over the last four decades (Jaacks et al. 2019). According to the Chinese General Social Survey (CGSS), about 31% of the population was overweight or obese (Zhou 2019). Studies (Piepoli et al. 2016, Oliveros et al. 2014) have reported that obesity is associated with LDL-C, HDL-C, TG, and TC as important risk factors for the development of dyslipidemia, hyperlipidemia, and atherosclerosis. As physical activity and GTE have been shown to be associated with the management of obesity and lipids cholesterol, there is great interest in

exercise and GTE modifications to help improve the body composition and treat chronic diseases.

Increased physical activity is an important component of therapy for obesity management. Brisk walking as a simple, economic, and safe form of exercise is the most popular moderate-intensity aerobic physical activity for both men and women, especially for most middle-aged and/or overweight and obese individuals (Siegel et al. 1995). A previous study showed that moderate exercise (energy expenditure 1200 to 2200 kcal/week of brisk walking or jogging) effectively elevate HDL-C levels from 2 to 8 mg/dl and lower TG levels by 5 to 38 mg/dl (Durstine et al. 2001). In addition, a meta-analysis review

(Kelley et al. 2006) showed that aerobic exercise increases the HDL-C levels and reduces the TG levels in patients with cardiovascular disease.

Green tea is a natural supplement, and its beneficial effects for promoting health have been intensively investigated in the past decade (Isemura et al. 2015, Giglio et al. 2018). Green tea is rich in catechins, and epigallocatechin-3-gallate (EGCG) is the most abundant and pharmacologically active of these catechins (Chen et al. 2016). Animal studies have shown that green tea or green tea extract (GTE) effectively regulates LDL -C oxidation and reduces the blood levels of cholesterol in rats/mice fed a high-fat diet. Recently, meta-analysis and systematic review studies (Kim et al. 2011, Onakpoya et al. 2014) reported that green tea or GTE has a significant effect on the LDL-C and TC levels in humans. The main mechanism underlying the effect of green tea or GTE on blood lipids is the inhibition of intestinal lipid absorption and reduction in cholesterol synthesis (Yang et al. 2016). However, these effects on the lipid profiles of humans are inconsistent and completely opposite results have been obtained in some studies, focusing on green tea or GTE in combination with exercise (Gahreman et al. 2016, Maki et al. 2009) . In addition, studies focus on the liver injury caused by tea catechins (Wolfram 2007), but catechins have a potential positive effect on the liver functional (Cheng et al. 2018).

The knowledge of a potential beneficial effects of GTE combined with brisk walking on the lipid profiles and liver functional in inactive overweight and obese adults' men is limited, especially those with under uncontrolled dietary condition. We hypothesized that GTE combined with brisk walking might improve the lipids cholesterol and liver function in overweight and obese men. Therefore, the present study aimed to examine the effects of GTE combined

with brisk walking on the change in (1) lipids cholesterol and (2) the liver function after 12 weeks plus 4-weeks follow-up in inactive overweight and obese men.

MATERIALS AND METHODS

Study design

This study is a randomized, double-blind, placebo-controlled trial. This study was approved by the Luohe Central Hospital of Clinical Research Ethics Committee (No. 2018-03-015-E01) and has also been registered as a clinical trial (Chinese Clinical Trials Registry, ChiCTR1900025134).

Participants

Twenty-four overweight and obese men were included in the study. According to the physical characteristics of Asian, the optimal BMI cut-off value is 24 kg/m² for men (Zeng et al. 2014). Those who met the following inclusion criteria were then included: male; between 25 and 59 years of age, BMI between 24 and 32, No participation in any clinical program within the previous 3 months. The exclusion criteria were as follows: regularly consume >2 cups of green tea/day, diabetic, history of medication usage in the last 4 weeks, history of serious cardiovascular disease, diagnosed with a mental illness or liver, renal or nerve disease.

Sample size and randomization

Twenty-four participants were included in this study. The sample size was determined from the sample size recommended in a clinical trial (total 20 participants, assuming a dropout rate of 20%) (Whitehead et al. 2016). Twenty-four participants were randomly divided into two groups: a GTE and placebo group. Randomization was performed by a randomized digital table sequence with stratified randomization accounting for the

potential bias factor of BMI (<27 and \geq 27 kg/m²). The GTE and placebo supplement, with A indicating the placebo supplement and B indicating the GTE supplement (determined by a coin toss). The alphabet was marked from the GTE Company, which was managed by another study assistant.

Procedures

The walking program consisted of four 60-min sessions per week, each including a 5 min warm-up and 5 min cool-down period. This walking program was performed under the supervision of the first author and a research assistant. Due to all subjects being physically inactive overweight or obese men, for the first 3 weeks (initiation phase), we set the target heart rate (HR) to 50%- 65% of the maximum HR for all participants and then gradually increased the intensity (65%-80%)(Murtagh EM et al. 2002). The maximum HR was calculated using the equation $HR_{max} = [220 > age]$. All participants were fitted with a heart rate monitor (FS1, Polar, Finland). The participants were also fitted with a pedometer (Power walker, EX-510; Yamax, Tokyo, Japan), which recorded the step counts during the exercise period. In the intervention period, all participants were asked to consume two GTE or placebo tablets daily (one after breakfast and one after dinner) and to drink a cup of water.

Supplements and diet

GTE (EGCG) and placebo were purchased from Damingtang Company (Hangzhou, China). Each GTE tablet contained 150 mg of EGCG (95% pure), isomaltitol, and microcrystalline cellulose (MC), and each placebo tablet contained neutral ingredients, such as corn starch, MC and isomaltitol.

Biochemical parameters

All participants visited the hospital 3 times: at baseline, at 12 weeks after the start, and at 16 weeks after the start (for the 4-week follow-up). Venous blood samples (5 mL) were obtained from all participants after fasting for 8-10 h (8:30-10:00 AM). Blood samples were analyzed for TC (mmol/L), LDL-C (mmol/L), HDL-C (mmol/L) and TG (mmol/L) as well as serum aminotransferases (alanine transaminase, [ALT], AST, γ -glutamyl transpeptidase [GGT], TBIL). All parameters were measure at the center laboratory, Luohe Central of Hospital.

Statistical analyses

All statistical analyses were performed using SPSS 20.0 software program (IBM SPSS Statistics, Armonk, USA). The intention-to-treat (ITT) efficacy analysis was used in this study. Results are reported as the mean \pm standard deviation (SD) for continuous variables unless otherwise stated. The Shapiro-Wilk normality test was used to test data normality. If the data were not normality distributed, a Wilcoxon's test was used within the groups. To examine the changes between the groups after the intervention, we used analysis of co-variance (ANCOVA) to analyze the data. After observing the normality, a parametric analysis was performed using a repeated measure analysis of variance (2 groups by 3 moments [baseline, post-test and follow-up]). For all comparisons, a p value of < 0.05 was considered significant.

RESULTS

Participants and baseline characteristics

An analysis of the baseline characteristics of participants showed no significant differences between the groups in the age, body weight, BMI, systolic blood pressure, or diastolic blood

pressure (Table I). There were also no significant differences in the LDL-C, HDL-C, TC, TG, ALT, AST, TBIL, or GGT between the groups at baseline.

The between-group ANCOVA

As shown in Table 2, there was a significant difference in the LDL-C levels between the GTE group and placebo group ($P < 0.05$). A comparison between the groups also showed a significant change in the TC levels ($P < 0.05$). There were no significant differences in the HDL-C and TG levels and the liver function (ALT, AST and TBIL levels) between the GTE group and placebo group. The GGT parameters did not conform to a normal distribution (Shapiro-Wilk normality test: $P = 0.000$), and therefore, the results of Wilcoxon's test showed that there were no significant differences either between or within the groups.

Repeat assessments

Table III shows the lipid profiles and the liver function parameters with three-fold

measurement for the two groups. Across both groups, significant time main effects were found in the LDL-C, HDL-C, TC, and TBIL levels. The placebo group showed increased in the LDL-C and TC levels and decreased in the TBIL levels. The GTE group, showed decreased LDL-C levels after intervention (although they increased after follow-up), and a significant decrease in the HDL-C levels. A significant group main effect was found for AST levels ($P = 0.024$) with the placebo group, and there was a significant difference in the AST levels between the two groups in the follow-up period. A group \times time effect was found for TC levels ($P = 0.013$), with the placebo group showing a dramatic increase and the GTE group a slight decrease. A significant group \times time effect was also found for the HDL-C levels ($P = 0.008$), with the GTE group showing a slight decrease and the placebo group consistently normal.

Table I. Subject characteristics at baseline.

Variable	GTE group (n= 12)	Placebo group (n= 12)
Age (years)	42.5±9.82	37.2±7.25
Body weight (kg)	83.0±9.37	82.3±8.46
Body mass index (BMI)	28.4±2.24	27.7±2.26
LDL-C (mmol/L)	2.91±0.89	2.82±0.84
HDL -C(mmol/L)	1.43±0.25	1.33±0.12
TC (mmol/L)	4.85±1.07	4.78±1.11
TG (mmol/L)	1.64±1.03	2.03±1.10
ALT (U/L)	35.2±15.52	45.8±22.67
AST (U/L)	23.0±6.38	26.6±9.22
TBIL (U/L)	18.9±5.24	17.7±4.38
GGT (U/L)	43.5±31.22	54.3±47.48

No difference between groups were statistically significant ($P < 0.05$).

Table II. Blood lipid profiles and liver function in ANCOVA after 12 weeks.

	GTE group (n= 12)				Placebo group (n= 12)				F	P
	Unadjusted		Adjusted		Unadjusted		Adjusted			
	Mean	SD	Mean	SE	Mean	SD	Mean	SE		
LDL-C(mmol/L)	2.68	0.708	2.64	0.11	3.14	0.928	3.18	0.11	12.651	0.002 [*]
HDL-C(mmol/L)	1.32	0.241	1.28	0.04	1.32	0.188	1.36	0.04	2.507	0.128
TC (mmol/L)	4.86	0.894	4.84	0.16	5.53	1.235	5.56	0.16	10.337	0.004 [*]
TG (mmol/L)	1.40	0.527	1.52	0.15	1.89	0.992	1.77	0.15	1.512	0.233
ALT (U/L)	30.8	15.97	33.6	3.68	42.7	15.56	39.9	3.68	1.433	0.245
AST (U/L)	18.7	5.65	19.6	1.29	23.7	6.26	22.7	1.29	2.717	0.114
TBIL (U/L)	13.9	5.22	13.5	0.98	12.5	3.35	12.8	0.98	0.271	0.608

* A statistically significant mean difference ($p < 0.05$).

Table III. Biochemical analysis of the participants during the experimental and follow-up periods.

Variable	GTE group (n= 12)			Placebo group (n= 12)			P-value		
	Pre	Post	Follow-up	Pre	Post	Follow-up	Group	Time	Group×Time
LDL-C	2.91±0.886	2.68±0.708 [*]	2.99±0.910 [§]	2.82±0.839	3.14±0.928 [*]	3.24±0.753 [#]	0.546	0.024	0.163
HDL-C	1.43±0.252	1.32±0.241 [*]	1.33±0.275 [#]	1.33±0.123	1.32±0.126	1.28±0.116	0.528	0.008	0.006
TG	1.64±1.026	1.40±0.527	1.52±0.497	2.03±1.100	1.89±0.992	1.88±1.106	0.222	0.428	0.910
TC	4.85±1.069	4.86±0.894	5.23±1.190 [#]	4.78±1.105	5.53±1.235 [*]	5.62±0.994 [#]	0.451	0.000	0.013
ALT	35.2±15.52	30.8±15.97	28.2±15.40	45.8±22.67	42.7±15.56	51.33±38.90	0.054	0.622	0.317
AST	23.0±6.38	18.7±5.65 [*]	17.3±5.68	26.6±9.22	23.7±6.26	27.3±12.56 [§]	0.024	0.124	0.181
TBIL	18.9±5.24	13.9±5.22 [*]	14.4±5.49 [#]	17.7±4.38	12.5±3.35 [*]	10.9±4.07 [#]	0.229	0.000	0.415
GGT	43.5±31.22	34.9±15.13	37.5±19.03	54.3±47.48	50.6±46.39	51.4±46.21	0.368	0.108	0.592

Values are expressed as mean ±SD. ^{*}, [#], [§] $p < 0.05$; ^{*} P -value for within-group comparisons in pre-and-post period; [#] P -value for within-group comparisons in post and follow-up; [§] P -value for within-group comparisons in pre and follow-up. [§] P -value for between group comparisons in follow-up period.

DISCUSSION

This study was designed to assess the effects of GTE combined with brisk walking on lipid profiles and liver function in inactive overweight and obese men. Compared with the placebo group, ingestion of GTE for 12 weeks significantly reduced the LDL-C levels in overweight and obese men. Worth noting is that there was no significant change in the TC levels in the GTE group even after 12 weeks' intervention; however, EGCG was able to significantly control the TC levels in overweight and obese men, and we observed a significant increase in the GTE group after 4 weeks follow-up. Recently, studies (Onakpoya et al. 2014, Cicero et al. 2017) reported that green tea results in a significant reduction in TC and LDL-C levels. Furthermore, in animal studies (Richard et al. 2009, Murase 2002), authors have reported that green tea or GTE significantly reduced LDL-C and TC levels in rats/mice. The mechanism underlying the reduction in plasma cholesterol levels by GTE might involve EGCG preventing the absorption of lipids and inhibiting digestive enzymes, with additional effects on the intestinal microbiota (Suzuki et al. 2016). The present findings are largely in agreement with those of previous studies, although there are some differences.

The discrepant results between the present study and the conclusion of the previous meta-analysis may be due to the following: first, as the table 3 shown, GTE have a positive effect on improvement the TC levels. The present study used a dose of only 300mg EGCG/day (Dekant et al. 2017); however, many, but not all, previous studies demonstrating significant changes in LDL cholesterol and TC levels used higher doses of GTE (EGCG). Study (Samavat et al. 2016) reported a significant reduction in LDL-C and TC levels in postmenopausal women after 1 year of consuming a supplement with

green tea catechins (1315 mg/day) compared to the baseline and placebo group. In addition, a 2-month randomized control trial of green tea (EGCG: 400mg/day and 800mg/day) found a significant change in the TC and LDL-C levels in healthy postmenopausal women (Brown et al. 2011).

Another important confounding factor may be the diet. Previous study (Li & Shi 2017) noted the over-consumption of high-fat and deep-fried foods in the Chinese dietary pattern over the past few decades. Data from animal studies (Wang et al. 2018, Huang et al. 2018) have indicated that a high-fat diet combined with GTE results in a significant change in the LDL cholesterol and TC levels in mice/rats. In another study (Jianrong 2004) reported that meat was consumed more frequently in the winter than in the summer for a large number of Chinese individuals, and the summer months showed a more balanced dietary pattern than the winter months (Zang et al. 2017). Our study was started in November and ended the following February, which is the winter period in north China. These findings indicate that a high-fat diet is an important confounding factor in the intervention period, and the season was also a confounding factor.

Although a previous study (Cai 2016) found that brisk walking significantly altered the HDL cholesterol and TG levels in humans, we noted no significant changes in the HDL cholesterol or TG levels in the placebo group in our present study, and while a significant decrease in the HDL-C levels was noted in the GTE group, there were no significant differences in the TG levels. The reason might be due to all participants were inactive overweight and obese men and the intensity of brisk walking. Study (Durstine et al. 2001) reported a significant increase in the HDL-C levels and a significant reduction in the TG levels, when subjects achieved 24-32 km per week of brisk walking or jogging and elicited between 1200

to 2200 kcal/wk. In the present study, although we set the target heart rate to 50% - 80% in the two phases during the intervention period, the participants were not achieved. Study by Andersen & Fernandez (2013) showed that dietary components have a significant effect on the HDL function. The over-consumption of cereal, meat, and poultry in Chinese dietary patterns might be the other reason (Song et al. 2017). The significant reduction in the HDL-C levels in the GTE group might be due to the mechanism of cholesterol metabolism; a recent animal study (Liao et al. 2016) found a significant reduction in the HDL-C levels after feeding green tea polyphenols to high-fat-diet mice for several weeks. The results of the present study are in agreement with those of that animal study. This outcome might be due to HDL-C playing a role in cholesterol metabolism. Another study (Cavallini et al. 2011) showed that HDL-C level is positively correlated with the *Bifidobacteria* populations in the gut. Furthermore, an animal study (Liao et al. 2016) found that EGCG significantly increased the number of *Bifidobacteria* in the gut and reduced the serum cholesterol level.

Finally, significant changes in AST levels but only a downward trend in ALT levels was noted in the GTE group. These findings for ALT levels in the present study demonstrate that GTE has a beneficial effect on the liver function. The effect of GTE on the liver function in this study agrees with the results of a recent meta-analysis of four trials, which showed a significant effect on the ALT and AST levels in cases of non-alcoholic fatty liver disease (NAFLD) (Mansour-Ghanaei et al. 2018). A daily consumption of 300mg EGCG combined with brisk walking for 12-week positively influenced the liver function biomarkers in overweight and obese men in the present study. We also found that a positive effect on the serum LDL-C and TC levels in overweight and obese adult men. However,

there was a negative effect on the HDL-C levels in the GTE group. Further studies on the effects of GTE combined with brisk walking on the HDL-C levels in overweight and obese men should be conducted.

Several limitations associated with the present study warrant mention. First, the sample size in this study was relatively small. Second, a relatively low dose GTE was used. Third, we did not control the diet in the present study. A multi-arm and larger-scale trial should be conducted in order to confirm the present study's results.

In conclusion, the results of this study provide evidence that GTE combined with brisk walking in inactive overweight and obese men for 12 weeks did not reduce the TG levels within the groups. However, this study confirmed that GTE combined with brisk walking was able to significantly control LDL-C and TC levels compared with the placebo group. In addition, our study shows that GTE combined with brisk walking is sufficient for improving the liver function than exercise alone. There was a downward trend in the lipid profiles in the follow-up period in both groups.

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Author contributions

Tengfei ZHANG conceived of this study, designed and coordinated this trial, performed statistical analysis, interpretation and wrote the initial draft of the manuscript; Si CHEN performed the randomization and statistical manuscript; Ningxia LI provided medical oversight. Zhenqing HOU performed the data entry. Atsushi SAITO provided scientific guidance for the design of the study.

