Clinical Nutrition 35 (2016) 592–599

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Therapeutic effect of high-dose green tea extract on weight reduction: A randomized, double-blind, placebo-controlled clinical trial

I-Ju Chen [a, b, c], Chia-Yu Liu [a, b, c], Jung-Peng Chiu [a, b, c], Chung-Hua Hsu [a, b, c, *]

[a] Branch of Linsen and Chinese Medicine, Taipei City Hospital, Taiwan
[b] Taiwan International Traditional Chinese Medicine Training Center, Taipei, Taiwan
[c] Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 7 October 2014
Accepted 12 May 2015

Keywords:
Green tea extract
Epigallocatechin gallate
EGCG 4
Obese women

SUMMARY

Background and aims: To examine the effect and safety of high-dose green tea extract (*Epigallocatechin gallate*, EGCG) at a daily dosage of 856.8 mg on weight reduction and changes of lipid profile and obesity-related hormone peptides in women with central obesity.

Methods: We conducted a randomized, double-blind trial registered under ClinicalTrials.gov Identifier no. NCT02147041. A total of 115 women with central obesity were screened at our clinic. 102 of them with a body mass index (BMI) $\geq$ 27 kg/m^2 and a waist circumference (WC) $\geq$ 80 cm were eligible for the study. These women were randomly assigned to either a high-dose green tea group or placebo group. The total treatment time was 12 weeks. The main outcome measures were anthropometric measurements, lipid profiles, and obesity related hormone peptides including leptin, adiponectin, ghrelin, and insulin.

Results: Significant weight loss, from 76.8 ± 11.3 kg to 75.7 ± 11.5 kg ($p = 0.025$), as well as decreases in BMI ($p = 0.018$) and waist circumference ($p = 0.023$) were observed in the treatment group after 12 weeks of high-dose EGCG treatment. This study also demonstrated a consistent trend of decreased total cholesterol, reaching 5.33%, and decreased LDL plasma levels. There was good tolerance of the treatment among subjects without any side effects or adverse events. Significantly lower ghrelin levels and elevated adiponectin levels were detected in the study group than in the placebo group.

Conclusion: 12 weeks of treatment with high-dose green tea extract resulted in significant weight loss, reduced waist circumference, and a consistent decrease in total cholesterol and LDL plasma levels without any side effects or adverse effects in women with central obesity. The antiobestic mechanism of high-dose green tea extract might be associated in part with ghrelin secretion inhibition, leading to increased adiponectin levels.

1. Introduction

Obesity has become a global health issue due to its alarmingly high and increasing prevalence rate worldwide [1]. According to estimates, around 12% of adults over the age of 20 were obese in 2008; this is nearly double the incidence estimated in 1980 [2]. Various studies have reported that obesity is a major risk factor for several debilitating and potentially fatal diseases like cardiovascular disease, hyperlipidemia, and diabetes mellitus, which lead to enormous health-care expenditure in many developing and developed countries [3–5]. Thus, strategies and policies for prevention and treatment of obesity have been deemed global priorities to reverse the trend of the global obesity epidemic [1].

Numerous anti-obesity interventions have been studied including lifestyle modification, behavioral therapy, pharmacological treatments, and surgery [6,7]. However, the limited efficacy and high incidence of adverse events with side effects observed in conventional therapies have motivated practitioners to investigate complementary and alternative medicine therapies for weight loss such as dietary supplements, herbal products, and acupuncture [8,9]. Green tea (*Camellia sinensis*), one of the most popular beverages in Asia [10], has been studied extensively for its beneficial effects on cardiovascular [11] and metabolic diseases [12].The main components of green tea include catechins, such as *Epigallocatechin gallate* (EGCG), epigallocatechin, *Epicatechin gallate*,

* Corresponding author. Institute of Traditional Medicine, National Yang-Ming University, 155, Li-Nong St, Sec 2, Peitou, Taipei, Taiwan. Tel.: +886 2 2826 7050; fax: +886 2 28201461.
E-mail address: owlherbs@yahoo.com.tw (C.-H. Hsu).

http://dx.doi.org/10.1016/j.clnu.2015.05.003

and epicatechin, which have been shown to be beneficial to human health [10]. Among the above catechins, EGCG is the most abundant green tea catechin and is considered the most bioactive component for reducing body weight, which it accomplishes by decreasing adipocyte differentiation and proliferation during lipogenesis [13].

However, equivocal results have been reported on the antiobestic effects of green tea [10]. On the one hand, Basu et al. [14] demonstrated that green tea extracts and beverages could cause reduction of body weight and body mass index in obese subjects in 8 weeks. On the other hand, in our previous study [15] we found that daily consumption of green tea extract containing 491 mg of catechins (302 mg EGCG) did not produce weight reduction in obese women, although significantly decreased plasma cholesterol and triglycerides were observed. Therefore, in this study we increased the concentration of EGCG to a daily dosage of 856.8 mg to examine the antiobestic effect of higher dosages of EGCG and to examine the effect of high-dosage GTE on obesity-related hormone peptides.

2. Method

2.1. Study design and participants

This was a single-center, placebo-controlled, double-blind study conducted from March 2012 to March 2014 at Taipei City Hospital in Taiwan. The inclusion criteria for this study was as follows: (1) women between the age of 20 and 60 years-old, (2) body mass index (BMI) $\geq$ 27 kg/m^2, (3) waist circumference (WC) $\geq$ 80 cm, and (4) willingness to fill out the questionnaires for this trial. Subjects with the following conditions were excluded: (1) heart failure, acute myocardial infarction, or stroke in the past year; (2) impaired liver and kidney function (serum alanine transaminase > 80 U/L, serum creatinine > 2.0 mg/dl); (3) breast feeding or pregnancy; (4) medicinally controlled endocrine disease (thyroid disease, pituitary disease, diabetes mellitus, etc.); and (5) any other conditions making the subject unsuitable for the trial, as evaluated by a physician. BMI was calculated as weight in kilograms divided by height in meters squared. We set the BMI cutoff value for obese as $\geq$ 27 kg/m^2 according to different metabolic responses to BMI across ethnicities.

Letters were sent to all the patients to explain the purpose of the study and to invite them to participate. A detailed explanation of the study design was given to the subjects before participating in this trial. Finally, 92 subjects were enrolled with written informed consent. The protocol was approved by the Human Ethics Committee of Taipei City Hospital and registered with ClinicalTrials.gov (NCT02147041).

2.2. Randomization and blindness

Subjects were randomly allocated to one of two arms for 12 weeks (Fig. 1) using a computer, which generated a random number from 0.0 to 0.99. The subjects given a random number between 0.00 and 0.49 were assigned to group A to receive decaffeinated GTE, and subjects given a number between 0.50 and 0.99 were assigned to group B to be given cellulose as a placebo treatment. Both decaffeinated GTE and cellulose were packed in the same opaque capsules for blinding. Treatments were administered by a blinded research assistant. The patients were told not to receive other types of obesity management and maintain their former diet, eating habits, and physical activity. Subjects were also told to take the record of their meals and physical activities every week and sent back to our assistant to make sure that their diet components compared to their former diet didn't differ much, so as physical activities. Regular consumption of other food and beverages containing caffeine dosage greater than 200 mg per day and health supplementary containing catechins or polyphenols during the study period were also prohibited. A list of foods containing catechins, polyphenols, or caffeine and dietary notes were given to each subject to record down everything they ate daily and monitor their compliance. All subjects were free to withdraw at any time during the course of the study.

2.3. Preparation of samples and treatment

The GTE samples, obtained from the Tea Research and Extension Station, Taiwan, were extracted from dried leaves of green tea. 90 °C pure water was used as solvent to extract from dry green tea leaves with ratio of tea leaves to solvents 1 to 20 repeatedly for 3 times with 20 min each time. Then the solvent was cooled down with catechins preserved by ethylacetate. Catechin-rich extract were isolated from 50 mL ethylacetate per gram by ceramic membrane filtration(molecular weight 90) repeatedly for 5 times. Bipolar ion exchange membrane was applied for decaffeination. The extract is concentrated under low pressure and temperature, and dried to a powder by spray-drying. Several tea catechins in addition to EGCG were also standardized in the component analysis of the decaffeinated GTE (Table 1). 500 mg of pure microcrystalline cellulose was capsulized as the placebo, as well as the decaffeinated GTE extracts capsulized 500 mg each. The treatment frequency for this trial was three times daily for 12 weeks with one capsule 30 min after meals. The total daily doses of GTE compounds taken by the treatment group are listed in Table 1, with the daily dose of EGCG amounting to 856.8 mg.

2.4. Outcome measurements

The percent reduction of body weight (BW), BMI, and WC of the two different treatments were used as major outcome measurements. Accurate and sensitive measurements of body weight and height were gauged using a standardized electronic beam scale to the nearest 0.1 kg and a wall-mounted stadiometer to the nearest 0.1 cm, respectively. Waist circumference was defined as midway between the lower rib margin and the iliac crest. Arterial blood pressure was obtained using an electronic digital sphygmomanometer. All measurements were done after an overnight fast and were performed once at the beginning of the study and once after 12 weeks of treatment.

2.5. Analysis of biochemical data and obesity-related hormone peptides

At the beginning and end of the study, an experienced nurse drew whole blood samples from patients the morning after 8–9 h of fasting to obtain laboratory data including measurements of blood sugar, plasma lipoproteins (for triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), and obesity-related hormone peptides including leptin, insulin, ghrelin, and adiponectin. The whole blood sample was centrifuged at 4 °C immediately with 15000 rpm for 20 min to separation with a 2 ml aliquot of serum and rapidly frozen at −80 °C for subsequent radioimmunoassay (RIA) analysis. The biochemical data was analyzed in clinical laboratories at the hospital. The Millipore Human Leptin assay (Millipore, St. Charles, MO, USA) using I125-labeled human leptin antiserum with a sensitivity limit of 0.5 ng/ml was used to detect plasma leptin levels. Plasma ghrelin and adiponectin were detected using Millipore Ghrelin and Adiponectin RIA Kits (Millipore, St. Charles) with sensitivity limits of 93 pg/ml and 1 ng/ml,

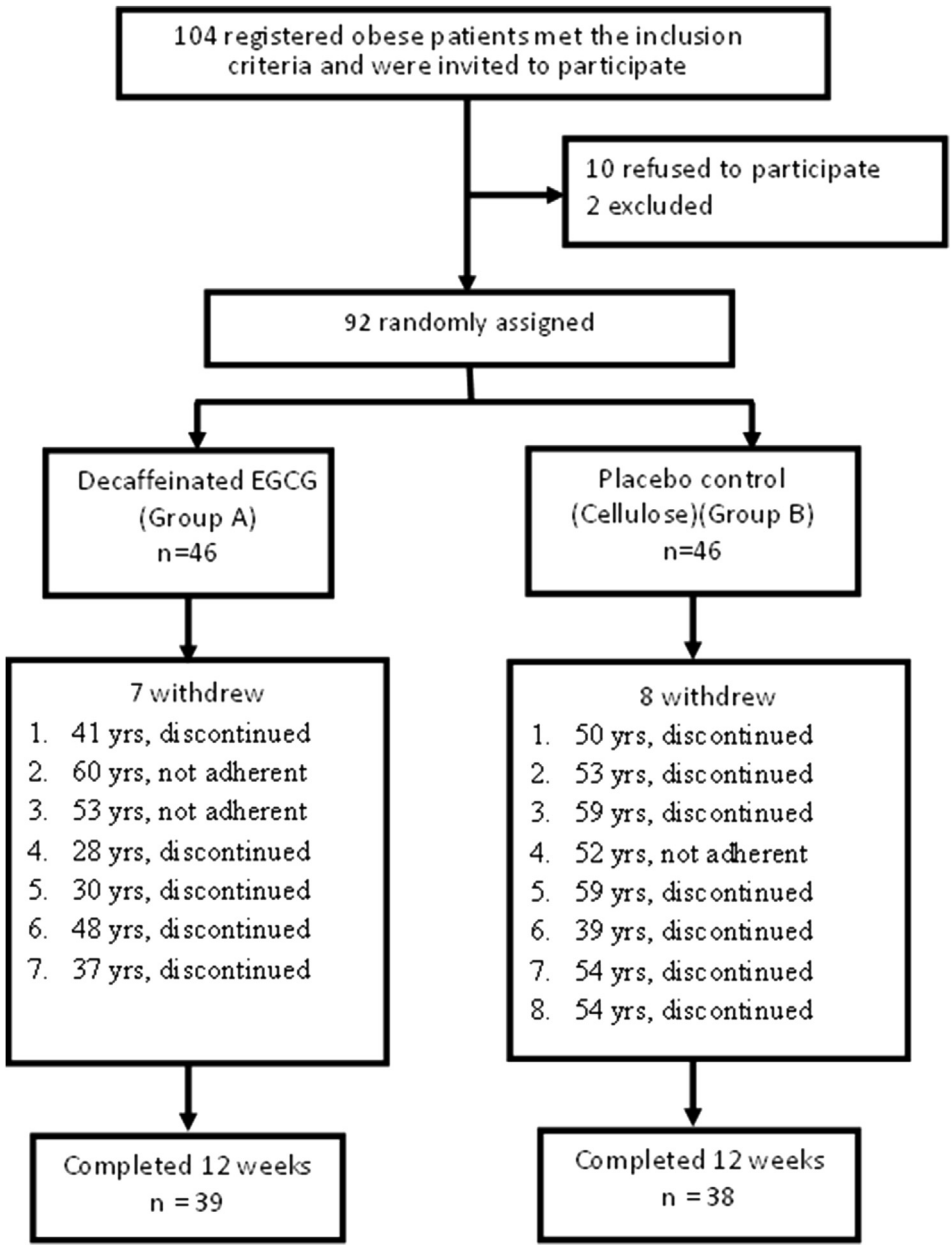

Fig. 1. Trial profile and design.

respectively. Plasma insulin levels were measured using a commercially available RIA (Linco Research, Inc.) with a sensitivity limit of 0.5 ng/ml. The homeostasis model assessment of insulin resistance (HOMA-IR) was applied to evaluate insulin resistance using the following calculation: HOMA-IR = insulin (mUI/L) × glucose (mmol/L/22.5). Values greater than 2.25 were considered to indicate insulin resistance [16].

Table 1
Green tea extract component analysis.

Component	% in weight	Daily dose (in mg)
EGCG (*Epigallocatechin gallate*)	57.12	856.8
ECG (*Epicatechin gallate*)	15.74	236.1
EGC (Epigallocatechin)	7.70	115.5
EC (Epicatechin)	4.80	71.9
GCG (*Gallocatechin gallate*)	4.25	63.7
GC (Gallocatechin)	<0.07	<1.05
Caffeine	<0.07	<1.05
Cellulose	10.3	155.0

2.6. EGCG dose analysis

For extraction, 100 ml of 50% methanol were mixed with the GTE sample for 10 min. 20 ml of the extracted solution were centrifuged at 10,000 rpm (Eppendorf Centrifuge 5402, MI, USA) for 10 min. The supernatant was filtrated with a 0.22-mm syringe filter (Millipore, Bedford, MA, USA). Then, 20 ml of the supernatant liquid were injected into the high performance liquid chromatography(HPLC) system for further analysis. HPLC analysis was performed by a Hitachi 7000 series module equipped with a photodiode array detector with wavelength set at 273 nm. Catechin, epicatechin, and EGCG were separated from the extract using a Merck Purospher STAR C-18 (50 × 4.6 mm i.d., 5 μm). The flow rate of the mobile

phase was 0.8 ml/min. All samples were analyzed at room temperature (25 °C).

2.7. Statistical analysis

The data were analyzed using SPSS software (version 16, Chicago, IL.). All evaluation data are presented as means with their standard errors. Independent Student's t-tests were employed for between-group analysis to examine the differences in anthropometric and biochemical characteristics, and obesity-related hormone peptides. Within-group analysis was analyzed by paired t-tests. All p-values were two-tailed and the α level of significance was set at 0.05.

3. Results

Experienced doctors at our outpatient clinic screened 115 women, and 104 subjects who met the inclusion criteria were invited to participate. Of these, 10 subjects refused to participate, while two subjects were excluded due to failure to attend study visits. In the end, 92 subjects were enrolled and were randomly allocated into either the decaffeinated EGCG group (group A) or the placebo control group (group B) (Fig. 1). Subjects were asked to return all packaging and unused capsules to monitor compliance. Each subject was supposed to take 252 capsules throughout the treatment, and if the subjects returned more than 14 capsules, they were considered non-adherent to the protocol. Five subjects in group A and seven subjects in group B discontinued the study due to personal reasons. There were two subjects in group A and one subject in group B who took less than 95% of the capsules and were thus deemed non-adherent to the protocol requirements. In the end, 77 subjects completed the study without any adverse events or side effects.

Baseline demographics and measurements including age, weight, BMI, waist circumference, and hip circumference showed no significant difference between the two groups (Table 2). There was also no significant difference detected between the two groups in terms of the biochemical factors analyzed including lipid profile, fasting sugar, and obesity-related hormones.

According to the within-group analyses, the body weight of group A decreased from 76.8 ± 11.3 kg to 75.7 ± 11.5 kg ($p = 0.025$) after 12 weeks of the EGCG treatment. BMI and waist circumference were reduced from 31.0 ± 3.8 kg/m^2 to 30.6 ± 3.9 kg/m^2 ($p = 0.018$) and 95.1 ± 9.6 cm to 92.8 ± 9.8 cm ($p = 0.023$), respectively (Table 3). In group B, only waist circumference and hip circumference reached significant reduction: from 95.7 ± 10.8 cm to 91.5 ± 8.2 cm ($p = 0.017$) and 107.2 ± 8.7 cm to 103.7 ± 6.9 cm ($p = 0.003$), respectively. No such difference was observed in weight or BMI.

From the biochemical data, group A exhibited significantly decreased cholesterol levels, from 198.8 ± 35.9 mg/dL to 183.9 ± 34.2 mg/dL ($p = 0.005$). A significant reduction in LDL levels was also detected, from 124.7 ± 30.5 mg/dL to 112.1 ± 27.0 mg/dL ($p = 0.006$). Group B, on the other hand, exhibited an elevation in LDL levels after the 12-week treatment. No difference was observed in fasting blood sugar, triglyceride, and HDL levels in group A after EGCG treatment, whereas HDL levels were significantly reduced in group B, from 50.8 ± 12.2 mg/dL to 46.7 ± 10.2 mg/dL. Taken together, these results suggest EGCG might have a beneficial effect on lipid profiles in obese subjects.

Liver function tests showed a significant increase in alanine aminotransferase from 27.2 ± 14.9 IU/mL to 33.6 ± 22.2 IU/mL in group A, but remained within normal range. No elevation of alanine transaminase was observed in group B. The hormone peptide analysis showed significant increases in adiponectin levels for both groups after 12 weeks: from 20.9 ± 11.0 ng/mL to 24.0 ± 10.7 ng/mL in group A and 15.6 ± 10.6 ng/mL to 19.8 ± 6.4 ng/mL in group B. However, no differences were found among the other hormone peptides of leptin, ghrelin, and insulin.

According to the post-treatment between-group analysis, no significant differences were detected in body weight, BMI, or waist circumference between group A and group B (Table 3).

Table 2
Demographic data of participants.

	Decaffeinated EGCG (n = 39)	Placebo(cellulose) (n = 38)	P-value
Age (years)	44.1(10.9)	44.9(11.9)	0.780
Height (cm)	157.1(5.0)	158.9(5.0)	0.119
Body weight (kg)	76.6(11.3)	75.8(10.6)	0.720
Body Mass Index (kg/m2)	31.0(3.8)	30.0(3.5)	0.217
Waist circumference(cm)	95.1(9.6)	95.7(10.8)	0.801
Hip circumference(cm)	106.6(7.6)	107.2(8.7)	0.736
Waist/Hip ratio	0.9(0.1)	0.9(0.1)	0.973
Systolic blood pressure, mmHg	139.0(15.4)	135.8(27.6)	0.223
Diastolic blood pressure, mmHg	83.2(13.7)	79.6(10.4)	0.335
Heart rate, bpm	77.2(10.7)	79.6(10.4)	0.532
Laboratory data			
Alanine transaminase (IU/L)	27.2(14.9)	27.6(11.9)	0.916
Creatinine, mg/dL	0.7(0.1)	0.6(0.1)	0.136
Triglyceride, mg/dL	129.9(49.4)	130.9(60.6)	0.938
Total cholesterol, mg/dL	198.8(35.9)	191.0(40.7)	0.381
Low density lipoprotein, mg/dL	124.7(30.5)	116.9(34.6)	0.305
High density of lipoprotein, mg/dL	49.2(9.6)	50.8(12.2)	0.517
LDL/HDL ratio	2.62(0.77)	2.43(0.86)	0.284
Fasting blood sugar, mg/dL	98.7(16.6)	105.8(27.7)	0.185
Glycemic hemoglobin, HbA1c, %	5.8(0.42)	6.2(1.1)	0.056
Hormone peptides			
Insulin, IU/L	19.6(12.2)	16.5(11.4)	0.306
HOMA-IR index	4.8(3.9)	4.4(3.9)	0.674
Leptin (ng/ml)	22.8(9.7)	21.7(9.1)	0.632
Ghrelin (pg/ml)	621.5(302.5)	599.0(250.0)	0.748
Adiponectin (mg/ml)	20.9(11.0)	15.6(10.6)	0.057

*$p < 0.05$; **$p < 0.001$.
Data expressed as a mean with standard deviation in parenthesis.

Table 3
Within-group anthropometric, blood pressure, and laboratory data at baseline and after 12 weeks.

Variable	Decaffeinated EGCG (n = 39)			Placebo (cellulose) (n = 38)			
	Baseline	After 12 weeks	p-value	Baseline	After 12 weeks	p-value	Post-treatment p-value
Anthropometric							
Weight, kg	76.8(11.3)	75.7(11.5)	0.025*	75.8(10.6)	73.8(9.4)	0.842	0.463
Body mass index, kg/m^2	31.0(3.8)	30.6(3.9)	0.018*	30.0(3.5)	29.1(3.6)	0.906	0.149
Waist circumference, cm	95.1(9.6)	92.8(9.8)	0.023*	95.7(10.8)	91.5(8.2)	0.017*	0.550
Hip circumference, cm	106.6(7.6)	106.1(8.7)	0.630	107.2(8.7)	103.7(6.9)	0.003*	0.209
Waist hip ratio	0.9(0.1)	0.9(0.1)	0.072	0.9(0.1)	0.9(0.1)	0.432	0.719
Systolic blood pressure, mmHg	139.0(15.4)	137.0(16.8)	0.795	135.8(27.6)	136.2(18.5)	0.689	0.864
Diastolic blood pressure, mmHg	83.2(13.7)	82.2(13.3)	0.929	82.4(11.7)	80.6(11.1)	0.495	0.610
Heart rate, bpm	77.2(10.7)	78.5(10.8)	0.518	79.6(10.4)	79.5(10.3)	0.984	0.696
Laboratory data							
Alanine transaminase (IU/L)	27.2(14.9)	33.6(22.2)	0.041*	27.6(11.9)	28.8(19.1)	0.295	0.362
Creatinine, mg/dL	0.7(0.1)	0.6(0.1)	0.304	0.6(0.1)	0.6(0.1)	0.019*	0.036*
Triglyceride, mg/dL	129.9(49.4)	132.0(46.4)	0.778	130.9(60.6)	157.6(109.6)	0.082	0.250
Total cholesterol, mg/dL	198.8(35.9)	183.9(34.2)	0.005*	191.0(40.7)	194.4(39.7)	0.828	0.272
Low density lipoprotein, mg/dL	124.7(30.5)	112.1(27.0)	0.006*	116.9(34.6)	119.2(32.7)	0.950	0.354
High density of lipoprotein, mg/dL	49.2(9.6)	47.0(9.6)	0.208	50.8(12.2)	46.7(10.2)	0.003*	0.903
LDL/HDL ratio	2.62(0.77)	2.46(0.71)	0.127	2.43(0.86)	2.63(0.82)	0.175	0.374
Fasting blood sugar, mg/dL	98.7(16.6)	101.7(21.3)	0.591	105.8(27.7)	107.3(30.2)	0.378	0.405
Glycemic hemoglobin, HbA1c, %	5.8(0.42)	5.8(0.5)	0.930	6.2(1.1)	6.3(1.0)	0.752	0.043*
Hormone peptides							
Insulin, IU/L	19.6(12.2)	17.4(9.8)	0.437	16.5(11.4)	14.2(6.8)	0.275	0.120
HOMA-IR index	4.8(3.9)	4.5(2.6)	0.436	4.4(3.9)	3.9(2.5)	0.254	0.434
Leptin (ng/ml)	22.8(9.7)	20.2(6.6)	0.523	21.7(9.1)	17.2(7.3)	0.348	0.101
Ghrelin (pg/ml)	621.5(302.5)	529.7(247.1)	0.220	599.0(250.0)	664.2(249.0)	0.704	0.032*
Adiponectin (mg/ml)	20.9(11.0)	24.0.0(10.7)	0.009*	15.6(10.6)	19.8(6.4)	0.015*	0.058

*p < 0.05; **p < 0.001.
Data expressed as a mean with standard deviation in parenthesis.

Unexpectedly, neither lipid profiles nor fasting blood sugar levels differed significantly between the post-treatment groups. However, glycohemoglobin levels from group A were obviously lower in comparison to group B after treatment but no difference was shown in HOMA-index or insulin. Significantly lower ghrelin levels and higher adiponectin levels were detected in group A compared to group B post-treatment.

Percentage reductions between groups in anthropometric measurements, blood pressure, and laboratory data were also analyzed (Table 4). The percentage reduction in total cholesterol levels reached 5.33% with a mean of 11.39 ± 21.45 mg/dL in group A. In comparison, group B demonstrated a mean 1.70% elevation in total cholesterol levels after treatment. No other differences in percentage reductions were found from anthropometric data, biochemical data, or obesity-related hormones.

3.1. Side effects

No subjects withdrew from the study because of discomfort or adverse events associated with the treatment. Three subjects had mild abdominal discomfort after GTE treatment, and one subject had abdominal discomfort after cellulose treatment. All the

Table 4
The reduction percentage between groups in anthropometric, blood pressure, and laboratory data at baseline and after 12 weeks.

Variable	Reduction %		
	Decaffeinated EGCG (n = 39)	Placebo (cellulose) (n = 38)	p-value
Anthropometric data			
Weight, kg	−0.85(2.13)	−0.05(8.15)	0.597
Body mass index, kg/m^2	−0.89(2.15)	−0.1(8.15)	0.579
Waist circumference, cm	−2.15(5.64)	−2.7(6.51)	0.713
Hip circumference, cm	−0.35(5.09)	−2.01(3.35)	0.118
Waist hip ratio	−1.75(6.06)	−0.68(6.23)	0.479
Systolic blood pressure, mmHg	−0.07(11.05)	−1.01(10.8)	0.740
Diastolic blood pressure, mmHg	0.35(11.58)	−0.80(10.19)	0.680
Heart rate, bpm	1.39(9.04)	1.64(11.12)	0.923
Biochemical data			
Triglyceride, mg/dL	2.37(29.93)	17.87(55.31)	0.186
Total cholesterol, mg/dL	−5.33(9.84)	1.79(14.65)	0.031*
Low density lipoprotein, mg/dL	−6.84(13.40)	2.23(21.65)	0.049*
High density of lipoprotein, mg/dL	−2.07(11.79)	−6.75(21.65)	0.136
Fasting blood sugar, mg/dL	2.45(16.64)	−1.45(9.38)	0.253
Glycemic hemoglobin, HbA1c, %	0.06(3.40)	−0.006(4.67)	0.948
Hormone peptides			
Leptin (ng/ml)	8.14(30.0)	−7.01(40.88)	0.127
Ghrelin (pg/ml)	5.61(85.16)	19.77(61.63)	0.480
Adiponectin (mg/ml)	2.82(34.12)	3.95(65.68)	0.673
Insulin, IU/L	0.73(41.2)	2.86(39.99)	0.847
HOMA-IR index	0.33(44.66)	4.92(43.10)	0.711

*p < 0.05; **p < 0.001.

symptoms were noted in the first week of treatment and resolved naturally without any other medication or treatment. There were no major adverse events noted during this trial.

4. Discussion

In this study, we demonstrated from within-group analyses that green tea extract with a high-dose of EGCG (daily dose of 856.8 mg) was able to not only decrease body weight and BMI in obese women after a 12-week treatment, but also led to significant reduction in waist circumference. This is in contrast to the lack of obvious body weight changes observed in our previous study, in which the treatment group was taking a lower dose of EGCG (360 mg daily) than in this study, although a lipid-lowering effect was observed [15]. When the concentration of EGCG was elevated to the daily dose of 856.8 mg in this study, it showed a more favorable effect on anthropometric measurements. This implies that the effect of EGCG on weight reduction might be dose-dependent.

The antiobestic effect of green tea has been discussed extensively over the past 10 years [17]. The effect of EGCG on body weight change in this study agrees with the result of a recent meta-analysis of 11 trials, which showed an average of 1.51 kg body weight loss in Asian participants [18]. The mechanisms of green tea extract in influencing body weight and body composition have been attributed to an increase in thermogenesis and fat oxidation [19,20]. This is accomplished by the caffeine component of green tea through enhancing sympathetic nervous system activity and regulating appetite [21], thus decreasing nutrient absorption and enzymes involved in hepatic lipid metabolism. However, some clinical studies could not demonstrate this antiobestic effect [10,12], perhaps because of confounding factors of ethnicity, habitual caffeine consumption, or catechol O-methyltransferase (COMT) concentration [10]. It is worth noting that the caffeine component of the GTE in this study was relatively low (daily dose of less than 1.05 mg) compared to other green tea extracts. No obvious side effects or discomfort from sympathetic system hyperactivity were noted in our study. Thus, the weight reduction effect noted in this study seems entirely attributable to the effects of EGCG, which has been proven to increase fat oxidation through the inhibition of COMT, an enzyme involved in the degradation of norepinephrine [22,23], and through the regulation of lipid-metabolism-related genes and transcription factor expression [24,25]. One *in vivo* study demonstrated EGCG dose-dependent inhibition of lipid accumulation in maturing preadipocytes [26]. More study is warranted to discover whether dose-dependent effects exist for EGCG in increasing fat oxidation and lipolysis or suppressing fatty acid synthesis.

In addition, it is interesting to note that even the placebo group showed significant body composition change with decreased waist and hip circumference, albeit without body weight change. In a study conducted by Du et al., the authors showed that fruit and vegetables containing high levels of cellulose more than 4 g/day could lead to waist circumference change without weight reduction, and concluded that higher intake of fiber has favorable effects on preventing abdominal obesity, probably through reduced total fat intake [27]. With our daily dosage of cellulose 1.5 g in the meal of subjects, the total cellulose amount per day could easily exceed 4 g. Thus, a possible reason for the lack of distinct differences in our between-group anthropometric measurement analysis could be that cellulose might also have beneficial effects on weight regulation with increased dosages and longer duration.

According to the lipid profiles, total cholesterol as well as low-density cholesterol levels obviously decreased after 12 weeks of EGCG treatment. In our previous study, taking 302 mg of EGCG per day for 12 weeks significantly reduced serum LDL-cholesterol and triglyceride levels with a marked increase in HDL-cholesterol levels [15]. However, we did not observe significant changes in triglyceride and HDL blood levels in this study. Our results corroborate those of Basu et al., who noted in their study that subjects with metabolic syndrome had a significant decreasing trend in blood LDL-cholesterol levels and LDL/HDL ratio after taking green tea extract for eight weeks [14]. The mechanism of the lipid-lowering effect of EGCG in plasma might be attributable to EGCG interrupting lipid absorption through competition with enzymes involved in lipid digestion and absorption [28,29]. Moreover, a dose-dependent effect of ECGC consumption was demonstrated in another *in vitro* study [30]. A meta-analysis conducted by Kim et al. also revealed that green tea catechins could reduce total cholesterol and LDL plasma levels without influencing HDL cholesterol or triglyceride levels [31]. Although Christine et al. demonstrated that GTE containing 25% EGCG can inhibit lipolysis of triglycerides and decrease triglyceride absorption in gastric and duodenal medium *in vitro*, Han et al. failed to observe any inhibitory effect of tea catechins on pancreatic lipase activity. In this study, the inconsistent results of EGCG on triglyceride levels might be related to the lower baseline triglyceride levels noted in this study group compared to our previous study groups. On the other hand, our subjects were asked to keep their former diet without any restriction, so perhaps the fasting and diet components of their lifestyle had a greater impact on plasma triglyceride levels than EGCG did, potentially leading to confounding effects. A larger-scale clinical trial or *in vitro* study is needed to determine the optimum dose of GTE to achieve healthy modulation of serum lipids. Surprisingly, significantly decreased HDL levels along with higher glycohemoglobin levels were also observed in the cellulose group compared with the GTE group. That being said, the cellulose group already showed a higher glycohemoglobin concentration compared to the GTE group before treatment, although this difference did not reach statistical significance. Therefore, cellulose treatment might not be able to reverse the trend of exacerbated insulin resistance without diet restriction or other treatment. According to a previous study, patients with insulin resistance exhibit increased HDL catabolism, which might explain the abnormal trend in HDL levels we observed in the cellulose group [32]. The influence of cellulose on sugar metabolism warrants further exploration.

GTE containing a high dose of EGCG was also observed to cause a significant increase in adiponectin levels after EGCG treatment, mirroring the results of our previous study. We also noted elevated adiponectin levels after EGCG treatment compared with the placebo group in the between-group analysis. Adiponectin, a protein that confers benefits via its anti-atherogenic and anti-diabetic properties, has been recognized as a key regulator of insulin sensitivity and tissue inflammation [33]. Several animal studies have reported that EGCG can increase adiponectin levels in a dose-dependent manner by up-regulating adiponectin expression in mouse preadipocyte cells [34–39]. However, the effect has been inconsistently replicated in clinical studies. In a study conducted by Basu et al., daily 400-mg EGCG supplementation for 8 weeks in obese subjects minimally affected adiponectin levels, which might be attributable to the lower dosage of EGCG given and ethnicity differences in comparison to this study, as well as its lack of a double-blind design [40].

Contrary to our previous results [15], we detected significantly lower ghrelin post-treatment in the EGCG group compared to the placebo group, but without significant changes in leptin levels. It has been reported that ghrelin levels decrease in humans with obesity and metabolic syndrome and increase during weight loss, suggesting it plays a role in energy adaptation [41] and

potentially facilitates weight regain over long-term follow-up [42]. However, other studies have revealed that biliopancreatic diversion with duodenal switch surgery leads to markedly suppressed ghrelin levels with significant weight reduction and long-lasting weight-reduction effects [43]. In our study, we observed significant weight reduction with decreased ghrelin levels after EGCG treatment compared with the placebo group, implying that a high dose of EGCG might not only increase energy metabolism and interrupt lipid accumulation but also directly inhibit the secretion of ghrelin. There has been no study to date, *in vivo* or *in vitro*, to explain this phenomenon or mechanism. Thus, a detailed mechanism and clinical implications should be explored in future studies.

There are some limitations to our study. First, we did not follow up regularly with these subjects during the study period to observe weight changes and trends of biochemical data and obesity-related hormones. Second, the bioavailability of high-dose EGCG was not investigated in our study. According to Chow's study [44], system availability of free EGCG increases more than 60% after a high daily dose of EGCG (800 mg of EGCG, once daily). In contrast, while the daily EGCG intake was similar in the present study (864 mg), it was spread over three doses per day, and bioavailability in this case of large doses after meals still needs to be determined. The optimum EGCG dosage and frequency for obesity control merits more in-depth investigation in further studies. Third, the duration of our treatment was not long enough to observe if weight reduction can be induced steadily without any liver function injury. Liver enzymes were obviously elevated after GTE treatment, although they remained within the normal range. Schmidt has reported that EGCG from GTE can induce acute cytotoxicity in liver cells that help command metabolism in the human body [45]. Some other case reports also uncovered the liver toxicity of green tea in the form of self-limited acute hepatitis and resolved after discontinuation of supplements containing green tea extract [46–48]. However, the mechanism of the liver toxicity is still unclear. Further studies are needed to monitor the optimum dosage that can be administered for obesity control without patients experiencing adverse effects. Careful monitoring the liver enzymes during high-dosage of green tea extract may be warranted. Finally, the placebo group had higher HbA1c and lower adiponectin levels at baseline, although the difference did not reach statistical significance. A larger-scale trial should be conducted to reduce sampling bias.

In conclusion, this study demonstrated significant weight loss and consistent decreases in total cholesterol and LDL plasma levels after 12 weeks of high-dose EGCG treatment in within-group analyses with good treatment tolerance among subjects without any side effects or adverse effects. The antiobestic mechanism might be partly associated with inhibition of the secretion of ghrelin, leading to an increase in adiponectin levels. This should be verified with a large-scale study with a longer follow-up in the future. The bioavailability and pharmacokinetics of high-dose EGCG in the human body also merits more exploration.

Conflict of interest

There is no conflict of interest of this manuscript.

Acknowledgment

This study was supported by a grant 10101-62-044 from Taipei City Hospital, Taiwan, ROC (NOS.).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2015.05.003.

References

[1] Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. Nat Rev Endocrinol 2013;9:13–27.
[2] Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet 2011;377:557–67.
[3] Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. Ann N Y Acad Sci 2012;1271:37–43.
[4] Apovian CM, Gokce N. Obesity and cardiovascular disease. Circulation 2012;125:1178–82.
[5] Nikolopoulou A, Kadoglou NP. Obesity and metabolic syndrome as related to cardiovascular disease. Expert Rev Cardiovasc Ther 2012;10:933–9.
[6] Lepor NE, Fouchia DD, McCullough PA. New vistas for the treatment of obesity: turning the tide against the leading cause of morbidity and cardiovascular mortality in the developed world. Rev Cardiovasc Med 2013;14:20–39. quiz 40.
[7] Gray LJ, Cooper N, Dunkley A, Warren FC, Ara R, Abrams K, et al. A systematic review and mixed treatment comparison of pharmacological interventions for the treatment of obesity. Obes Rev Off J Int Assoc Study Obes 2012;13:483–98.
[8] Perrio MJ, Wilton LV, Shakir SA. The safety profiles of orlistat and sibutramine: results of prescription-event monitoring studies in England. Obesity 2007;15:2712–22.
[9] Ioannides-Demos LL, Proietto J, McNeil JJ. Pharmacotherapy for obesity. Drugs 2005;65:1391–418.
[10] Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: a literature review. Chin Med 2010;5:13.
[11] Wolfram S. Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr 2007;26:373S–88S.
[12] Kim HM, Kim J. The effects of green tea on obesity and type 2 diabetes. Diabetes Metab J 2013;37:173–5.
[13] Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res 2006;50:176–87.
[14] Basu A, Sanchez K, Leyva MJ, Wu M, Betts NM, Aston CE, et al. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr 2010;29:31–40.
[15] Hsu CH, Tsai TH, Kao YH, Hwang KC, Tseng TY, Chou P. Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial. Clin Nutr 2008;27:363–70.
[16] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[17] Rains TM, Agarwal S, Maki KC. Antiobesity effects of green tea catechins: a mechanistic review. J Nutr Biochem 2011;22:1–7.
[18] Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes 2009;33:956–61.
[19] Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, et al. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr 1999;70:1040–5.
[20] Boschmann M, Thielecke F. The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. J Am Coll Nutr 2007;26:389S–95S.
[21] Belza A, Frandsen E, Kondrup J. Body fat loss achieved by stimulation of thermogenesis by a combination of bioactive food ingredients: a placebo-controlled, double-blind 8-week intervention in obese subjects. Int J Obes 2007;31:121–30.
[22] Klaus S, Pultz S, Thone-Reineke C, Wolfram S. *Epigallocatechin gallate* attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes 2005;29:615–23.
[23] Borchardt RT, Huber JA. Catechol O-methyltransferase. 5. Structure-activity relationships for inhibition by flavonoids. J Med Chem 1975;18:120–2.
[24] Yan J, Zhao Y, Zhao B. Green tea catechins prevent obesity through modulation of peroxisome proliferator-activated receptors. Sci China Life Sci 2013;56:804–10.
[25] Huang J, Zhang Y, Zhou Y, Zhang Z, Xie Z, Zhang J, et al. Green tea polyphenols alleviate obesity in broiler chickens through the regulation of lipid-metabolism-related genes and transcription factor expression. J Agric Food Chem 2013;61:8565–72.
[26] Lin JK, Lin-Shiau SY. Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. Mol Nutr Food Res 2006;50:211–7.
[27] Du H, Boshuizen HC, Forouhi NG, Wareham NJ, Halkjær J, Tjønneland A, et al. Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. Am J Clin Nutr 2010;91:329–36.

[28] Wu X, He W, Yao L, Zhang H, Liu Z, Wang W, et al. Characterization of binding interactions of (-)-epigallocatechin-3-gallate from green tea and lipase. J Agric Food Chem 2013;61:8829–35.
[29] Walkowiak J, Bajerska J, Kargulewicz A, Lisowska A, Siedlerski G, Szczapa T, et al. Single dose of green tea extract decreases lipid digestion and absorption from a test meal in humans. Acta Biochim Pol 2013;60:481–3.
[30] Raederstorff DG, Schlachter MF, Elste V, Weber P. Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem 2003;14:326–32.
[31] Kim A, Chiu A, Barone MK, Avino D, Wang F, Coleman CI, et al. Green tea catechins decrease total and low-density lipoprotein cholesterol: a systematic review and meta-analysis. J Am Dietetic Assoc 2011;111:1720–9.
[32] Rashid S, Patterson BW, Lewis GF. Thematic review series: patient-oriented research. What have we learned about HDL metabolism from kinetics studies in humans? J Lipid Res 2006;47:1631–42.
[33] Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab 2007;9:282–9.
[34] Shimada M, Mochizuki K, Sakurai N, Goda T. Dietary supplementation with *Epigallocatechin gallate* elevates levels of circulating adiponectin in non-obese type-2 diabetic Goto-Kakizaki rats. Biosci Biotechnol Biochem 2007;71:2079–82.
[35] Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin–a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2006;8:264–80.
[36] Sung HY, Hong CG, Suh YS, Cho HC, Park JH, Bae JH, et al. Role of (-)-epigallocatechin-3-gallate in cell viability, lipogenesis, and retinol-binding protein 4 expression in adipocytes. Naunyn Schmiedeberg's Arch Pharmacol 2010;382:303–10.
[37] Lee H, Bae S, Yoon Y. The anti-adipogenic effects of (-)*Epigallocatechin gallate* are dependent on the WNT/beta-catenin pathway. J Nutr Biochem 2013;24:1232–40.
[38] Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, et al. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 2007;292:E1378–87.
[39] Cho SY, Park PJ, Shin HJ, Kim YK, Shin DW, Shin ES, et al. (-)-Catechin suppresses expression of Kruppel-like factor 7 and increases expression and secretion of adiponectin protein in 3T3-L1 cells. Am J Physiol Endocrinol Metab 2007;292:E1166–72.
[40] Basu A, Du M, Sanchez K, Leyva MJ, Betts NM, Blevins S, et al. Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. Nutrition 2011;27:206–13.
[41] Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev Off J Int Assoc Study Obes 2007;8:21–34.
[42] Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med 2011;365:1597–604.
[43] Kotidis EV, Koliakos GG, Baltzopoulos VG, Ioannidis KN, Yovos JG, Papavramidis ST. Serum ghrelin, leptin and adiponectin levels before and after weight loss: comparison of three methods of treatment–a prospective study. Obes Surg 2006;16:1425–32.
[44] Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of *Epigallocatechin gallate* and polyphenon E in healthy individuals. Clin Cancer Res Off J Am Assoc Cancer Res 2003;9:3312–9.
[45] Schmidt M, Schmitz HJ, Baumgart A, Guedon D, Netsch MI, Kreuter MH, et al. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 2005;43:307–14.
[46] Gloro R, Hourmand-Ollivier I, Mosquet B, Mosquet L, Rousselot P, Salamé E, et al. Fulminant hepatitis during self-medication with hydroalcoholic extract of green tea. Eur J Gastroenterol Hepatol 2005;17:1135–7.
[47] Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C, et al. Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 2009;65:331–41.
[48] Molinari M, Watt KD, Kruszyna T, Nelson R, Walsh M, Huang WY, et al. Acute liver failure induced by green tea extracts: case report and review of the literature. Liver Transplant 2006;12:1892–5.