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Effect of *Zingiber officinale* Supplementation on Obesity Management with Respect to the Uncoupling Protein 1 -3826A>G and ß3-adrenergic Receptor Trp64Arg Polymorphism

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The present study aimed to investigate the effect of ginger (*Zingiber officinale)* supplementation on some obesity-associated parameters, with nutrigenetics approach. Accordingly, 80 eligible obese women (aged 18–45 years) were randomly assigned to receive either ginger (2-g ginger rhizomes powder as two 1-g tablets per day) or placebo supplements (corn starch with the same amount) for 12 weeks. Subjects were tested for changes in body weight, body mass index, waist and hip circumferences, body composition, appetite score, and dietary intake. Moreover, participants were genotyped for the -3826A>G and Trp64Arg polymorphisms of uncoupling protein 1 and ß3-adrenergic receptor genes, respectively. Over 12 weeks, ginger supplementation resulted in a slight but statistically significant decrease in all anthropometric measurements and total appetite score as compared with placebo group, which were more pronounced in subjects with the AA genotype for uncoupling protein 1 and Trp64Trp genotype for ß3-adrenergic receptor gene. However, there was no significant difference in changes of body composition and total energy and macronutrients intake between groups. In conclusion, our findings suggest that ginger consumption has potential in managing obesity, accompanying with an intervention–genotype interaction effect. However, further clinical trials need to explore ginger's efficacy as an anti-obesity agent in the form of powder, extract, or its active components. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: Zingiber officinale Roscoe; obesity; polymorphism; UCP1; ß3ADR.

INTRODUCTION

Ginger (*Zingiber officinale Roscoe*, family Zingiberaceae) is extensively used both as a spice and medicinal plant around the world (Ali *et al*., 2008). Traditionally, ginger has been used as a remedy to treat a wide variety of ailments including vomiting, indigestion, asthma, joint, and muscle pain (Li *et al*., 2012). Moreover, scientific studies have demonstrated its various pharmacological activities such as antiemetic (Palatty *et al*., 2013), antiinflammatory and antioxidant (Grzanna *et al*., 2005; Mashhadi *et al*., 2013), glucose and lipid lowering (Li *et al*., 2012; Mahluji *et al*., 2013), and anticancer effects (Pereira *et al*., 2011; Jeena *et al*., 2014). In addition, the anti-obesity effect of ginger and its compounds has been recently noticed (Goyal and Kadnur, 2006; Pulbutr *et al*., 2011; Malik and Sharmaa, 2011; Ahn and Oh, 2012; Mahmoud and Elnour, 2013; Li *et al*., 2014; Saravanan *et al*., 2014).

Obesity, the excessive accumulation of fat, has reached pandemic proportions within the past two decades and is a major contributor to the increasing prevalence of chronic diseases (Racette *et al*., 2003; Aggarwal, 2010). This has led to continuous attempts to search effective agents for weight management. Among them, the use of medicinal plants has been further examined mostly for the possibility of fewer adverse effects. It was hypothesized that the rhizomes of ginger (*Z. officinale*) and its extract could influence many key features of the obesity through different mechanisms. In this regard, evidences from the *in vitro* and experimental studies support the weight lowering and anti-obesity effects of ginger (Pulbutr *et al*., 2011; Malik and Sharmaa, 2011; Ahn and Oh, 2012; Mahmoud and Elnour, 2013; Saravanan *et al*., 2014), but scientific research in humans is very limited (Atashak *et al*., 2011; Mansour *et al*., 2012).

Obesity is a multifactorial disease that results from the complex interaction of various factors, including genetic, environmental, behavioral, and cultural factors. Concerning genetic factors, uncoupling protein 1 (UCP1) and ß3-adrenergic receptor (ß3ADR) genes have been considered as important candidate genes for obesity, because of their roles in the regulation of thermogenesis, metabolism, and adipose tissue lipolysis (Tsunekawa *et al*., 2011). The presence of the -3826A>G single-nucleotide polymorphism in the promoter region of the UCP1 gene has been associated with higher fat accumulation, reduced postprandial thermogenesis, and resistance to weight loss during a low-calorie diet and lipid/lipoprotein metabolism (Kotani *et al*., 2008; Jia *et al*., 2010). Likewise, the missense single-nucleotide mutation in the β3ADR gene (Trp64Arg polymorphism) was reported to be related with increasing weight gain, abdominal obesity, difficulty in weight loss, lower

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basal metabolic rate, and insulin resistance (Tsunekawa *et al*., 2011; Mirrakhimov *et al*., 2011; Rudkowska and Pérusse, 2012).

To the best of our knowledge, the effect of ginger consumption on obesity-related features has been investigated just in a few pilot studies. Moreover, its efficacy by considering individuals' genetic differences has not been ever studied. Accordingly, the present randomized double-blind, placebo-controlled study was carried out to investigate the effect of ginger supplementation on some obesity-related features and appetite suppression with nutrigenetics approach, considering the polymorphism of UCP1 and ß3ADR genes.

METHODS AND MATERIALS

Subjects. Through a general call schedule across the city of Tabriz, Iran, 80 eligible healthy obese women, aged 18–45 years and with body mass index (BMI) of 30–40 kg/m^2, were voluntarily recruited in our study. The exclusion criteria were as follows: clinically diagnosed diabetes mellitus, cardiovascular disease, gallstone, hypothyroidism or hyperthyroidism, deep depression, pregnancy, breastfeeding or menopause, being on a weight-lowering diet, subjects with high physical activity, smoking, taking medications that could influence weight change, taking nutritional supplements, and being hypersensitive to ginger.

Supplements preparation. Dried rhizomes of ginger (*Z. officinale Roscoe*, Chinese yellow ginger) were purchased from a local market in Tabriz. They were identified and authenticated by morphologic comparison with different standard texts by Nutrition Research Center, Tabriz University of Medical Science (Tabriz, Iran). The ginger rhizomes were finely ground and then prepared as tablets containing 1-g ginger powder in each (Pharmaceutics Laboratory, Faculty of Pharmacy, Tabriz University of Medical Science). Likewise, the placebo tablets consisted of corn starch and other excipients in order to match the weight of ginger tablet. The tablets were placed in identical bottles and were labeled with two codes by a third person not directly involved in our study. And a slight amount of ginger powder was added to the placebo tablets containers to give ginger odor.

Study design. The present study was a randomized, double-blind, placebo-controlled clinical trial. All eligible subjects signed a written consent form, at the beginning of the study. Subjects ($N = 80$) were randomly assigned to the ginger or placebo group ($N = 40$) using a random number table and underwent 12 weeks of intervention with ginger or placebo tablets. Subjects were instructed to take two tablets per day (30 min before meal). They were also instructed to maintain their dietary and exercise pattern throughout the study. Tablets were being given to the participants monthly.

The study was approved by the Ethics Committee of Tabriz University of Medical Science (reference number 92154), and the study was registered on the Iranian Registry of Clinical Trials (http://www.irct.ir) with the identification No. 201311172017N18.

Anthropometric and body composition measurements. Subject's height was measured without shoes, using a standard tape with 0.1 cm precision. The body weight was determined with light clothing without shoes on a calibrated Seca weight scale, accurate to 0.1 kg (Seca 762; Vogel & Halke, Hamburg, Germany). BMI was calculated using the following formula: weight (kg)/height (m^2). The waist circumference (WC) was measured in a standing position at the narrowest point between the lowest rib and the iliac crest, whereas the hip circumference (HC) was measured as the greatest gluteal circumference. Waist-to-hip ratio and waist-to-height ratio (WHtR) were also calculated. Body composition consists of total body fat percentages, total body fat mass, total body fat free mass, and total body water was assessed by an 8-electrode bioelectrical impedance analyzer, accurate to 0.1 kg (Tanita BC-418 MA; Tanita Co., Tokyo, Japan), using the standardized protocol of device. All measurements were taken every 4 weeks by the same examiner between 8 and 9 AM and after an overnight fasting (water permitted).

Appetite and dietary intake assessment. For dietary intake assessments, the subjects were instructed to precisely record their food intake for three consecutive days (2 days a week and one weekend) at baseline, weeks 6 (midpoint) and 12 (overall three times). Dietary data (energy values and macronutrient content) were analyzed using Nutritionist 4 software (First Databank; Hearst, San Bruno, CA, USA). Moreover, subjective feelings of appetite were assessed at baseline and monthly, using a five-item validated questionnaire based on the visual analog scales with 0–10 scores (Flint *et al*., 2000). Participants were asked with the following. Generally, (i) how is your appetite? (ii) How hungry do you feel? (iii) How full do you feel? (iv) How satiated do you feel? (v) How is your desire to eat the next food?

Genotyping. Genomic DNA was isolated from blood samples that had been kept in EDTA tubes at −70 °C, using the conventional phenol chloroform extraction method. The single-nucleotide polymorphisms of UCP1 -3826A>G (rs1800592) and β3ADR Trp64Arg (rs4994) were genotyped by polymerase chain reaction–restriction fragment length polymorphism method as previously described in detail (Kotani *et al*., 2008; Kwon *et al*., 2012). The polymerase chain reaction product of UCP1 gene was digested with a restriction enzyme, BclI (Thermo Scientific, Vilnius, Lithuania), and the β3ADR amplified product was digested with BstNI enzyme (Thermo Scientific), according to the enzymes instructions. Afterwards, the digestion products were separated on a 3% agarose gel and visualized using SYBR green I safe staining (Invitrogen Corp., Carlsbad, USA) under ultraviolet excitation. The UCP1 polymorphism genotyping resulted in 157-bp and 122-bp fragments for the AA homozygotes; 279-bp, 157-bp, and 122-bp products for the AG heterozygotes; and a single 279-bp product for the GG homozygotes (Fig. 1). Moreover, genetic

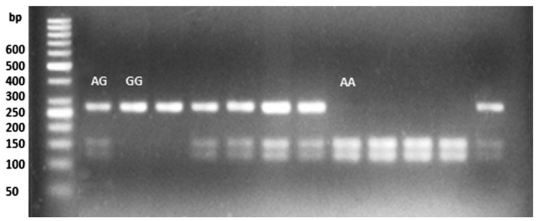

Figure 1. Polymerase chain reaction-based restriction fragment length polymorphism analysis of the uncoupling protein 1 gene (-3826A>G single-nucleotide polymorphism): 50-bp DNA ladder.

analysis of β3ADR polymorphism led to 97-bp, 61-bp, and 31-bp fragments as Trp64Trp carriers and 158-bp, 97-bp, 61-bp, and 31-bp fragments as Trp64Arg heterozygotes (Fig. 2). However, we did not find the Arg64Arg polymorphism in our study population.

Statistical analyses. Data were analyzed using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA). All variables were normally distributed as tested by the Kolmogorov–Smirnov test and considering the mean and SD. Possible differences at baseline among the treatment groups were assessed by an independent sample *t*-test. Differences between groups from baseline to weeks 4, 8, and 12 were analyzed using analysis of covariance with the baseline values and energy intake differences employed as covariates. Additionally, for analyzing body composition data, total body water difference (between two measurements) was used along with the baseline values and energy intake differences as covariates. Moreover, a repeated measures analysis of variance was performed to assess within-group differences in time trend. In addition, considering the effect of genetic variations of participants on the intervention outcomes, the analysis of covariance was further performed by subgroups analysis based on the polymorphism of each gene. The chi-square test was also used to compare polymorphisms' frequency between two groups. Results are reported as mean ± SD and mean difference (95% confidence interval), and $p < 0.05$ was considered as statistically significant.

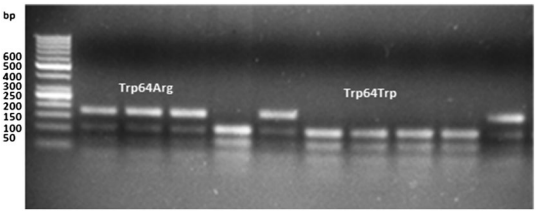

Figure 2. Polymerase chain reaction-based restriction fragment length polymorphism analysis of β3-adrenergic receptor gene (Trp64Arg single-nucleotide polymorphism): 50-bp DNA ladder.

RESULTS

Overall, one person from the ginger group and nine participants from the placebo group dropped out from the study for reasons such as pregnancy (four persons), using antidepressant agents (two persons), diagnosed hypothyroidism (one person), and others (three persons), as shown in Fig. 3. None of the subjects discontinued the study because of the adverse effects of ginger or placebo.

The baseline characteristics of the participants are shown in Table 1. There was no significant difference between groups in any of the parameters measured, at the beginning of the study. Table 2 represents the data of subjects in both groups during the study, irrespective of their genotypes. Ginger supplement given to obese women for 12 weeks slightly but significantly reduced the body weight ($p = 0.023$), BMI ($p = 0.019$), WC ($p = 0.011$), HC ($p = 0.003$), and WHtR ($p = 0.012$) as compared with the placebo, while there was no significant difference in the changes of body composition and total energy and macronutrients intake between groups ($p > 0.05$, Table 2). Ginger versus placebo consumption was also significantly decreased, the total appetite score during 8 weeks of intervention (0.028, Table 2). According to our results, there were no statistically significant differences between groups in changes of serum glucose and lipid profile, except for a significant decrease of

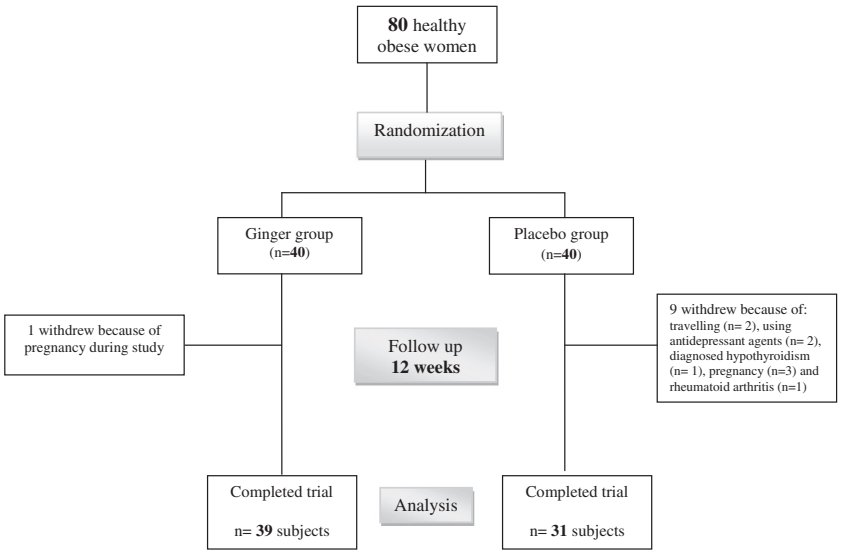

Figure 3. Study participant flow diagram.

Table 1. Baseline characteristics of the participants in both groups

Parameters	Ginger group (n = 39)*	Placebo group (n = 31)*	p-value**
Age (y)	35.25 ± 7.30	34.54 ± 7.91	0.699
Height (cm)	160.84 ± 5.41	158.87 ± 6.39	0.166
Body weight (kg)	88.75 ± 9.18	89.62 ± 11.02	0.721
BMI (kg/m^2)	34.34 ± 3.61	35.46 ± 3.41	0.192
Waist circumference (cm)	102.60 ± 7.41	104.50 ± 9.43	0.349
Hip circumference (cm)	119.70 ± 7.16	120.10 ± 8.33	0.828
Waist-to-hip ratio (cm)	0.85 ± 0.04	0.87 ± 0.05	0.306
Waist-to-height ratio (cm)	0.63 ± 0.05	0.65 ± 0.06	0.143
Body fat (%)	41.73 ± 3.01	42.04 ± 4.38	0.744
Body fat mass (kg)	36.63 ± 6.14	37.50 ± 7.80	0.605
Body fat free mass (kg)	50.58 ± 3.65	50.84 ± 4.22	0.781
Appetite total score (0–50)	29.30 ± 5.27	26.30 ± 9.34	0.117

BMI, body mass index.

Values are mean ± SD.

*Baseline data are shown just for the study subjects who completed the study.

**p-values are based on the independent sample t-test.

serum triglyceride (TG) in ginger group compared with the placebo (data not shown).

Moreover, the within-group analyses indicated that ginger consumption resulted in a statistically significant decrease of all anthropometric parameters, total appetite score, and total energy and macronutrients intake (Table 2) along with a significant decrease of serum glucose, TG, and total cholesterol (data not shown). Although in the placebo group, there was only a significant reduction in WC, waist-to-hip ratio, WHtR, appetite score, and total energy and macronutrients intake (Table 2) as well as serum glucose, TG, and cholesterol (data not shown) during the 12 weeks, albeit these decreases were more pronounced in the ginger group than those in the placebo group.

Furthermore, we investigated the influence of UCP1 and β3ADR genotypes on participants' response to the intervention. Comparing genotype allelic frequencies of study subjects showed that the distribution of β3ADR polymorphism was significantly different between groups ($p = 0.006$, Table 3). The intervention–genotype interactions are also shown in Table 4. Comparing two study groups with considering subjects genotype demonstrated that significant reductions of body weight, BMI, WC, HC, and WHtR by ginger supplement versus placebo were significantly more noticeable in subjects with the AA genotype for UCP1 and Trp64Trp genotype for β3ADR gene.

DISCUSSION

The present study was designed to determine whether the 12-week supplementation with *Z. officinale* would influence some parameters associated with the obesity. According to our results, body weight, BMI, WC, and HC were slightly reduced in ginger versus placebo group, whereas it was statistically significant. However, body fat mass and fat free mass did not change by intervention. The weight-lowering effect is supported by findings from animal studies. More recently, Saravanan *et al.* (2014) reported that oral feeding of high-fat-diet-induced obese rats with different doses of gingerol for 30 days significantly decreased the body weight and tissue lipids, dose dependently. Likewise, Li *et al.* (2014) found that ginger extract treatment (200 mg/kg) slightly decreased weight gain in the high-fat, high-carbohydrate-diet-fed rats over 10 weeks, which was statistically non-significant. Furthermore, significant lower weight gain and adiposity by ginger treatment in high-fat-diet-fed rats or mouse have been shown in other studies (Goyal and Kadnur, 2006; Malik and Sharmaa, 2011; Mahmoud and Elnour, 2013). In contrast, Wadikar and Premavalli (2008) showed that ginger juice significantly increased food consumption and weight gain in rats. However, it seems that the amount of weight loss in these experimental studies is more than our study. To the best of our knowledge, the effect of ginger on anthropometric parameters and body composition in obese subjects was assessed just in one study with low sample size (Atashak *et al.*, 2011). According to their results, ginger consumption alone (1 g/day) for 10 weeks did not cause any significant change in obese men.

Overall, *Z. officinale* could influence body weight and body composition through some default mechanisms, especially to the following: (i) the inhibition of intestinal absorption of dietary fat (Platel and Srinivasan, 2004; Mahmoud and Elnour, 2013), (ii) the suppression of adipocyte differentiation and lipid accumulation (Ahn and Oh, 2012), (iii) increasing lipolysis (Pulbutr *et al.*, 2011), (iv) increasing thermogenesis and energy expenditure (Mansour *et al.*, 2012), and (v) controlling appetite as will be discussed in detail in the succeeding texts.

The other finding of our study was a significant reduction of total appetite score in both groups, while there was no difference between groups in energy and macronutrients intake during the study. This is in agreement with that obtained by Mansour *et al.* (2012). They revealed that a single-dose hot ginger beverage (with 2-g ginger powder) intake before a standard breakfast meal would decrease appetite and increase satiety and fullness of overweight men, compared with the control group based on visual analog scales data. However, they have no objective measurement of food intake. In contrast, improved food consumption by ginger consumption has been also reported (Ueki *et al.*, 2008; Wadikar and Premavalli, 2008). It seems that there are

Table 2. Effect of the 12-week ginger or placebo supplementation on some parameters in obese women

Parameters	Study groups	Time of intervention: Week 4 Mean ± SD	Week 4 Mean difference (95% CI)	Week 4 *p* value*	Week 8 Mean ± SD	Week 8 Mean difference (95% CI)	Week 8 *p* value*	Week 12 Mean ± SD	Week 12 Mean difference (95% CI)	Week 12 *p* value*	*p*-value**
Body weight (kg)	Ginger	88.33 ± 8.74	−0.57 (−1.16, 0.01)	0.056	88.00 ± 8.77	−0.74 (−1.43, −0.06)	0.032	87.69 ± 8.90	−0.95 (−1.77, −0.13)	0.023	<0.001
	Placebo	89.76 ± 11.39			89.59 ± 11.19			89.50 ± 11.24			0.691
BMI (kg/m^2)	Ginger	34.18 ± 3.43	−0.23 (−0.46, −0.001)	0.049	34.05 ± 3.47	−0.30 (−0.57, −0.03)	0.029	33.92 ± 3.48	−0.39 (−0.71, −0.06)	0.019	<0.001
	Placebo	35.51 ± 3.51			35.43 ± 3.40			35.41 ± 3.49			0.717
Waist circumference	Ginger	101.56 ± 7.14	−0.74 (−1.4, −0.06)	0.032	100.76 ± 7.21	−1.00 (−1.82, −0.18)	0.017	99.89 ± 7.32	−1.25 (−2.20, −0.30)	0.011	<0.001
(cm)	Placebo	104.14 ± 9.37			103.59 ± 9.27			102.94 ± 9.14			<0.001
Hip circumference	Ginger	119.23 ± 7.00	−0.48 (−1.03, 0.05)	0.078	118.75 ± 7.16	−0.87 (−1.5, −0.180)	0.014	118.50 ± 7.08	−1.13 (−1.85, −0.40)	0.003	<0.001
(cm)	Placebo	120.12 ± 8.70			120.02 ± 8.42			120.04 ± 8.77			0.960
Waist-to-hip ratio	Ginger	0.85 ± 0.04	−0.003 (−0.008, 0.003)	0.390	0.84 ± 0.05	−0.001 (−0.008, 0.006)	0.793	0.844 ± 0.04	−0.003 (−0.01, 0.005)	0.511	<0.001
	Placebo	0.86 ± 0.05			0.86 ± 0.05			0.85 ± 0.05			<0.001
Waist-to-height ratio	Ginger	0.63 ± 0.04	−0.005 (−0.009, −0.001)	0.023	0.62 ± 0.04	−0.006 (−0.012, −0.001)	0.015	0.62 ± 0.05	−0.008 (−0.01, −0.002)	0.012	<0.001
	Placebo	0.65 ± 0.05			0.65 ± 0.05			0.64 ± 0.05			<0.001
Body fat (%)	Ginger	42.08 ± 2.97	−0.08 (−0.51, 0.35)	0.713	42.20 ± 3.00	−0.29 (−0.70, 0.12)	0.164	42.14 ± 2.96	−0.02 (−0.57, 0.51)	0.925	0.078
	Placebo	42.02 ± 4.34			42.55 ± 4.25			42.11 ± 4.20			0.211
Body fat mass (kg)	Ginger	36.94 ± 5.89	−0.13 (−0.70, 0.44)	0.643	37.07 ± 5.92	−0.37 (−0.93, 0.19)	0.191	37.09 ± 6.01	−0.44 (−1.23, 0.35)	0.272	0.094
	Placebo	37.59 ± 7.82			38.19 ± 7.78			38.11 ± 7.84			0.054
Body fat free mass	Ginger	50.47 ± 3.67	0.10 (−0.20, 0.40)	0.509	50.34 ± 3.54	0.14 (−0.14, 0.42)	0.329	50.34 ± 3.72	0.05 (−0.34, 0.44)	0.797	0.582
(kg)	Placebo	51.08 ± 4.58			50.73 ± 4.53			51.11 ± 4.52			0.376
Appetite total score	Ginger	24.29 ± 5.40	−2.37 (−4.13, −0.61)	0.009	23.29 ± 5.03	−2.41 (−4.56, −0.26)	0.028	22.75 ± 5.60	−2.19 (−4.53, 0.14)	0.066	<0.001
(0–50)	Placebo	23.98 ± 9.48			23.09 ± 10.17			22.38 ± 10.03			<0.001
Total dietary energy	Ginger	1687.84 ± 498.85	90.34 (−143.45, 324.13)	0.443	1667.22 ± 412.02	58.03 (−135.47, 251.54)	0.550	1480.65 ± 378.63	15.94 (−146.98, 178.87)	0.845	<0.001
intake (kcal)[a]	Placebo	1597.50 ± 453.49			1535.12 ± 524.45			1395.55 ± 374.98			0.004
Total carbohydrate	Ginger	270.88 ± 86.14	9.26 (−31.80, 50.34)	0.654	270.67 ± 72.68	12.86 (−21.23, 46.96)	0.453	236.24 ± 69.68	0.76 (−32.38, 33.90)	0.963	0.003
intake (g)[a]	Placebo	261.61 ± 81.72			248.86 ± 92.40			230.56 ± 77.20			0.016
Total protein	Ginger	60.27 ± 17.33	1.16 (−7.07, 9.39)	0.779	62.34 ± 15.61	3.26 (−4.35, 10.88)	0.394	57.43 ± 13.10	3.18 (−2.98, 9.34)	0.305	0.038
intake (g)[a]	Placebo	59.11 ± 16.30			59.12 ± 17.44			51.97 ± 13.04			0.005
Total fat intake (g)[a]	Ginger	44.25 ± 17.57	5.06 (−3.47, 13.61)	0.241	39.95 ± 14.80	2.00 (−5.23, 9.24)	0.582	35.76 ± 13.64	2.46 (−3.64, 8.57)	0.422	0.001
	Placebo	39.19 ± 17.46			35.63 ± 14.08			30.96 ± 9.67			0.010
Total dietary fiber	Ginger	14.56 ± 5.21	0.74 (−1.57, 3.06)	0.523	15.35 ± 5.10	0.31 (−2.32, 2.94)	0.813	14.12 ± 5.03	0.49 (−2.21, 3.20)	0.715	0.173
intake (g)[a]	Placebo	13.81 ± 4.09			14.53 ± 6.15			13.27 ± 5.64			0.100

CI, confidence interval; BMI, body mass index.

Ginger group: $n = 39$; placebo group: $n = 31$.

[a]Data from baseline, week 6 (midpoint) and week 12 intervention, respectively.

*p-values indicate the comparison between the groups by analysis of covariance with the baseline values and energy intake differences, employed as covariates for anthropometric parameters, and the baseline values, energy intake differences and total body water differences, as covariates for body composition data.

**p-values indicate within-group comparison by repeated measures analysis of variance.

Table 3. Genotype allelic frequencies of the study subjects regarding the single-nucleotide polymorphism of UCP1 and ß3ADR genes

	UCP1 -3826A>G polymorphism				β3ADR Trp64Arg polymorphism			
Study group	AA	AG	GG	*p*-value*	Trp64Trp	Trp64Arg	Arg64Arg	*p*-value*
Ginger group (n = 39)	21 (53.8%)	12 (30.8%)	6 (15.4%)	0.576	37 (94.9%)	2 (5.1%)	0 (0%)	0.006
Placebo group (n = 31)	13 (41.9%)	11 (35.5%)	7 (22.6%)		22 (71.0%)	9 (29.0%)	0 (0%)	
Total (n = 70)	34 (48.6%)	23 (32.9%)	13 (18.6%)	—	59 (84.3%)	11 (15.7%)	70 (100%)	—

UCP1, uncoupling protein 1; β3ADR, β3-adrenergic receptor.

*p-values indicate the comparison of polymorphism between the two study groups using the chi-square test.

Table 4. The intervention–genotype interactions as comparing the effect of ginger versus placebo at the end of intervention with respect to the UCP1 and β3ADR polymorphisms

	Ginger versus placebo group regarding the UCP1 -3826A>G polymorphism			Ginger versus placebo group regarding the β3ADR Trp64Arg polymorphism	
Parameters	AA	AG	GG	Trp64Trp	Trp64Arg
Body weight (kg)	−1.47* (−2.79, −0.15)	−0.14 (−1.38, 0.86)	−1.38 (−3.30, 0.53)	−1.13* (−2.02, −0.24)	1.57 (−1.27, 4.41)
BMI (kg/m^2)	−0.65* (−1.16, −0.13)	−0.02 (−0.60, 0.56)	−0.54 (−1.31, 0.21)	−0.50* (−0.85, −0.15)	0.46 (−1.01, 1.94)
Waist circumference (cm)	−1.80* (−3.41, −0.18)	−0.96 (−2.35, 0.43)	−0.58 (−2.77, 1.61)	−1.45* (−2.52, −0.39)	−1.10 (−5.19, 2.98)
Hip circumference (cm)	−1.89* (−3.04, −0.701)	−0.49 (−1.77, 0.82)	−0.54 (−1.95, 0.95)	−1.16* (−1.98, −0.35)	−0.20 (−3.18, 2.77)
Waist-to-hip ratio (cm)	−0.002 (−0.016, 0.013)	−0.006 (−0.01, 0.005)	−0.002 (−0.019, 0.015)	−0.004 (−0.01, 0.005)	0.001 (−0.035, 0.036)
Waist-to-height ratio (cm)	−0.01* (−0.02, −0.001)	−0.005 (−0.01, 0.003)	−0.004 (−0.018, 0.011)	−0.009* (−0.01, −0.002)	−0.007 (−0.03, 0.01)
Body fat (%)	−0.33 (−1.12, 0.45)	0.46 (−0.44, 1.37)	−1.12 (−2.94, 0.69)	0.11 (−0.50, 0.72)	−0.19 (−2.16, 1.76)
Body fat mass (kg)	−0.50 (−1.72, 0.71)	−0.39 (−1.95, 1.17)	−0.89 (−2.70, 0.91)	−0.51 (−1.40, 0.37)	−0.21 (−3.29, 2.87)
Body fat free mass (kg)	0.11 (−0.41, 0.63)	0.002 (−0.722, 0.727)	1.03 (−0.70, 2.78)	−0.05 (−0.54, 0.42)	0.06 (−0.06, 0.19)
Appetite total score (0–50)	−2.80 (−6.61, 1.01)	−0.36 (−4.75, 4.03)	−5.40 (−8.57, −2.23)	−2.46 (−5.02, 0.10)	1.01 (−10.54, 12.57)

UCP1, uncoupling protein 1; β3ADR, β3-adrenergic receptor; BMI, body mass index.

Ginger group: n = 39; placebo group: n = 31.

Values are the mean difference (95% confidence interval).

*$p < 0.05$, significant differences by comparing two study groups at the end of intervention using the analysis of covariance test (baseline value as covariate).

two conflicting hypotheses about ginger's mechanism of action on appetite. One widely discussed is that *Z. officinale* has a modulatory effect on 5-hydroxytryptamine (serotonin) and its receptors, which play a crucial role in controlling appetite (Goyal and Kadnur, 2006; Mansour *et al*., 2012; Palatty *et al*., 2013). According to the other hypothesis, ginger is a digestive stimulant that increases gastrointestinal tract secretions and peristalsis and decreases food transit time (Platel and Srinivasan, 2004) and maybe acts as an appetizer.

Another important finding in our study suggests the intervention–genotype interactions. In this regard, significant reductions of the body weight, BMI, WC, HC, and WHtR by ginger supplement versus placebo were significantly more noticeable in subjects with the AA genotype for UCP1 and Trp64Trp genotype for β3ADR gene.

Uncoupling protein 1 is a proton transporter in mitochondrial inner membrane that dissipates the proton electrochemical gradient from Adenosine triphosphate (ATP) synthesis and wastes energy through the heat, mainly in brown adipose tissue. Hence, UCP1 and its gene play important roles in energy homeostasis and body weight regulation (Jia *et al*., 2010). It has been reported that the presence of mutant G allele of the UCP1 gene (-3826A>G polymorphism) is associated with increased weight gain over time, reduced resting energy expenditure, and resistance to weight loss during a controlled-energy diet (Kotani *et al*., 2008; Tsunekawa *et al*., 2011).

The other important obesity candidate gene – the β3-adrenergic receptor (ADRB3) – which is abundant in visceral adipose tissue, is involved in the regulation of catecholamine-induced lipolysis and non-shivering

thermogenesis by increasing UCP1 expression and activity (Mattsson *et al*., 2011; Rudkowska and Pérusse, 2012).

The Trp64Arg polymorphism of this gene may promote the development of obesity through decreased lipolysis (Mirrakhimov *et al*., 2011). There are also some evidences suggesting that subjects carrying the mutant Arg allele of β3ADR gene may have a reduced capacity to lose weight and/or body fat in response to diet (Tsunekawa *et al*., 2011; Rudkowska and Pérusse, 2012). Accordingly, our results are in agreement with these earlier reports, indicating that carriers of the mutant allele for the UCP1 gene (G allele) and β3AR gene (Arg allele) had lower weight, BMI, WC, HC, and WHtR loss compared with the wild-type genotypes, during ginger versus placebo supplementation.

Taken together, this is the first clinical trial with almost a large population that addressed the question whether administration of only the ginger supplement has benefits on some obesity-associated parameters? And also, are there any subjective differences based on genetic factors, affecting the intervention outcome?

A potential limitation of this study is that we cannot generalize our results to men. The other limitation of this study was that the number of subjects in the genotype subgroups of the Trp64Arg polymorphism might not be sufficient to completely identify the effect of gene–intervention interaction on weight loss or other parameters, particularly that we did not find the Arg64Arg polymorphism in our study population. However, its frequency has often been reported to be low in other populations (Mirrakhimov *et al*., 2011; Tsunekawa *et al*., 2011). Moreover, because there was no other intervention such as weight-lowering diet across the study, it might be better to design an intervention with higher dose of ginger powder (i.e., 3 g) to reach more efficacy of intervention, just as a suggestion for future researches, because it is a safe herbal medicine according to existing data and also the FDA's report on its safety (Li *et al*., 2012).

In conclusion, our results demonstrated the beneficial effects of ginger supplementation on anthropometric measures and the appetite feeling in healthy obese women, with nutrigenetics approach, because the carriers of the mutant allele for the UCP1 gene (G allele) and β3ADR gene (Arg allele) had more weight, BMI, WC, HC, and WHtR loss compared with the wild-type genotypes.

Accordingly, *Z. officinale* can be considered as an adjunct to lifestyle changes for prevention or treatment of obesity. However, further clinical trials need to explore ginger's efficacy and utility as an anti-obesity agent in the form of powder, extract, or active components.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Aggarwal BB. 2010. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. *Annu Rev Nutr* **30**: 173–199.

Ahn EK, Oh JS. 2012. Inhibitory effect of galanolactone isolated from *Zingiber officinale Roscoe* extract on adipogenesis in 3 T3-L1 Cells. *J Korean Soc Appl Biol Chem* **55**: 63–68.

Ali BH, Blunden G, Tanira MO, Nemmar A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. *Food Chem Toxicol* **46**: 409–420.

Atashak S, Peeri M, Azarbayjani MA, Stannard SR, Mosalman Haghighi M. 2011. Obesity-related cardiovascular risk factors after long- term resistance training and ginger supplementation. *J Sports Sci Med* **10**: 685–691.

Flint A, Rabin A, Blundell JE, Strop A. 2000. Reproducibility, power and validity of visual analogue in assessment of appetite sensation in single test meal studies. *Int J Obes Relat Metab Disord* **24**: 38–48.

Goyal RK, Kadnur SV. 2006. Beneficial effects of *Zingiber officinale* on goldthioglucose induced obesity. *Fitoterapia* **77**: 160–163.

Grzanna R, Lindmark L, Frondoza CG. 2005. Ginger--an herbal medicinal product with broad anti-inflammatory actions. *J Med Food* **8**(2): 125–132.

Jeena K, Liju VB, Viswanathan R, Kuttan R. 2014. Antimutagenic potential and modulation of carcinogen-metabolizing enzymes by ginger essential oil. *Phytother Res* **28**(6): 849–855.

Jia JJ, Tian YB, Cao ZH, *et al.* 2010. The polymorphisms of UCP1 genes associated with fat metabolism, obesity and diabetes. *Mol Biol Rep* **37**(3): 1513–1522.

Kotani K, Sakane N, Saiga K, *et al*. 2008. Relationship between A-3826G polymorphism in the promoter of the uncoupling protein-1 gene and high-density lipoprotein cholesterol in Japanese individuals: a cross-sectional study. *Arch Med Res* **39**(1): 142–146.

Kwon DH, Bose S, Song MY, *et al*. 2012. Efficacy of Korean red ginseng by single nucleotide polymorphism in obese women: randomized, double-blind, placebo-controlled trial. *J Ginseng Res* **36**(2): 176–189.

Li Y, Tran VH, Duke CC, Roufogalis BD. 2012. Preventive and protective properties of *Zingiber officinale* (ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: a brief review. *Evid Based Complement Alternat Med* **2012**: 516870.

Li Y, Tran VH, Kota BP, Nammi S, Duke CC, Roufogalis BD. 2014. Preventative effect of *Zingiber officinale* on insulin resistance in a high-fat high-carbohydrate diet-fed rat model and its mechanism of action. *Basic Clin Pharmacol Toxicol* **115**(2): 209–215.

Mahluji S, Ebrahimzade Attari V, Mobasseri M, Payahoo L, Ostadrahimi A, Golzari S. 2013. Effects of ginger (*Zingiber officinale*) on plasma glucose level, HbA1c and insulin sensitivity in type 2 diabetic patients. *Int J Food Sci Nutr* **64**(6): 682–686.

Mahmoud RH, Elnour WA. 2013. Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. *Eur Rev Med Pharmacol Sci* **17**(1): 75–83.

Malik ZA, Sharmaa PL. 2011. Attenuation of high-fat diet induced body weight gain, adiposity and biochemical anomalies after chronic administration of ginger (*Zingiber officinale*) in Wistar rats. *Int J Pharmacol* **7**: 801–812.

Mansour MS, Ni YM, Roberts AL, Kelleman M, Roychoudhury A, St-Onge MP. 2012. Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: a pilot study. *Metabolism* **61**(10): 1347–1352.

Mashhadi NS, Ghiasvand R, Askari G, *et al*. 2013. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. *Int J Prev Med* **4**(S1): 36–42.

Mattsson CL, Csikasz RI, Chernogubova E, *et al*. 2011. β1-Adrenergic receptors increase UCP1 in human MADS brown adipocytes and rescue cold-acclimated β3-adrenergic receptor-knockout mice via nonshivering thermogenesis. *Am J Physiol Endocrinol Metab* **301**(6): 1108–1118.

Mirrakhimov AE, Kerimkulova AS, Lunegova OS, *et al*. 2011. An association between TRP64ARG polymorphism of the B3 adrenoreceptor gene and some metabolic disturbances. *Cardiovasc Diabetol* **10**: 89.

Palatty PL, Haniadka R, Valder B, Arora R, Baliga MS. 2013. Ginger in the prevention of nausea and vomiting: a review. *Crit Rev Food Sci Nutr* **53**(7): 659–669.

Pereira MM, Haniadka R, Chacko PP, Palatty PL, Baliga MS. 2011. *Zingiber officinale Roscoe* (ginger) as an adjuvant in cancer treatment: a review. *J BUON* **16**(3): 414–424.

Platel K, Srinivasan K. 2004. Digestive stimulant action of spices: a myth or reality? *Indian J Med Res* **119**(5): 167–179.

Pulbutr P, Thunchomnang K, Lawa K, Mangkhalathon A, Saenubol P. 2011. Lipolytic effects of zingerone in adipocytes isolated from normal diet fed rats and high fat diet fed rats. *Int J Pharmacol* **7**: 629–634.

Racette SB, Deusinger SS, Deusinger RH. 2003. Obesity: overview of prevalence, etiology, and treatment. *Phys Ther* **83**: 276–288.

Rudkowska I, Pérusse L. 2012. Individualized weight management: what can be learned from nutrigenomics and nutrigenetics? *Prog Mol Biol Transl Sci* **108**: 347–382.

Saravanan G, Ponmurugan P, Deepa MA, Senthilkumar B. 2014. Anti-obesity action of gingerol: effect on lipid profile, insulin, leptin, amylase and lipase in male obese rats induced by a high-fat diet. *J Sci Food Agric* **94**(14): 2972–2977.

Tsunekawa K, Yanagawa Y, Aoki T, *et al*. 2011. Association between accumulation of visceral fat and the combination of β3 adrenergic receptor Trp64Arg, β2 adrenergic receptor Arg16Gly and uncoupling protein 1 -3826A>G polymorphisms detected by Smart Amplification Process 2. *Endocr J* **58**(12): 1079–1086.

Ueki S, Miyoshi M, Shido O, Hasegawa J, Watanabe T. 2008. Systemic administration of [6]-gingerol, a pungent constituent of ginger, induces hypothermia in rats via an inhibitory effect on metabolic rate. *Eur J Pharmacol* **584**: 87–92.

Wadikar DD, Premavalli KS. 2011. Appetizer administration stimulates food consumption, weight gain and leptin levels in male Wistar rats. *Appetite* **57**(1): 131–133.

