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Research article

Free-fatty-acid-regulating effects of fermented red ginseng are mediated by hormones and by the autonomic nervous system

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ABSTRACT

Background: Understanding what causes changes in the flux of free fatty acids (FFA) is important to elucidate the etiology of metabolic syndrome. The first aim of this study was to test whether or not hormones and the autonomic nervous system influence blood FFA levels. A secondary aim was to test by means of a multiple group path analysis whether the consumption of fermented red ginseng (FRG; *Panax ginseng*) would influence those causal relationships.
Methods: Ninety-three postmenopausal women (age 50–73 yr) were randomly divided into two groups. One group (44 women; age, 58.4 ± 5.9 yr; body mass index, 23.6 ± 2.5 kg/m^2) was supplied placebo capsules and the other group (49 women, age 58.4 ± 5.5 yr; body mass index, 22.9 ± 2.4 kg/m^2) was supplied FRG capsules. Both prior to and after the study (2 wk), blood samples were collected from the participants and several blood variables were measured and analyzed.
Results: Squared multiple correlations of FFA were 0.699 in the placebo group and 0.707 in the FRG group. The unstandardized estimate of estradiol (E2) for FFA was 0.824 in both groups.
Conclusion: The path coefficients of cortisol and the branchial pulse for FFA were significantly different between the FRG group and the placebo group.

1. Introduction

Of the primary energy sources in the human body (carbohydrates, proteins, and lipids), lipids are the most efficient type of energy storage (9 kcal/g) and are hence much more prevalent than carbohydrates or proteins as a form of storage [1]. This makes the process of lipid release a crucial component in understanding human energy metabolism and pathology. Several studies have reported that the incidence of metabolic syndrome in postmenopausal women is higher than in premenopausal women and that the causes are related not only to estrogen levels but also to the levels of other hormones related to lipid metabolism [2].

The chronic override of free fatty acids (FFA) in the blood may be a risk factor in human energy metabolism. A high level of FFA often correlates with type 2 diabetes, hypertension, dyslipidemia, insulin resistance, hyper uric acid, and abnormal fibrinolysis [3]. Obese individuals commonly show insulin resistance; correspondingly, their levels of fatty acids are also elevated. The most common cause of the positive correlations between FFA and several diseases is the competition between override FFA and carbohydrates in the energy oxidation process [4]. Boden et al [5] reported that after lipids were administered to test volunteers, lipid oxidation increased and carbohydrate oxidation decreased simultaneously. Compared to healthy volunteers, diabetic patients showed a 40–55% decrease in their insulin-stimulated glucose absorption rates [6].

Energy metabolism differs between the postprandial and fasting states. In the postprandial state, carbohydrates are used as a major energy source and insulin is released. In the fasting state, adipocytes release triglycerides, which are broken down into FFA and glycerol, which then enter the circulatory system. During the overnight fasting period, the burst size of FFA during the daily cycle is maximized [7].

In a fasting state, over the long term, basal metabolic lipolysis occurs when insulin levels and catecholamine levels decrease.

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In the short term, acute lipolysis occurs in “fight or flight” (emergency) states. In this state, catecholamines are triggered by the sympathetic nerve system [8]. In cell membranes, those catecholamine signals stimulate β-adrenoreceptors, which activate adenylyl cyclase via simultaneous G-protein coupled receptors. Adenylyl cyclase then transforms adenosine triphosphate into cyclic adenosine monophosphate (cAMP). The cAMP then binds to the regulatory module of the protein kinase A, activating it, which then phosphates hormone-sensitive lipase (HSL) [9].

Both long- and short-term lipolyses are affected by several hormones. Glucocorticoid [10], adrenocorticotropic hormone (ACTH) [11], thyroid hormone, dehydroepiandrosterone [12], insulin [7], and estrogen [13] have all been shown to influence lipolysis through the functioning of β-adrenergic receptors, the production of adenylyl cyclase, the activities of G-proteins, or changes in cAMP production.

The lipolysis of white adipose tissue is influenced by the autonomic nervous system as well as the central nervous system. For example, when the sympathetic nerve directly stimulates the adrenal medulla, it causes catecholamine to be released. The catecholamine then stimulates adipocytes to trigger lipolysis. In addition to this catecholamine pathway, sympathetic nerves directly innervate within adipose tissue. Cousin et al [14] reported that the level of lipids in the peritoneum increased after denervation. This suggests that the sympathetic nervous system influences the activity or differentiation of white adipose cells.

Parasympathetic nerves as well as sympathetic nerves showed a relation with adipose tissue. Kreier et al [15] reported that when the parasympathetic nerve was removed, the HSL activity in white adipose tissue increased. However, the absorption of FFA and blood glucose decreased.

Given that the direct measurement of autonomic nervous activity by micrography is not feasible in a large epidemiological study, heart rate variability (HRV) is used as the measurement method for the autonomic nervous system [16]. HRV is measured by the variation of the beat-to-beat interval. The average R-R is calculated by 60 divided by the average heart rate in beats/min. Chang et al [17] showed that HRV is related to several component of metabolic syndrome (MtS). When they separated 1,298 individuals into four groups based on the components of MtS, those who had one or more components of MtS showed a lower standard deviation of the R-R interval compared to a healthy control group.

The recorded use of ginseng dates back 2,000 years. It has been one of the most popular herbal supplements in Asia, especially in Korea, China, and Japan. In the USA, ginseng ranked as one of the top-10 selling herbal supplements in 2003 [18]. The primary effective components of ginseng are known as ginsenosides, and these include compound K (CK), Rg3, Rk1, and Rg5, all of which have a steroidal skeleton.

In the results of this study, CK served as the ligand of glucocorticoid receptor (GR) [19] and Rg3 functioned as the ligand of estrogen receptor (ER) [20], which implies a possible effect of ginseng on lipolysis. In fact, when CK was administered to a 3T3-L1 adipocyte cell line, the storage of triglycerides was suppressed. On the other By contrast, Rg1 stimulated triglyceride storage in adipocytes [21]. Rg3, Rk1, and Rg5 treatments in 3T3-L1 suppressed lipid accumulation [22].

As well as the reported effects of ginseng on FFA, red ginseng has also been shown to have a beneficial effect on insulin and glucose regulation. Vuksan et al [23] reported that red ginseng consumption improved insulin and glucose regulation in type 2 diabetes patients. Lee et al [24] showed that red ginseng has a beneficial effect on insulin sensitivity. We also reported that fermented red ginseng (FRG) showed a serial causal effect on the level of hormones, insulin resistance, and insulin levels. In an analysis of the effects of hormones on glucose blood levels, the difference between the FRG group and the placebo group was due to the level of aldosterone [25].

According to an experiment with mice, ginsenosides stimulated an acetylcholine release in the terminal of cholinergic neurons [26]. In a human study with 120 adult men, wild ginseng increased the activity of the autonomic nerves and increased the heart rate [27].

The first aim of this study was to test the hypothesis that hormones (including insulin) and the branchial pulse rate (the autonomic nervous system activity) affected the flux of FFA in the blood. For this analysis, a path model was established and estimates of the model fit and the hypothesis were then tested. The second aim of this study was to test whether FRG consumption affects the relationship between the independent variables of several hormones and the autonomic nervous system and the dependent variable of FFA.

The study hypotheses were: (1) ACTH, growth hormone (GH), E2, glucocorticoid, tri-iodothyronine (T3), thyroid-stimulating hormone, and/or insulin influence the release of FFA; (2) the brachial pulse rate, which represents the activity of the autonomic nervous system and affects the release of FFA from adipocytes; and (3) the consumption of FRG changes the rate of FFA release, and this release is mediated by FRG on ER or GR.

2. Materials and methods

2.1. Participants and study design

This study was approved by the Institutional Review Board of Sahmyook University (Seoul, Korea). The study participants were 117 postmenopausal women (age 50–73 yr) who were recruited from four Catholic churches. Participants with any disease, including diabetes, cardiovascular disease, dyslipidemia, and kidney disease, were excluded. None of the study participants took any supplements for 2 wk prior to or during the experiment.

Anthropometric parameters were used to evaluate and categorize the 117 participants, who then had their brachial and ankle blood pressure and brachial and ankle blood pulse measured twice, once in the supine position and again after a 10-min rest period. Although the brachial and ankle pressures and pulse rate vary according to the spectrum of life activity, the pressure and the pulse in the supine position can be considered as the pressure and the pulse of a participant in a resting state.

After overnight fasting, blood and urine samples from the 117 participants were collected from 8:00 AM to 10:00 AM. The study participants were then divided into two groups according to the double-blind method of drawing lots. One group was supplied with capsules containing FRG powder (Bifido Inc., Gangwon-do, Korea), and the other group was supplied with placebo capsules containing edible starch for 2 wk. Because a hypothesis of this study was that ginsenosides are ligands of nuclear receptors and that the effects of a nuclear receptor can begin within 2 h, we considered that 2 wk of FRG consumption was sufficient.

The ingredients of the FRG capsules were as follows: crude saponin, 258.6 mg/g; compound K, 57.05 mg/g; Rg3, 53.85 mg/g; Rh2, 11.97 mg/g; Rg2, 5.72 mg/g; Rh1, 2.99 mg/g; and Rb1, 0.023 mg/g. The total weight of the FRG capsule powder was 2.1 g. After 2 wk, 24 women dropped out of the study; therefore, 93 women (49 in the FRG group and 45 in the placebo group) participated in the second blood sample collection. The reported cause of departure for 23 of the women was individual personal reasons not related to FRG consumption. One woman left the experiment after reporting insomnia associated with her consumption of FRG (Fig. 1).

Blood samples were measured at the Green Cross Reference Laboratory (Gyeonggi-do, Korea). The methods of sample analysis are listed in Appendix I.

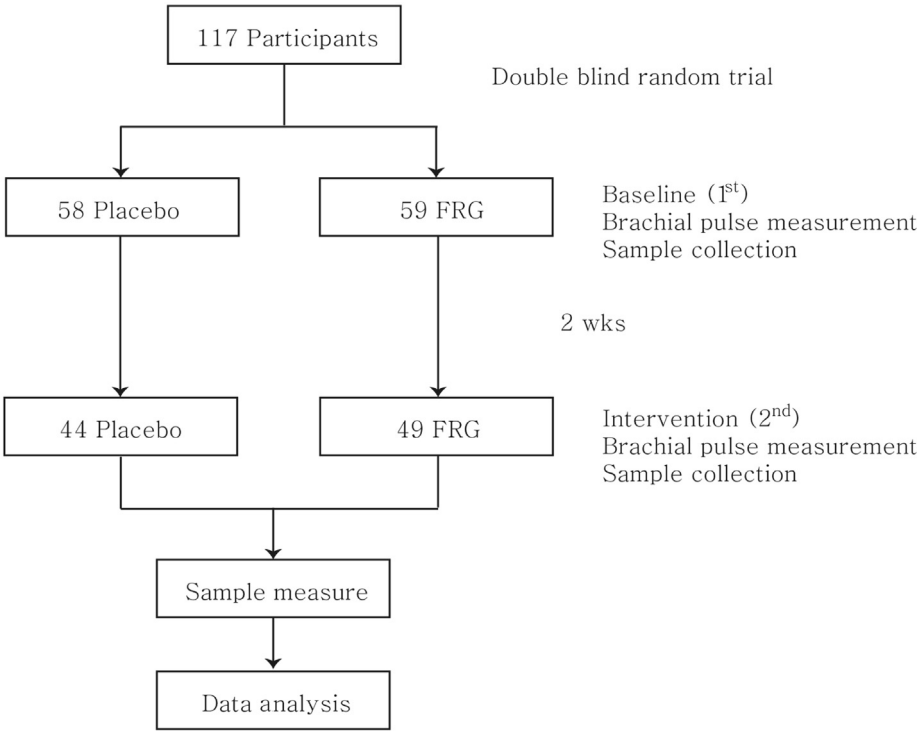

Fig. 1. Flow of this study. The 117 volunteers who participated in the first blood sample collection were divided into two groups with random double-blind trials. The blood samples were collected between 8:00 AM and 10:00 AM. One group took fermented red ginseng (FRG) capsules three times/day (2.1 g/d) for 2 wk. The other group took a placebo containing starch. Ninety-three women participated in the second blood sample collection. Ninety of the 93 participants were postmenopausal, and three were perimenopausal.

Blood samples from 20 women/group were further collected and matched according to age, height, weight, and body mass index.

2.2. Statistical analyses

The arithmetic means of the variables from both groups were analyzed by SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The outliers of insulin and E2 were excluded and considered as missing values. Unmeasured variables were considered as random missing values and 10 datasets were generated by a multiple imputation method [28].

Path analysis has several advantages in that several variables and multiple groups can be analyzed simultaneously; moreover, the effects of decomposition and model fitness can be assessed. We used path analysis as well as traditional statistics including mean comparisons in this study. The path model was analyzed with Mplus 6.11 (Muthén & Muthén, Los Angeles, CA, USA). The data in this report are part of an FRG study that was conducted in Seoul, Korea in 2010. Only the data relevant to this analysis are presented in this report.

3. Results

3.1. Anthropometric data

There were no significant differences in age, weight, height, and body mass index between the FRG group and the placebo group (Table 1).

3.2. Arithmetic mean comparisons

Hormones showed circadian variation and seasonal variation. Despite the fact that a double-blind random sampling method was utilized in this study, there was sampling error. Therefore, the analyses of the hormones and other variables required crosstalk validation and a comprehensive assessment. We analyzed the mean comparisons of samples between the FRG group and the placebo group with three statistical methods: an analysis of covariance (ANCOVA) in the second samples (ANCOVA comparison), independent *t* tests of the second samples (second sample *t* test), and independent *t* tests of the differences between the second and first samples (difference *t* test; Table 2). In the ANCOVA comparison, the mean values of ACTH, cortisol, T3, and FFA did not show a significant difference between the two groups, whereas the level of insulin was lower in the FRG group than it was in the placebo group ($p = 0.04$). In the difference *t* test, the level of insulin was found to be lower in the FRG group than in the placebo group ($p = 0.01$). In the ANCOVA comparison, the level of dehydroepiandrosterone was higher in the FRG group than it was in the placebo group ($p = 0.05$), and the same result was shown in the difference *t* test ($p = 0.03$). In the ANCOVA comparison, the levels of E2 ($p = 0.06$) and GH ($p = 0.06$) were higher in the FRG group than in the placebo group, but the differences were not statistically significant (Table 2).

3.3. Path model

The baseline model was established based on reports in the literature. The Wald test was conducted to test the hypothesis of

Table 1
Anthropometric Data of Participants

	Mean ± SD		*p*
	FRG ($n = 49$)	Placebo ($n = 44$)	
Age (yr)	58.5 ± 5.5	58.6 ± 5.8	0.944
Weight (kg)	56.9 ± 6.7	57.7 ± 6.9	0.559
Height (cm)	157.7 ± 5.3	156.6 ± 5.4	0.311
BMI (kg/m^2)	22.88 ± 2.39	23.55 ± 2.51	0.189
Waist circumference (cm)	32.7 ± 2.3	33.1 ± 2.4	0.397
Hip circumference (cm)	36.9 ± 2.0	37.3 ± 2.1	0.284
Waist/hip	0.89 ± 0.03	0.89 ± 0.04	0.965

BMI, body mass index; FRG, fermented red ginseng; SD, standard deviation.

Table 2
Comparison of Hormones and Free Fatty Acid Between the Fermented Red Ginseng (FRG) Group and the Placebo Group

Markers	Sample	Groups	Mean ± SD (*n*)	$p^{1)}$ ($p^{2)}$)	$\Delta 2^{nd} - 1^{st}$	
					Mean ± SD (*n*)	$p^{1)}$
ACTH (pg/mL)	1st	Placebo	19.6 ± 10.1 (43)	0.712		
		FRG	20.6 ± 13.2 (49)			
	2nd	Placebo	20.4 ± 10.1 (44)	0.321	2.3 (43)	0.52
		FRG	22.7 ± 11.8 (49)	(0.34)	0.9 (49)	
Cortisol (μg/dL)	1st	Placebo	11.4 ± 3.3 (43)	0.064		
		FRG	9.9 ± 4.2 (49)			
	2nd	Placebo	11.2 ± 3.5 (44)	0.531	−0.2 (43)	0.13
		FRG	10.8 ± 3.8 (49)	(0.52)	0.9 (49)	
Estradiol (pg/mL)	1st	Placebo	22.6 ± 17.3 (20)	0.693		
		FRG	20.6 ± 15.1 (20)			
	2nd	Placebo	14.5 ± 7.3 (19)	0.083	−7.4 (19)	0.06*
		FRG	18.3 ± 7.3 (19)	(0.06*)	1.0 (19)	
GH (ng/mL)	1st	Placebo	1.4 ± 1.3 (20)	0.424		
		FRG	1.9 ± 2.6 (20)			
	2nd	Placebo	0.9 ± 1.2 (20)		−0.40 (20)	0.50
		FRG	1.8 ± 1.4 (20)	(0.06*)	−0.06 (20)	
DHEAS (μg/dL)	1st	Placebo	71.5 ± 28.9 (43)	0.412		
		FRG	66.7 ± 26.8 (49)			
	2nd	Placebo	65.2 ± 31.4 (44)	0.871	−6.2 (43)	0.03**
		FRG	66.2 ± 29.1(49)	(0.05*)	0.5 (49)	
Insulin (μU/mL)	1st	Placebo	6.5 ± 7.2 (43)	0.667		
		FRG	7.1 ± 6.2 (49)			
	2nd	Placebo	6.4 ± 3.6 (43)	0.838	1.0 (43)	0.01**
		FRG	6.3 ± 2.9 (48)	(0.04**)	−0.2 (48)	
Glucose (mg/dL)	1st	Placebo	91.9 ± 8.9 (43)	0.211		
		FRG	89.9 ± 6.8 (49)			
	2nd	Placebo	89.6 ± 6.8 (44)	0.128	2.2 (43)	0.85
		FRG	87.4 ± 7.1 (49)	(0.31)	2.5 (49)	
FSH (mIU/mL)	1st	Placebo	73.8 ± 25.6 (20)	0.261		
		FRG	65.6 ± 19.6 (20)			
	2nd	Placebo	74.0 ± 24.3 (20)	0.387	0.1 (20)	0.44
		FRG	68.0 ± 18.5 (20)	(0.68)	2.4 (20)	
LH (mIU/mL)	1st	Placebo	37.8 ± 14.7 (20)	0.068*		
		FRG	30.6 ± 8.8 (20)			
	2nd	Placebo	38.8 ± 14.8 (20)	0.036**	1.0 (20)	0.56
		FRG	30.3 ± 9.0 (20)	(0.30)	−0.3 (20)	
TSH (μIU/mL)	1st	Placebo	2.7 ± 1.6 (20)	0.058*		
		FRG	1.9 ± 1.0 (20)			
	2nd	Placebo	2.8 ± 1.8 (20)	0.298	0.1 (20)	0.41
		FRG	2.3 ± 1.2 (20)	(0.80)	0.4 (20)	
T3 (pg/dL)	1st	Placebo	—			
		FRG	—			
	2nd	Placebo	912.1 ± 912 (20)		0.12 (20)	0.33
		FRG	935.6 ± 123 (20)	(0.86)	0.06 (20)	
FFA (μEq/L)	1st	Placebo	662.4 ± 232.1 (43)	0.901		
		FRG	656.8 ± 197.3 (49)			
	2nd	Placebo	640.0 ± 223.7 (44)	0.653	−23.5 (43)	0.61
		FRG	660.3 ± 217.0 (49)	(0.61)	3.8 (49)	
Brachial Pulse rate (beats/min)	1st	Placebo	70.1 ± 8.4 (48)	0.654		
		FRG	69.4 ± 9.5 (44)			
	2nd	Placebo	68.5 ± 8.5 (48)	0.957	−0.6 (47)	0.494
		FRG	68.4 ± 8.5 (43)	(0.829)	−1.7 (44)	

* $p < 0.1$.
** $p < 0.05$.
*** $p < 0.01$.
$\Delta 2^{nd} - 1^{st}$, second sample minus first sample; (*n*), number of samples; ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone; FSH, follicle stimulating hormone; GH, growth hormone; LH, luteinizing hormone; SD, standard deviation; T3, tri-iodothyronine; TSH, thyroid-stimulating hormone.
1) Independent *t* test.
2) Analysis of covariance.

whether or not there was a significant difference between groups for each path. The path of cortisol on FFA and the path of the brachial pulse rate on FFA both showed a significant difference between the two groups (Table 3).

The final model was then established (Fig. 2 and Table 4). The path of cortisol on FFA and the path of the brachial pulse rate on FFA were measured freely, whereas the other paths were analyzed with equality constraints (Fig. 2). Therefore, the values of the unstandardized coefficients of the path of cortisol on FFA and the values of the unstandardized coefficients of the path of the brachial pulse rate on FFA were two in both cases, and the values of the other unstandardized coefficients were one (Fig. 2). The final model's goodness of fit was good, as the root mean square error of approximation was 0.000 and the comparative fit index was 1.000.

When the effects of several independent variables on the FFA levels were compared with standardized coefficients, the path coefficients of E2 on FFA were highest at 0.678 in the FRG group and 0.656 in the placebo group.

The standardized coefficients of cortisol on FFA were 0.387 in the placebo group, whereas it was −0.233 in the FRG group. Therefore, when cortisol increased by a standardized deviation (3.5 μg/dL), the level of FFA increased by 0.387 standard deviations

Table 3
Path Coefficients of Baseline Model and the Values of Wald Test

Path		Group	Unstandardized estimate	Standardized estimate	Wald test
From	To				χ^2D(1)
ACTH	Cortisol	FRG	0.448 ± 0.096***	0.556 ± 0.099***	0.056
		Placebo	0.413 ± 0.114***	0.479 ± 0.116***	
TSH	T3	FRG	0.537 ± 0.202 ***	0.475 ± 0.168***	2.566
		Placebo	0.135 ± 0.119	0.217 ± 0.194	
FSH	LH	FRG	0.610 ± 0.216***	0.506 ± 0.135***	0.712
		Placebo	0.920 ± 0.269***	0.600 ± 0.128***	
FSH	Estradiol	FRG	−0.473 ± 0.309	−0.311 ± 0.195	0.091
		Placebo	−0.328 ± 0.498	−0.252 ± 0.359	
LH	Estradiol	FRG	−0.208 ± 0.243	−0.160 ± 0.184	0.631
		Placebo	0.102 ± 0.255	0.125 ± 0.271	
Insulin	FFA	FRG	−0.347 ± 0.114***	−0.338 ± 0.101***	2.644
		Placebo	−0.007 ± 0.158	−0.007 ± 0.132	
Cortisol	FFA	FRG	−0.253 ± 0.144*	−0.250 ± 0.140*	10.133***
		Placebo	0.457 ± 0.152***	0.386 ± 0.127***	
Estradiol	FFA	FRG	0.865 ± 0.153***	0.701 ± 0.106***	0.095
		Placebo	0.795 ± 0.165***	0.667 ± 0.107***	
GH	FFA	FRG	−0.347 ± 0.186**	−0.298 ± 0.141**	3.304*
		Placebo	0.144 ± 0.234	0.088 ± 0.140	
ACTH	FFA	FRG	0.068 ± 0.111	0.085 ± 0.139	0.452
		Placebo	0.181 ± 0.128	0.177 ± 0.125	
T3	FFA	FRG	0.056 ± 0.291	−0.032 ± 0.178	0.188
		Placebo	0.086 ± 0.273	0.037 ± 0.114	
DHEAS	FFA	FRG	0.296 ± 0.108***	0.240 ± 0.097**	2.287
		Placebo	−0.016 ± 0.160	−0.016 ± 0.128	
TSH	FFA	FRG	−0.370 ± 0.282	−0.206 ± 0.165	3.147*
		Placebo	0.196 ± 0.241	0.132 ± 0.168	
Brachial Pulse rate	FFA	FRG	0.677 ± 0.199***	0.462 ± 0.110***	5.152*
		Placebo	0.107 ± 0.168	0.071 ± 0.111	
SMC of FFA		FRG		0.760***	
		Placebo		0.733***	

* $p < 0.1$.
** $p < 0.05$.
*** $p < 0.01$.
ACTH, adrenocorticotropic hormone; DHEAS, Dehydroepiandrosterone; FFA, free fatty acid; FRG, fermented red ginseng; FSH, follicle stimulating hormone; GH, growth hormone; LH, luteinizing hormone; SMC, squared multiple correlations (R^2_{smc}); T3, tri-iodothyronine; TSH, thyroid-stimulating hormone.

(0.387 × 232.1 μEq/L = 89.8 μEq/L) in the placebo group, whereas when cortisol increased by a standardized deviation (3.8 μg/dL), the level of FFA decreased by 0.233 standard deviations (0.233 × 217.0 μEq/L = 50.6 μEq/L) in the FRG group (Table 4).

Squared multiple correlation (SMC; R^2_{smc}) refers to the square value of the standardized estimate and SMC signifies the explanation ability of the independent variables on the fluctuation of the dependent variables. For example, the standardized estimate of the brachial pulse rate on FFA was 0.081 and the SMC of the brachial rate on FFA was 0.01 ($1\% = 0.081^2$) in the placebo group, whereas in the FRG group the estimate of the brachial pulse rate on FFA was 0.464 and the SMC of the brachial rate on FFA was 0.215 ($21.5\% = 0.464^2$). The standardized estimates of ACTH on FFA and T3 on FFA were both below 0.1, demonstrating no significant influence on the concentration of FFA in the final model (Table 4).

The SMC values of FFA were 0.699 ($p < 0.01$) in the placebo group and 0.707 ($p < 0.01$) in the FRG group. When the brachial pulse variable was excluded from the final model, the SMC of FFA changed to 0.671, which did not show a significant change in the placebo group. However, the SMC of FFA in the FRG group decreased by 0.500, which implies the importance of the brachial pulse rate on FFA release in the FRG group.

4. Discussion

4.1. E2 and FFA

The accumulation pattern for postmenopausal women is different from that for men [29]. In females, the major locations of fat accumulation are in the lower body and in subcutaneous tissue, whereas in males fat accumulates in the upper body and in the organs of the abdominal cavity (visceral fat). The fat accumulation area is important in relation to the onset of MtS [30] because released FFA from abdominal adipocytes are directly transported to the liver via the hepatic portal vein, resulting in a decrease in insulin clearance and an increase in the synthesis of triglycerides and very low density lipoprotein [31]. Therefore, the movement and accumulation effect of lipids by E2 are important for a proper understanding of the lipid metabolic process.

The effects of E2 on lipolysis are different between subcutaneous adipocytes and abdominal adipocytes. For example, E2 treatment decreased the level of lipolysis in the adipocytes, which mediated an increased number of α2A–adrenergic receptors, whereas E2 treatment did not show any effect on the lipolysis of the abdominal adipocytes [32]. In addition, abdominal adipocytes showed a low level of α-adrenoreceptors and a high level of β-adrenoreceptors when compared to the level of β-adrenoreceptors in subcutaneous adipocytes [33]. These differences in the ratio with regard to the adrenoreceptor type may help to explain differences in gender-dependent spatial fat accumulation.

In the present study, the positive relationship between the concentrations of E2 and FFA may have been due to the fasting times and the lowered E2 levels of the postmenopausal women in the present study design. Because blood samples were collected after 8 h of overnight fasting, the migration effect of FFA by lipoprotein lipase from the circulatory system to the adipocytes can be ignored. However, it was possible to infer that genome independent lipolysis by E2 could stimulate HSL and inositol triphosphate activities. Even though it is well known that Rg3 acts as the ligand of ERs and Rg3 was a high ratio of ginsenosides in this study, the effect of E2 on FFA did not show a significant difference between the groups.

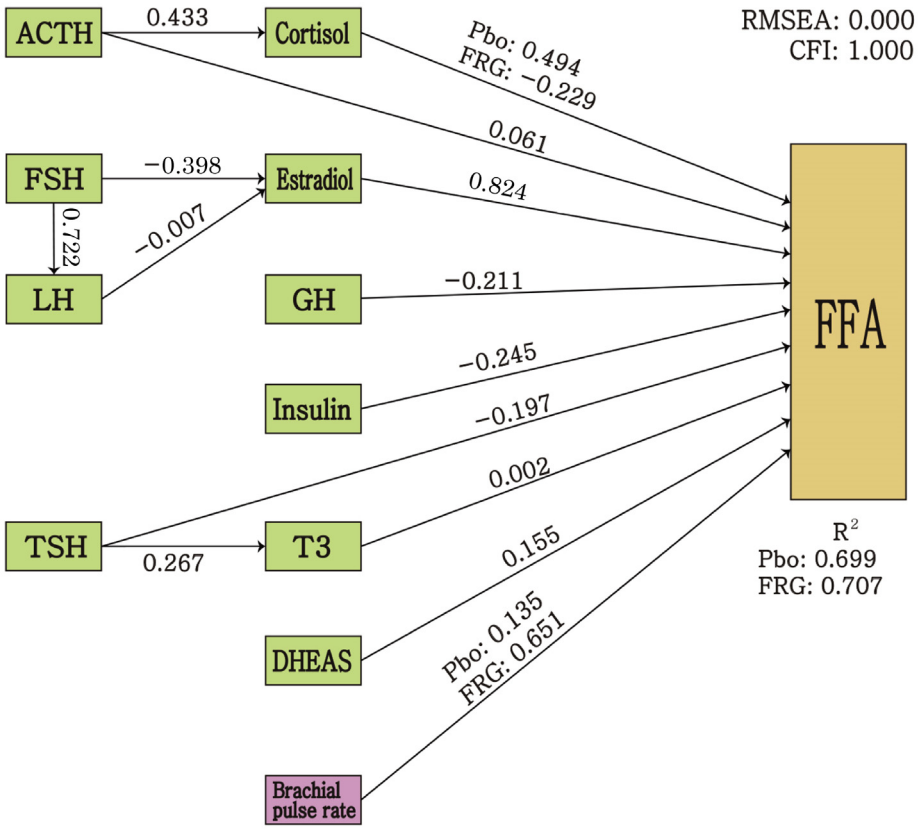

Fig. 2. The equality constrained final model. The path of cortisol on free fatty acid (FFA) and the path of brachial pulse rate on FFA were freely analyzed and the other paths were constrained for equality between the fermented red ginseng (FRG) group and the placebo group. The numbers on the arrow line represent the unstandardized estimates of path. ACTH, adrenocorticotropic hormone; CFI, comparative fit index, DHEAS, dehydroepiandrosterone; FFA, free fatty acid; FSH, follicle stimulating hormone; GH, growth hormone; LH, luteinizing hormone; R^2, squared multiple correlations (SMC); RMSEA, root mean square error of approximation; T3, tri-iodothyronine; TSH, thyroid-stimulating hormone.

4.2. Cortisol and FFA

Djurhuus et al [34] reported that when a physiologically high level of cortisol was injected into the adipose tissue, the level of blood FFA increased by 60%, as mediated by lipolysis stimulation. In the final model here, the path coefficient value of cortisol on FFA was positive ($p = 0.002$) in the placebo group, whereas the path coefficient value was negative ($p = 0.082$) in the FRG group. Therefore, it may be presumed that CK consumption acts as a competitive inhibitor with cortisol of the GR in this study.

In a postprandial state, insulin is released and suppresses the functions of HSL and lipolysis in adipocytes. In a fasting state, however, the level of insulin decreases, and the levels of cortisol and growth hormone increase, which in turn stimulates the expression of HSL [35]. The proper expression of HSL is important in the regulation of blood glucose. HSL-deficient mice cannot release a proper level of FFA and thus enter into an insulin-resistant state [36]. However, in the present study, the growth hormone and FFA showed a significant negative relationship. These results may be due to the decreased level of GH of the postmenopausal women owing to the natural process of aging. In fact, the explanation ability levels (SMC) of GH on the FFA concentration results were only 1% in the placebo group and 2% in the FRG group.

4.3. Brachial pulse rate and FFA

Although several studies have reported that the activity of the sympathetic nervous system is related to MtS [17,37], the exact mechanism of this has yet to be elucidated. Jeon et al [38] reported that when crude saponin, including ginsenoside, was intravenously injected into rats, their heart rates increased. Because GR and ER are present in the brain stem area, it may be presumed that CK and Rg3, ligands of GR and ER, regulate the autonomic nervous system via the central nervous system. Therefore, consecutively, brain stems that have GR and ER influenced by CK and Rg3 could have an effect on how FFA is released in adipocytes. If so, it would be of interest to assess whether CK or Rg3 has the strongest effect on the brachial pulse rate in this study. ER-α is present in the autonomic nerve center of the brain stem, which regulates the cardiovascular system [38]. When estrogen was administered into this area, autonomic nerve regulation of the heart improved and the level of sympathetic activity decreased [39]. Furthermore, when estrogen was injected into the brain of an ovariectomized rat, its heart rate decreased [40].

GR is highly expressed in the dorsal hindbrain area and is especially prominent in the nucleus of the solitary tract [41]. These areas are centers of cardiovascular regulation. When cortisol was injected into the dorsal hindbrain of a rat, its heart rate increased within 3 days [42]. Therefore, because the autonomic effect on FFA was increased in the FRG group, CK was shown to have a stronger effect in the FRG group as compared to the placebo group.

4.4. Counteraction between cortisol and the brachial pulse

In the final path model (Fig. 2 and Table 4), two paths showed significant differences between two groups, and the significance levels were changed between the two paths and two groups. In this case, the significance levels of the path coefficients of cortisol to FFA were significant in the placebo group ($p = 0.002$) but were not significant in the FRG group ($p = 0.082$). However, the significant level of the brachial pulse on the FFA path was not significant in the

Table 4
Path Coefficients of Equality Constrained Final Model

Path		Group	Unstandardized estimate		Standardized estimate	
From	To		Estimates ± SE	*p*	Estimates ± SE	*p*
ACTH	GC	Placebo	0.433 ± 0.073***	0.000	0.497 ± 0.084***	<0.001
		FRG			0.543 ± 0.085***	<0.001
TSH	T3	Placebo	0.267 ± 0.117**	0.022	0.383 ± 0.135***	0.005
		FRG			0.255 ± 0.134**	0.057
FSH	LH	Placebo	0.722 ± 0.164***	0.000	0.504 ± 0.105***	<0.001
		FRG			0.568 ± 0.105***	<0.001
FSH	E2	Placebo	−0.398 ± 0.329	0.227	−0.291 ± 0.242	0.230
		FRG			−0.276 ± 0.230	0.230
LH	E2	Placebo	−0.007 ± 0.164	0.968	−0.002 ± 0.165	0.989
		FRG			−0.006 ± 0.142	0.967
Insulin	FFA	Placebo	−0.245 ± 0.096*	0.011	−0.188 ± 0.074*	0.011
		FRG			−0.250 ± 0.097*	0.010
GC	FFA	Placebo	0.494 ± 0.163***	0.002	0.387 ± 0.114***	0.001
		FRG	−0.229 ± 0.132*	0.082	−0.233 ± 0.135*	0.083
E2	FFA	Placebo	0.824 ± 0.102***	0.000	0.656 ± 0.078***	<0.001
		FRG			0.678 ± 0.081***	<0.001
GH	FFA	Placebo	−0.211 ± 0.169	0.213	−0.108 ± 0.087	0.215
		FRG			−0.188 ± 0.146	0.197
ACTH	FFA	Placebo	0.061 ± 0.085	0.471	0.053 ± 0.077	0.485
		FRG			0.079 ± 0.110	0.469
T3	FFA	Placebo	0.002 ± 0.209	0.994	0.002 ± 0.090	0.980
		FRG			0.000 ± 0.125	0.997
DHEAS	FFA	Placebo	0.155 ± 0.104	0.136	0.114 ± 0.077	0.136
		FRG			0.131 ± 0.091	0.151
TSH	FFA	Placebo	−0.197 ± 0.184	0.286	−0.122 ± 0.117	0.297
		FRG			−0.112 ± 0.107	0.294
Brachial	FFA	Placebo	0.135 ± 0.171	0.428	0.081 ± 0.101	0.423
Pulse rate		FRG	0.651 ± 0.171***	0.000	0.464 ± 0.106***	<0.001
SMC of		Placebo			0.699 ± 0.097***	<0.001
FFA		FRG			0.707 ± 0.087***	<0.001

* $p < 0.1$.
** $p < 0.05$.
*** $p < 0.01$.
ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone; E2, estradiol; FFA, free fatty acid; FRG, fermented red ginseng; FSH, follicle stimulating hormone; GC, glucocorticoid; GH, growth hormone; LH, luteinizing hormone; SE, standard error; SMC, squared multiple correlations (R^2_{smc}); T3, tri-iodothyronine; TSH, thyroid-stimulating hormone.

placebo group ($p = 0.428$), although it was significant in the FRG group ($p < 0.001$). These results may help researchers establish the homeostasis levels of essential components such as the major energy source, FFA, in human physiology.

In the change of significance levels, one possible cause of the "rise and fall" phenomenon between the two groups is the nature of the glucocorticoid receptors (GR). GRs can be influenced by genetic variations, redundancies, synergy, crosstalk with other nuclear receptors, and by other types of cell signaling. Therefore, in the path of cortisol to FFA, some test participants showed a significant effect of FRG consumption, whereas others did not show a strong effect. However, for the complimentary path of cortisol to FFA, the path of the brachial pulse rate to the FFA level showed that the "rise and fall" phenomenon or the "seesaw" phenomenon between the cortisol level and the brachial pulse rate was related to the homeostasis of FFA.

Regarding the methodology, these results are good examples that show that path analysis may be a useful tool for the simultaneous analysis and comparison of the effects of several independent variables on dependent variables with multiple groups.

5. Conclusions

Among the several variables in this study, estrogen best explained FFA fluctuations. The brachial pulse provided a better explanation of FFA variance in the FRG group than in the placebo group. Cortisol had a strong effect on FFA release in the placebo group, but it did not have this effect in the FRG group. These "seesaw" effects between the brachial pulse rate and cortisol imply multiple routes of human physiology as regards the homeostasis of FFA. In conclusion, FRG consumption changed the effect of cortisol on FFA levels from peripheral tissues to the autonomic nervous system, whereas the level of FFA and the effects of other variables on FFA remained unchanged.

The effect of ginsenosides on human physiology depends on the ratio, dose, and treatment period of the ginsenosides. A study with a single type of ginsenoside in different environments would improve our understanding of the effects of hormones on FFA levels.

Conflicts of interest

The contributing authors declare no conflicts of interest.

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Appendix I

Reagents and Instruments

Test name		Glucose(S)	Insulin	Cholesterol total	LDL Cholesterol	FFA
Test method		enzymatic method	ECLIA	Enzymatic, colorimetry	Enzymatic, colorimetry	ACS-ACOD (colorimetry method)
Reagent	Kit name	Glucose Hexokinase	Insulin	Cholesterol reagent	LDL-Cholesterol	NEFA HR.II
	Company, nationality	SIEMENS, NY, USA	Roche, Mannheim, Germany	SIEMENS, NY, USA	SIEMENS, NY, USA	Wako, Osaka, Japan
Analytical instrument	Instrument name	ADVIA	MODULAR ANALYTICS	ADVIA	ADVIA	Hitachi
	Model name	ADVIA 1650	E170	ADVIA 1650	ADVIA 1650	HITACHI 7180
	Company, nationality	SIEMENS, NY, USA	Roche, Mannheim, Germany	SIEMENS, NY, USA	SIEMENS, NY, USA	HITACHI, Tokyo Japan
Test name		TSH	T3	FSH	LH	
Test method		CLIA	CLIA	CLIA	CLIA	
Reagent	Kit name	TSH	T3	ADVIA CENTAUR FSH	ADVIA CENTAUR FSH	
	company, nationality	SIEMENS, NY, USA	SIEMENS, NY, USA	SIEMENS, NY, USA	SIEMENS, NY, USA	
Analytical instrument	Instrument name	Centaur	Centaur	ADVIA CENTAUR	ADVIA CENTAUR	
	Model name	ADVIA CENTAUR	ADVIA CENTAUR	ADVIA CENTAUR	ADVIA CENTAUR	
	Company, nationality	SIEMENS, NY, USA	SIEMENS, NY, USA	SIEMENS, NY, USA	SIEMENS, NY, USA	
Test name		Cortisol	ACTH	DHEA-S	Estrogen (E2)	HGH(S)
Test method		RIA	IRMA	RIA	CLIA	CLIA
Reagent	Kit name	Coat-A-count Cortisol	ACTH IRMA	Coat-A-Count DHEA-Sulfate	ADVIA Centaur Estradiol	Immulite 2000 GH
	company, nationality	SIEMENS, LA, USA	BRAHMS, Berlin, Germany	SIEMENS, LA, USA	SIEMENS, NY, USA	SIEMENS, LA, USA
Analytical instrument	Instrument name	r-counter	r-counter	r-counter	Centaur	Immulite
	Model name	COBRA 5010 QUANTUM	COBRA5010 Quantum	COBRA5010 Quantum	ADVIA Centaur	Immulite 2000
	Company, nationality	PACKARD, Meriden, USA	PACKARD, Meriden, USA	PACKARD, Meriden, USA	SIEMENS, NY, USA	SIEMENS, LA, USA

ACTH; Adrenocorticotropic hormone, HGH; human growth hormone, DHEAS; Dehydroepiandrosterone, FFA; free fatty acid, FSH; follicle stimulating hormone , LH; luteinizing hormone, T3; triiodothyronine, TSH; thyroid-stimulating hormone.

References

[1] Cahill Jr GF. Starvation in man. N Engl J Med 1970;282:668–75.
[2] Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann N Y Acad Sci 1998;851:311–35.
[3] Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595–607.
[4] Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1:785–9.
[5] Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, Smith C. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 1991;88:960–6.
[6] Boden G, Chen X. Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. T J Clin Invest 1995;96:1261–8.
[7] Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest 1989;84:205–13.
[8] Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res 1993;34:1057–91.
[9] Miyoshi H, Perfield 2nd JW, Souza SC, Shen WJ, Zhang HH, Stancheva ZS, Kraemer FB, Obin MS, Greenberg AS. Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated lipolysis in adipocytes. J Biol Chem 2007;282:996–1002.
[10] Ottosson M, Lonnroth P, Bjorntorp P, Eden S. Effects of cortisol and growth hormone on lipolysis in human adipose tissue. J Clin Endocrinol Metab 2000;85:799–803.
[11] Katocs Jr AS, Largis EE, Allen DO. Role of Ca2+ in adrenocorticotropic hormone-stimulated lipolysis in the perfused fat cell system. J Biol Chem 1974;249:2000–4.
[12] Tagliaferro AR, Ronan AM, Payne J, Meeker LD, Tse S. Increased lipolysis to beta-adrenergic stimulation after dehydroepiandrosterone treatment in rats. Am J Physiol 1995;268:R1374–80.
[13] D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. J Biol Chem 2005;280:35983–91.
[14] Cousin B, Casteilla L, Lafontan M, Ambid L, Langin D, Berthault MF, Penicaud L. Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. Endocrinology 1993;133:2255–62.
[15] Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, et al. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat–functional implications. J Clin Invest 2002;110:1243–50.
[16] American Heart Association. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043–65.
[17] Chang CJ, Yang YC, Lu FH, Lin TS, Chen JJ, Yeh TL, Wu CH, Wu JS. Altered cardiac autonomic function may precede insulin resistance in metabolic syndrome. Am J Med 2010;123:432–8.
[18] Mintel Reporter. Complementary and alternative medicines—US—July 2008. Chicago, IL: Mintel Intl; 2008.
[19] Yang CS, Ko SR, Cho BG, Shin DM, Yuk JM, Li S, Kim JM, Evans RM, Jung JS, Song DK, et al. The ginsenoside metabolite compound K, a novel agonist of glucocorticoid receptor, induces tolerance to endotoxin-induced lethal shock. J Cell Mol Med 2008;12:1739–53.
[20] Hien TT, Kim ND, Pokharel YR, Oh SJ, Lee MY, Kang KW. Ginsenoside Rg3 increases nitric oxide production via increases in phosphorylation and expression of endothelial nitric oxide synthase: essential roles of estrogen receptor-dependent PI3-kinase and AMP-activated protein kinase. Toxicol Appl Pharmacol 2010;246:171–83.
[21] Huang YC, Lin CY, Huang SF, Lin HC, Chang WL, Chang TC. Effect and mechanism of ginsenosides CK and Rg1 on stimulation of glucose uptake in 3T3-L1 adipocytes. J Agric Food Chem 2010;58:6039–47.
[22] Kim SN, Lee JH, Shin H, Son SH, Kim YS. Effects of *in vitro*-digested ginsenosides on lipid accumulation in 3T3-L1 adipocytes. Planta Med 2009;75:596–601.
[23] Vuksan V, Sung MK, Sievenpiper JL, Stavro PM, Jenkins AL, Di Buono M, Lee KS, Leiter LA, Nam KY, Arnason JT, et al. Korean red ginseng (*Panax ginseng*) improves glucose and insulin regulation in well-controlled, type 2 diabetes: results of a randomized, double-blind, placebo-controlled study of efficacy and safety. Nutr Metab Cardiovasc Dis 2008;18:46–56.
[24] Lee HJ, Lee YH, Park SK, Kang ES, Kim HJ, Lee YC, Choi CS, Park SE, Ahn CW, Cha BS, et al. Korean red ginseng (*Panax ginseng*) improves insulin sensitivity and attenuates the development of diabetes in Otsuka Long-Evans Tokushima fatty rats. Metabolism 2009;58:1170–7.
[25] Lee KJ, Lee SY, Ji GE. Diabetes-ameliorating effects of fermented red ginseng and causal effects on hormonal interactions: testing the hypothesis by multiple group path analysis. J Med Food 2013;16:383–95.

[26] Su CF, Cheng JT, Liu IM. Increase of acetylcholine release by *Panax ginseng* root enhances insulin secretion in Wistar rats. Neurosci Lett 2007;412:101–4.
[27] Yook T, Yu J, Lee H, Song B, Kim L, Roh J, Shin J, Lim S. Comparing the effects of distilled *Rehmannia glutinosa*, Wild Ginseng and *Astragali Radix* pharmacopuncture with heart rate variability (HRV): a randomized, sham-controlled and double-blind clinical trial. J Acupunct Meridian Stud 2009;2:239–47.
[28] Rubin DB. Multiple imputation for nonresponse in surveys. New York: John Wiley & Sons; 1987.
[29] Mattiasson I, Rendell M, Tornquist C, Jeppsson S, Hulthen UL. Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. Horm Metab Res 2002;34:583–8.
[30] Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, Adams PW. Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 1982;54:254–60.
[31] Arner P. Differences in lipolysis between human subcutaneous and omental adipose tissues. Ann Med 1995;27:435–8.
[32] Lindberg UB, Crona N, Silfverstolpe G, Bjorntorp P, Rebuffe-Scrive M. Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. Horm Metab Res 1990;22:345–51.
[33] Lafontan M, Berlan M. Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. Endocr Rev 1995;16:716–38.
[34] Djurhuus CB, Gravholt CH, Nielsen S, Mengel A, Christiansen JS, Schmitz OE, Moller N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab 2002;283:E172–7.
[35] Degerman E, Belfrage P, Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). J Biol Chem 1997;272:6823–6.
[36] Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, Sattler W, Magin TM, Wagner EF, Zechner R. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J Biol Chem 2002;277:4806–15.
[37] Grassi G, Dell'Oro R, Quarti-Trevano F, Scopelliti F, Seravalle G, Paleari F, Gamba PL, Mancia G. Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. Diabetologia 2005;48:1359–65.
[38] Jeon BH, Kim CS, Park KS, Lee JW, Park JB, Kim KJ, Kim SH, Chang SJ, Nam KY. Effect of Korea red ginseng on the blood pressure in conscious hypertensive rats. Gen Pharmacol 2000;35:135–41.
[39] Cherney A, Edgell H, Krukoff TL. NO mediates effects of estrogen on central regulation of blood pressure in restrained, ovariectomized rats. Am J Physiol Regul Integr Comp Physiol 2003;285:R842–9.
[40] Saleh MC, Connell BJ, Saleh TM. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. Brain Res 2000;879:105–14.
[41] Härfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikström AC, Okret S, Yu ZY, Goldstein M, Steinbusch H, et al. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc Natl Acad Sci U S A 1986;83:9779–83.
[42] Bechtold AG, Scheuer DA. Glucocorticoids act in the dorsal hindbrain to modulate baroreflex control of heart rate. Am J Physiol Regul Integr Comp Physiol 2006;290:R1003–11.