Effects of Korean Red Ginseng on Mitochondria and Steroidogenesis in Male Patients with Metabolic Syndrome

Dong-Hyuk Jung

Department of Medicine

The Graduate School, Yonsei University

Effects of Korean Red Ginseng on Mitochondria and Steroidogenesis in Male Patients with Metabolic Syndrome

Directed by Professor Chun-Bae Kim

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Dong-Hyuk Jung

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This certifies that the Doctoral Dissertation of Dong-Hyuk Jung is approved.

[Signature]

Thesis Supervisor : Chun-Bae Kim

[Signature]

Thesis Committee Member#1: Jong-Ku Park

[Signature]

Thesis Committee Member#2: Jang-Young Kim

[Signature]

Thesis Committee Member#3: Kyu-Sang Park

[Signature]

Thesis Committee Member#4: Yong-Jae Lee

[three signatures total in case of Master's]

The Graduate School
Yonsei University

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"The fear of the Load is the beginning of wisdom; all who follow his precepts have good understanding. To him belongs eternal praise." (Psalms 111: 10)

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ABSTRACT

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Dong-Hyuk Jung

Department of Medicine
The Graduate School, Yonsei University

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Background: It has been observed that mitochondrial dysfunction is associated with an increased risk of metabolic syndrome. There is growing evidence that hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis and hormone (testosterone and growth hormone) deficiency may lead to metabolic syndrome. Recent studies have reported that ginseng treatment improves mitochondrial and HPA-axis function and increases anabolic hormone secretion.

Objectives: The objective of this study was to investigate the effect of Korean red ginseng (KRG) on metabolic syndrome, hormones, and mitochondrial function using leukocyte mitochondrial DNA copy number in men with metabolic syndrome.

Methods: We performed a randomised, double-blind, placebo-controlled study in 62 subjects who were not taking drugs that could affect their metabolic function. A total of 62 men with metabolic syndrome were randomly assigned to either an KRG group (3.0 g/d) or a placebo group for 4 weeks. We analysed changes in metabolic syndrome components, leukocyte mitochondrial DNA copy number, hormones (total testosterone, IGF-1, cortisol, and DHEAS) and inflammatory markers (C-reactive protein, ferritin) from baseline to 4 weeks.

Results: Significant improvement in mitochondrial function (P=0.024) and an increase in total testosterone and IGF-1 levels (P=0.032, P=0.013) were observed in the KRG group compared with the placebo group. Diastolic blood pressure (P=0.004) and serum cortisol (P=0.005) were significantly decreased in

the KRG group.

Conclusions: We found evidence that KRG had a favourable effect on mitochondrial function and hormones in men with metabolic syndrome.

Key words: ginseng, mitochondria, steroidogenesis and metabolic syndrome

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I. INTRODUCTION

Metabolic syndrome represents a cluster of risk factors including insulin resistance, high blood pressure (BP), obesity, and dyslipidaemia. Metabolic syndrome also increases the risk of cardiovascular disease, as well as type 2 diabetes mellitus and chronic kidney disease.[1-3] Various pathophysiological processes have been reported to contribute to metabolic syndrome; however, the key pathophysiologic mechanism has not been clearly elucidated. Recent studies have suggested that the hypothalamus-pituitary-adrenal (HPA) axis and hormones play a critical role in obesity and insulin resistance, which are etiologically linked to the development of metabolic syndrome.[4] While acute stress leading to increased cortisol is a physiologic phenomenon, persistent stress is related to HPA abnormalities and persistent hypercortisolaemia,[5,6] a condition that contributes to visceral obesity, insulin resistance, increased BP, and dyslipidaemia.[7] These contributions may be responsible for the association of HPA abnormalities with metabolic syndrome.[8]

Several endocrine mechanisms are associated with the pathogenesis of metabolic syndrome in men, and several studies have reported that low testosterone and insulin-like growth factor 1 (IGF-1) are associated with metabolic syndrome.[9,10] Recent studies also reported that serum testosterone and

IGF-1 are linked with lipolysis, reduce fatty acid production, and decrease circulating glucose.[11,12] Mitochondrial dysfunction has been implicated in a variety of diseases, such as cancer, aging, neurodegeneration, and metabolic syndrome.[13] Altered mitochondrial DNA (mtDNA) copy number regulation can lead to diseases such as neurogenic disorders and multiple sclerosis.[14] Additionally, a decrease in mtDNA copy number has been related with type 2 diabetes and dyslipidaemia.[15,16] Korean Red ginseng (KRG) is one of the most popular Asian herbal medicines. KRG contains many bioactive compounds, including glycan, peptides, and ginsenosides.[17] Several studies have suggested that KRG has anti-obesity and hypoglycaemic effects.[18] One of its mechanisms of action is related to the improvement of mitochondrial function by increasing mitochondrial number and fusion.[19] Certain studies have also reported that ginseng treatment leads to HPA-axis changes and an increase in anabolic hormone secretion.[20,21] To our knowledge, there is no systemic clinical study aimed at investigating changes in mitochondrial function, hormones, and metabolic components after the administration of KRG in male metabolic syndrome patients. We tested the hypothesis that KRG has an ameliorating effect on metabolic syndrome via improvement of mitochondrial and HPA-axis function and hormone secretion.

II. MATERIALS AND METHODS

1. Study design and participants

This study was conducted as a single centre, double-blind, placebo-controlled, randomised clinical trial. The study was approved by the Institutional Review Board (IRB) of Wonju Severance Hospital, Yonsei University College of Medicine in Wonju, Korea (IRB no.YWMR-13-0-049) The study complied with the code of ethics of the World Medical Association(Declaration of Helsinki) with all subjects receiving informed consent before participation.

The study population was recruited from the general population through advertisements. All participants were male patients with metabolic syndrome between 35 and 70 years old. Metabolic syndrome was defined as three or more of the following risk factors according to the modified National Cholesterol Education Program Adult Treatment Panel Ⅲ[22] and the Korean Society for the Study of Obesity Criteria [23]: 1) waist circumference ≥ 90 cm for men, 2) high triglyceride ≥ 150 mg/dL, 3) low high-density lipoprotein (HDL)-cholesterol ≤ 40 mg/dL, 4) elevated systolic BP ≥ 130 mmHg or elevated diastolic BP ≥ 85 mmHg, and 5) high fasting plasma glucose (FPG) ≥ 100 mg/dL. Subjects were excluded if they were taking drugs that could affect their vascular and metabolic function including hypoglycaemic medication and antihypertensive medication. Men with cardiovascular disease were also excluded.

A total of 72 male patients with metabolic syndrome participated in this study. After the initial screening visit, subjects were randomly assigned in a double-blind fashion to receive either 3.0 g of KRG or an identical-appearing placebo with ginseng flavour. Thirty-six patients were randomly assigned to the KRG group, and 36 were placed in the placebo group. We used computer randomization and an allocation ratio of 1:1. The KRG group took three capsules two times a day (KRG 1.5 g × 2) for 4 weeks and the placebo group was provided with identically shaped capsules containing over 90% corn starch for 4 week (Figure1). The KRG group was provided a soft capsule, and each capsule

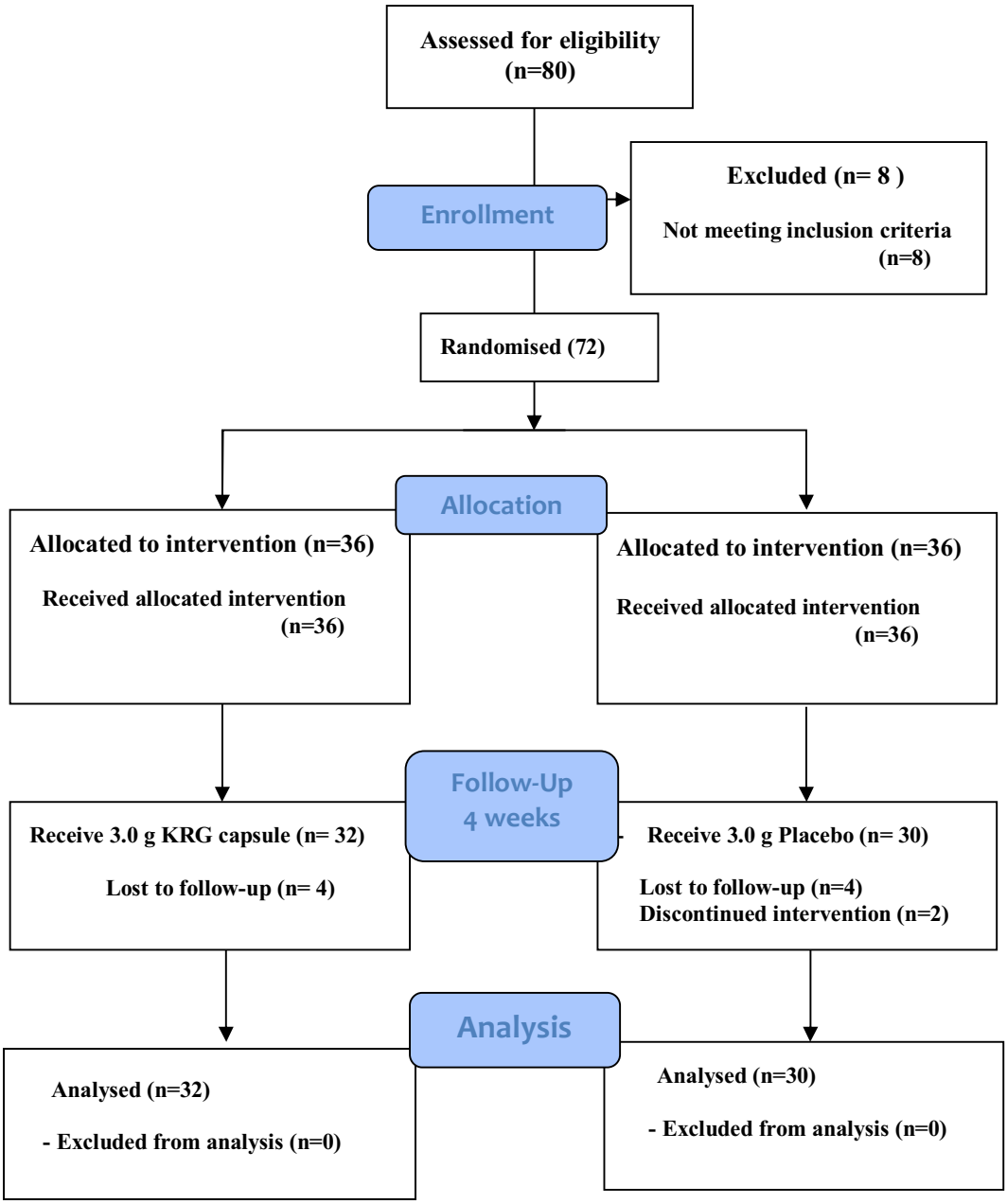

Figure 1. Flow chart of the study design and subject participation.

contained 500 mg of KRG. KRG contains the following major ginsenosides, Rg1: 2.89%, Re: 2.16%, Rf: 0.93%, Rh1: 0.13%, Rg2: 0.58%, Rb1: 5.16%, Rc: 2.22%, Rb2: 1.82%, Rd: 0.47%, Rg3: 0.22%, and other minor ginsenosides.

2. Anthropometric and laboratory measurement

Self-reported cigarette smoking and drug history were determined from questionnaires. Smoking status was categorized as nonsmoker and current smoker. Height and weight were measured using an automatic scale. Body mass index (BMI; kg/m^2) was calculated as weight (in kilograms) divided by the height squared (in meters). BP was measured on the right arm using a standard mercury sphygmomanometer (Baumanometer, USA). Two systolic and diastolic BP readings were recorded at 5-min intervals and averaged for analysis. Due to the diurnal variation of cortisol, venous blood was collected once between 08:00 to 10:00 from an antecubital vein in each subject after a 12-hour fast. All hormonal determinations were carried out during the same assay. Plasma ACTH, DHEAS, cortisol, testosterone and IGF-1 concentrations were determined using commercially available radioimmuno-assay kits with a Modular Analytics E170 system (Roche Diagnostic Systems, Basel, Switzerland). The intra-assay coefficient of variation for the measurements of hormone concentrations using this method was less than 10%. C-reactive protein was measured using a latex-enhanced immunoturbidimetric method. FPG, total cholesterol, triglyceride and HDL cholesterol levels, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by enzymatic methods using a Hitachi 7600-110 automated chemistry analyser (Hitachi, Tokyo, Japan). Diabetes was defined as a self-reported history of the disorder, a history of taking anti-diabetic drugs, or a fasting plasma glucose level $\geq$ 126 mg/dL. Hypertension was defined as a self-reported history of the disorder, a history of taking anti-diabetic drugs, a systolic BP $\geq$ 140 mmHg or a diastolic BP $\geq$ 90 mmHg.

3. DNA extraction

Blood from the peripheral vein was collected in tubes containing EDTA. The DNA was extracted from lymphocytes using the Genomic Blood Spin Mini Kit (Nucleogen, Korea) according to the manufacturer's instructions. The quantity and quality of DNA were measured by the Epoch spectrophotometer (BioTek, Winooski, VT, USA).

4. Real-time quantitative PCR assay

The mtDNA content was measured by real-time quantitative PCR (qPCR) of mitochondrial encoded NADH dehydrogenase 1 (*MT-ND1*) normalized by nuclear DNA encoded haemoglobin beta (*HBB*) genes. qPCR was performed using the CFX96 Real-time System (Bio-Rad, Singapore). Fifteen microliters of the reaction mixture contained 10 ng of template DNA, 1X Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan) and 7.5 p/mole of primers. Thermal cycling conditions consisted of one cycle at 95°C for 60 sec, followed by 35 cycles of 95°C for 5 sec, 61°C for 10 sec and 72°C for 20 sec. After the PCR reaction, a dissociation curve analysis was performed from 61°C to 95°C (increments of 0.5°C) to confirm specific amplification. To assess the efficiency of all primer pairs, the standard curves were derived from seven serial dilutions of reference DNA (40, 20, 10, 5, 2.5, 1.25 and 0.625 ng) using Bio-Rad CFX Manager version 1.6 software (Bio-Rad). The primers used for *ND1* were 5'-GACCCTACTTCTAACCTCCCTGT-3' and 5'-TAGGAGGTGTATGAG TTGGTCGT-3'. The *HBB* primers were designed as 5'-ACCCAAGAG TCTTCTCTGTCTCCA-3' and 5'-TCTGCCGTTACTGCCCTGTG-3'. We also screened the UCSC database (http://genome.ucsc.edu/) to confirm the unique sequence without any repeat sequences in the primers. All of the experiments contained reference DNA, were repeated three times and the threshold lines for threshold cycles (Ct) were automatically determined for each primer. mtDNA quantification was performed by the ΔCt method as described elsewhere.[24,25]

5. Statistical analysis and Sample size estimation

Demographic and biochemical characteristics of the study population were summarised. All continuous variables are presented as mean ± SD, and the categorical variables are summarized as percentages for the KRG and placebo groups. Baseline characteristics between the KRG and placebo groups were compared using *t*-tests for the continuous variables and Fisher's exact test for categorical variables in cells with an expected count of less than five. A paired *t*-test was applied when comparing baseline data with changes at 4 weeks for each group. The independent *t*-test was used to compare changes from baseline to 4 weeks between the KRG placebo groups. Multiple co-variant ANOVA was applied when comparing KRG and placebo groups to control for liver enzyme. All analyses were conducted using SPSS statistical software version 15.0 (SPSS, Inc., Chicago, IL, USA). All statistical tests were 2-sided, and statistical significance was defined as a p-value <0.05. The total sample size of 60 (30 + 30) was derived using the parameter values of alpha = 0.05 and power = 90%, where the expected change was 6.5 mmHg and the standard deviation was 11.5 mmHg. The change value was the placebo-adjusted treatment effect in systolic blood pressure in the 4 weeks from baseline. As there was no reference paper that detected an improvement of mitochondria function with mtDNA copy number, we assumed the expected change value based on a reference paper by Jovanovski et al., who detected a blood pressure change after ginseng administration.[26] In the reference paper, a significant systolic blood pressure change of 5.0 mmHg occurred after the administration of ginsenoside Rg3 (enriched KRG) 400 mg to 23 subjects for 3 hours. Our experiment utilised a high dose and long duration of RG intervention; thus, the drop-out rate was assumed to be less than 20% based on good tolerability of the nutraceutical combination (NC).

III. RESULTS

1. Clinical characteristics of study participants

A total of 72 participants were initially included in this clinical study, and they were assigned to either the KRG group or the placebo group; ultimately, 62 subjects completed the study.

There were no significant side effects related to the red ginseng. Eight subjects were lost to follow-up, and two subjects included in placebo group were later disqualified due to taking a medicine that could influence mitochondria function. Baseline characteristics of all subjects are presented in Table 1. There were no differences between the two participant groups in mean age, metabolic syndrome components, and underlying diseases, including hypertension and diabetes. Only mean AST and ALT levels were significantly higher in the RG group than in the placebo group.

2. Metabolic components, hormones and leukocyte mtDNA copy number

Table 2 summarizes the inter- and intra-group differences in metabolic components and inflammatory markers. Mean SBP, DBP and serum ferritin level decreased significantly in the KRG group ($P < 0.001$, $P < 0.001$ and $P = 0.016$, respectively). However, the extent of change of the DBP was the only significant difference between the ginseng and the placebo groups. There was no significant difference between the two groups with respect to the changes in the HOMA-IR, total cholesterol, triglyceride and HDL-cholesterol. After 4 weeks, the mtDNA copy number in leukocytes (measured as the ratio of mtDNA to nuclear DNA) increased significantly from 67.2 ± 30.4 to 97.5 ± 33.4 in the KRG group compared with the placebo group, which increased from 81.1 ± 51.6 to 86.2 ± 40.9 ($P = 0.024$). In the KRG group, the serum IGF-1 level significantly increased from 157.7 ± 43.2 to 170.1 ± 39.7 compared with the placebo group, which increased from 180.1 ± 43.3 to 174.9 ± 51.6 ($P = 0.013$), and the total testosterone level also increase significantly from 396.2 ± 152.9 to 422.7± 162.2 compared with the placebo group, which increased from 431.4 ± 95.8 to 419.2 ±

Table 1. Clinical characteristics of the subjects at baseline

	Korean red ginseng group	Placebo group	*P*
Number	32	30	
Age (years)	48.2 ± 10.9	45.2 ± 9.7	0.283
BMI (kg/m^2)	29.7 ± 6.9	27.9 ± 6.8	0.324
SBP (mmHg)	139.7 ± 12.7	134.1 ± 12.4	0.083
DBP (mmHg)	94.6 ± 10.7	90.6 ± 10.1	0.132
FPG (mg/dL)	128.4 ± 22.4	111.0 ± 20.2	0.160
Total cholesterol (mg/dL)	190.3 ± 40.1	198.5 ± 33.1	0.311
Triglyceride (mg/dL)	159 (113-204)	137 (99.5-201)	0.274
HDL-Cholesterol	47.2± 11.6	44.8± 10.5	0.414
HOMA-IR	2.34	2.09	0.436
AST (IU)	29.5 ± 13.0	22.6 ± 6.8	0.019
ALT (IU)	41.9 ± 22.3	29.9 ± 13.1	0.021
Current smokers (%)	4 (12.5)	4 (13.3)	0.378
Hypertension† (%)	7 (21.8)	5(12.5)	0.496
Diabetes§ (%)	4 (12.5)	3 (12.5)	0.591

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; AST, aspartate alaninetransferase; ALT, alanine aminotransferase;

101.9 (P=0.032). At 4 weeks, the serum cortisol level in the KRG group decreased from 14.3 ± 5.5 to 12.7 ± 4.4, whereas it increased from 10.5 ± 4.4 to 12.2 ± 4.8 in the placebo group. There were significant differences between the two groups regarding serum cortisol. In the KRG group, serum DHEAS levels dropped after the 4 week regimen, but the decrease was not significant (Table 3). The mean change of mtDNA copy number was higher for the KRG group than for the placebo group, independent of liver enzyme and age (Figure 2).

Table 2. Changes in metabolic components and inflammatory markers after the administration of the KRG or placebo for 4 weeks

	Korean red ginseng group (N_1 =32)			Placebo group (N_2 =30)			P^b
	Baseline	Changes	P^a	Baseline	Changes	P^a	
BMI (kg/m^2)	29.7 ± 6.9	-1.7 ± 7.0	0.185	27.9 ± 6.8	0.01 ± 0.8	0.780	0.177
SBP (mmHg)	139.7 ± 12.7	-8.6 ± 8.9	<0.001	134.1 ± 12.4	3.0 ± 8.8	0.082	0.468
DBP (mmHg)	94.6 ± 10.7	-5.1 ± 7.5	0.001	90.6 ± 10.1	0.5 ± 7.0	0.690	0.004
FPG (mg/dL)	128.4 ± 22.4	-4.0 ± 16.7	0.226	111.0 ± 20.2	4.3 ± 14.1	0.117	0.051
Total cholesterol (mg/dL)	190.3 ± 40.1	5.0 ± 27.1	0.340	198.5 ± 33.1	4.4 ± 29.7	0.441	0.940
Triglyceride (mg/dL)	159 (113-204)	-7.2 ± 118.0	0.752	137 (99-201)	-7.2 ± 43.0	0.379	0.544
HDL-cholesterol (mg/dL)	47.2± 11.6	1.4 ± 6.7	0.276	44.8± 10.5	2.8 ± 6.5	0.034	0.468
HOMA-IR	2.33 ± 1.3	0.1 ± 0.7	0.495	2.11 ± 1.1	- 0.2 ± 0.6	0.099	0.112
AST (IU)	29.5 ± 13.0	1.6 ± 12.5	0.493	22.6 ± 6.8	1.9 ± 7.9	0.231	0.921
ALT (IU)	41.9 ± 22.3	14.1 ± 98.5	0.463	29.9 ± 13.1	1.0 ± 11.5	0.643	0.514
C-reactive protein (mg/L)	1.4 ± 1.0	0.2 ± 1.2	0.441	1.9 ± 2.8	-0.6 ± 1.8	0.104	0.071
Ferritin	287.1 ± 207.8	- 25.4 ± 53.1	0.016	188.5 ± 89.4	-15.8 ± 49.9	0.111	0.495
WBC count (10 3 cells/)	6.7 ± 1.9	- 0.3 ± 1.5	0.301	6.5 ± 2.1	0.5 ± 1.9	0.185	0.091

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment ;AST, aspartate alaninetransferase; ALT, alanine aminotransferase;

[†]Data are expressed as means ± SD, median (IQR), or percentage.

[a] Paired test comparing mean at baseline with mean at 4 weeks between KRG group and placebo group.

[b] Independent test comparing KRG effects between two groups at 4 weeks.

Table 3. Change in peripheral blood mitochondrial DNA copy number and hormone at baseline and end point of study

	Korean red ginseng group (N_1 =32)			Placebo group (N_2 =30)			*P*
	Baseline	Changes	*P* [a]	Baseline	Changes	*P* [a]	
DNA copy number	67.2 ± 30.4	30.3 ± 32.1	<0.001	81.1 ± 51.6	5.1 ± 52.1	0.599	0.024
ACTH	17.7 ± 15.1	0.5 ± 15.2	0.860	14.9 ± 7.6	2.2 ± 7.0	0.121	0.235
DHEAS	227.1 ± 106.3	- 12.6 ± 36.3	0.052	116.6 ± 21.6	3.8 ± 56.5	0.717	0.169
Cortisol	14.3 ± 5.5	-1.6 ± 4.6	0.050	10.5 ± 4.4	1.7 ± 4.4	0.048	0.005
Cortisol / DHEAS ratio	8.1 ± 7.1	- 0.2 ± 4.6	0.801	4.9 ± 3.0	1.4 ± 3.8	0.052	0.136
IGF-1	157.7 ± 43.2	12.4 ± 26.7	0.012	180.1 ± 43.3	-5.2 ± 27.4	0.316	0.013
Total testosterone	396.2 ± 152.9	26.5 ± 58.5	0.013	431.4 ± 95.8	-12.2± 81.1	0.425	0.032
Insulin	7.2 ± 3.1	0.4 ± 2.2	0.333	7.7 ± 3.0	-0.2 ± 4.0	0.795	0.477

ACTH, Adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone sulfate; IGF-1, insulin-like growth factor.

[†]Data are expressed as means ± SD.

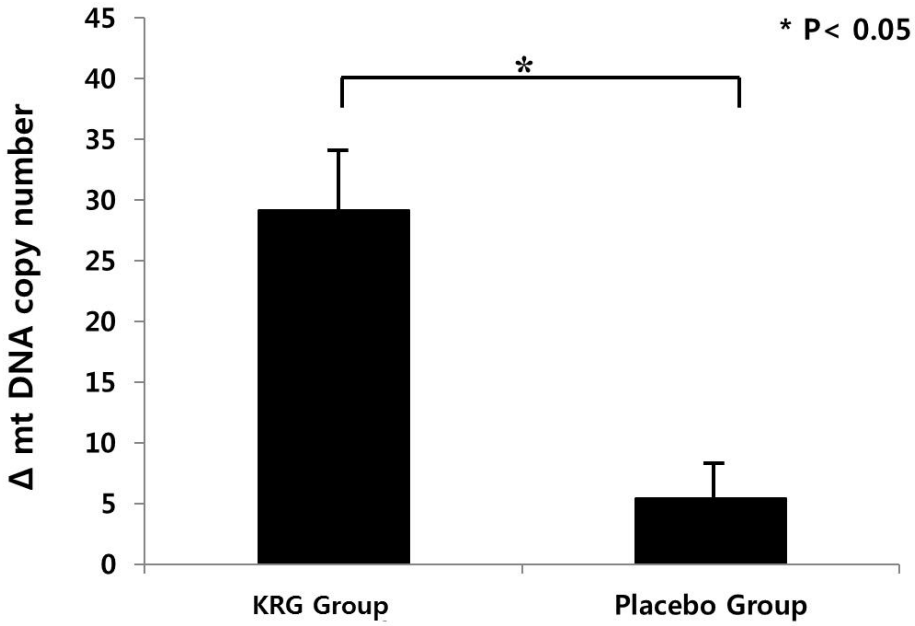

Figure 2. Mean change of leukocyte mtDNA copy number for the study population adjusted for liver enzyme and age, between KRG group and placebo group after 4 weeks treatment.

IV. DISCUSSION

In this double-blind, randomised, placebo-controlled study, we examined the effects of KRG on mitochondrial function in men with metabolic syndrome using mtDNA copy number and its effects on metabolic syndrome components and hormones. The results of our study show that RG increases mtDNA copy number, which suggests that KRG has a beneficial effect on metabolic syndrome by improving mitochondrial function, as a low mtDNA copy number is associated with high BP, insulin resistance, and obesity.[27,28] However, there was no difference in metabolic syndrome components, except diastolic BP after 4 weeks treatment, between the RG and placebo groups.

To our knowledge, this is the first study to examine the effects of KRG on mitochondria, metabolic syndrome, and related hormones simultaneously. Recent studies have reported that mitochondrial dysfunction is etiologically associated with the development of metabolic syndrome.[29] Mozhey et al. reported reduced amounts of mtDNA in peripheral blood from patients with metabolic syndrome.[30] Lee et al. reported that mtDNA decreased before the development of diabetes.[15] A decrease in ATP synthesis and reduced mitochondrial biogenesis are the features of mitochondrial dysfunction, which is closely associated with insulin resistance and atherosclerosis, a key pathogenic factor in the development of metabolic syndrome.[28] Furthermore, the underlying mechanism of metabolic syndrome is very complex and includes not only mitochondrial dysfunction but also inflammation and hormonal changes related to aging or stress.[31]

Thus, we assessed the systemic effect of KRG by performing blood tests to evaluate mitochondria function, inflammatory state, and hormones and by checking blood pressure.

In our study, the cause of increased mtDNA is thought to be multifactorial in patients with metabolic syndrome after 4 weeks of treatment. Several studies have shown that the effect of KRG could stem from either an improvement in antioxidant capacity or increased mitochondrial biogenesis. Chattopadhyay et al. reported that excessive formation of reactive oxygen species was found in

patients with metabolic syndrome, which could aggravate mitochondria function.[32] Scavengers such as catalase and MnSOD protect mitochondria from free radical damage, and an increase in catalase and MnSOD was observed in an exploratory study of ginseng treatment. In addition, Rg3 (ginsenoside) treatment also increased PGC-1α protein levels, supporting the concept that ginseng improves mitochondrial function.[33] Collectively, KRG supplementation appears to lead to improvements in mitochondrial function, and this finding indicates that ginseng may be an attractive candidate for the improvement of metabolic syndrome.

Recent studies have linked hormonal changes to metabolic syndrome, especially low testosterone and low IGF-1.[34,35] Hypogonadism is a common feature of metabolic syndrome.[36] Testosterone improves muscle function and increases glucose burning, and testosterone treatment has been shown to improve insulin resistance in humans.[37,38] Some animal studies have reported that ginseng increases blood testosterone levels.[20] In this study, we confirmed the effect of KRG on testosterone levels in men with metabolic syndrome. Mitochondria are key controllers of steroid hormone synthesis.[39] Thus, the improvement of mitochondrial function by administration of KRG may be associated with an increase in testosterone. Taken together, this mechanism may explain the interaction between hormones and mitochondria, and ultimately the alleviation of metabolic syndrome, by ginseng. More research is required to establish the cellular and molecular mechanism of the metabolic activities of ginseng.

In this clinical study, there was a significant change in the IGF-1 level after the administration of ginseng between the KRG and placebo groups. Although some studies reported that ginsenosides have an effect on the IGF-1 receptor and enhance the signal pathway of the IGF-1 receptor *in vitro*,[40] there are no reports of an effect of KRG on serum IGF-1 levels. Unlike testosterone, the relationship of IGF-1 and metabolic syndrome is less clear; many epidemiology studies have demonstrated that not only a low IGF-1 level but also a high IGF-1 level can be a risk factor for metabolic syndrome and mortality.[35] However, the average IGF-1 serum level of participants was 157 mg/dl, which is within the range of

replacement treatment; thus, an increase in the IGF-1 level may be beneficial for improving metabolic syndrome.

Chronic stress leads to consistent hyperactivity of the HPA axis.[41] HPA axis hyperactivity is a feature that has been demonstrated in cardiovascular disease, nervous disease and endocrine disease.[42,43] A recent epidemiologic study also suggested that alterations of HPA activity are observed in patients with hypertension, diabetes, and metabolic syndrome.[44] It has been proposed that KRG has a corticosteroid-like action. In an animal study, administration of ginseng decreased stress-induced plasma cortisol levels.[45] In this clinical study, the serum cortisol level also decreased in the KRG group after administration of KRG. Although the mechanism of action of RG on the HPA axis is not fully understood, ginseng may be beneficial in treating HPA-axis hyperactivity, which can be a risk factor for metabolic syndrome. This study had several limitations. One limitation of our study was the short duration. We hypothesized that daily supplementation of 3 g KRG for 4 weeks could improve metabolic syndrome and related risk factors based on a preliminary study (not published). Previous clinical studies associated with KRG involved various treatment durations from 2 weeks to 12 weeks. The optimal duration and schedule for KRG treatment requires further investigation. Another limitation was that we assessed peripheral leukocytes instead of muscle biopsies to measure mitochondrial function, which have often been investigated using muscle biopsies in disease studies. Although mtDNA copy number may vary among different organs, previous research has reported that changes in mtDNA levels of blood leukocytes reflect comparable processes in other tissues, such as muscle and hepatocytes. Thus, we hypothesized that the mtDNA copy number of peripheral leukocytes may be an indicator of mitochondrial function.

V. CONCLUSION

In conclusion, KRG had a favourable effect on anabolic hormones, and mitochondrial function in men with metabolic syndrome. However, we did not observe an improvement of metabolic syndrome components after 4 weeks of treatment, with the exception of diastolic BP. Although further studies are required to identify the optimal administration schedule and detailed mechanism, the use of KRG as a nutritional support is expected to be a clinically valuable option for mitochondria dysfunction and patients with metabolic syndrome.

Conflict of interest

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REFERENCES

1. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB: Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* 2005, 112:3066-3072. Epub 2005 Nov 3067.
2. Russo A, Autelitano M, Bisanti L: Metabolic syndrome and cancer risk. *Eur J Cancer* 2008, 44:293-297. Epub 2007 Dec 2004.
3. Sowers JR: Metabolic risk factors and renal disease. *Kidney Int* 2007, 71:719-720.
4. Wallerius S, Rosmond R, Ljung T, Holm G, Bjorntorp P: Rise in morning saliva cortisol is associated with abdominal obesity in men: a preliminary report. *J Endocrinol Invest* 2003, 26:616-619.
5. Bruehl H, Rueger M, Dziobek I, Sweat V, Tirsi A, Javier E, et al: Hypothalamic-pituitary-adrenal axis dysregulation and memory impairments in type 2 diabetes. *J Clin Endocrinol Metab* 2007, 92:2439-2445. Epub 2007 Apr 2410.
6. Vinson GP: Angiotensin II, corticosteroids, type II diabetes and the metabolic syndrome. *Med Hypotheses* 2007, 68:1200-1207. Epub 2006 Nov 1228.
7. Weigensberg MJ, Toledo-Corral CM, Goran MI: Association between the metabolic syndrome and serum cortisol in overweight Latino youth. *J Clin Endocrinol Metab* 2008, 93:1372-1378.
8. Krikorian A, Khan M: Is metabolic syndrome a mild form of Cushing's syndrome? *Rev Endocr Metab Disord* 2010, 11:141-145.
9. Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT: Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. *Int J Epidemiol* 2011, 40:189-207.
10. Jorgensen JO, Moller L, Krag M, Billestrup N, Christiansen JS: Effects of growth hormone on glucose and fat metabolism in human subjects. *Endocrinol Metab Clin North Am* 2007, 36:75-87.
11. De Pergola G: The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone. *Int J Obes Relat Metab Disord* 2000,

24:S59-63.

12. Haring R, Volzke H, Steveling A, Krebs A, Felix SB, Schofl C, et al: Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. *Eur Heart J* 2010, 31:1494-1501.
13. Blokhin A, Vyshkina T, Komoly S, Kalman B: Variations in mitochondrial DNA copy numbers in MS brains. *J Mol Neurosci* 2008, 35:283-287.
14. Macmillan CJ, Shoubridge EA: Mitochondrial DNA depletion: prevalence in a pediatric population referred for neurologic evaluation. *Pediatr Neurol* 1996, 14:203-210.
15. Lee HK, Song JH, Shin CS, Park DJ, Park KS, Lee KU, et al: Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 1998, 42:161-167.
16. Lee JE, Park H, Ju YS, Kwak M, Kim JI, Oh HY, et al: Higher mitochondrial DNA copy number is associated with lower prevalence of microalbuminuria. *Exp Mol Med* 2009, 41:253-258.
17. Yin J, Zhang H, Ye J: Traditional chinese medicine in treatment of metabolic syndrome. *Endocr Metab Immune Disord Drug Targets* 2008, 8:99-111.
18. Yun SN, Moon SJ, Ko SK, Im BO, Chung SH: Wild ginseng prevents the onset of high-fat diet induced hyperglycemia and obesity in ICR mice. *Arch Pharm Res* 2004, 27:790-796.
19. Feng Z, Bai L, Yan J, Li Y, Shen W, Wang Y, et al: Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol. *Free Radic Biol Med* 2011, 50:1437-1446.
20. Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W, Hafez ES: Effect of Panax ginseng on testosterone level and prostate in male rats. *Arch Androl* 1982, 8:261-263.
21. Xu Y, Ding J, Ma XP, Ma YH, Liu ZQ, Lin N: Treatment with Panax ginseng antagonizes the estrogen decline in ovariectomized mice. *Int J Mol Sci* 2014, 15:7827-7840.

22. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005, 112:2735-2752. Epub 2005 Sep 2712.
23. Kim JH, Im JA, Lee DC: The relationship between leukocyte mitochondrial DNA contents and metabolic syndrome in postmenopausal women. *Menopause* 2012, 19:582-587.
24. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001, 25:402-408.
25. Wang YC, Lee WC, Liao SC, Lee LC, Su YJ, Lee CT, et al: Mitochondrial DNA copy number correlates with oxidative stress and predicts mortality in nondiabetic hemodialysis patients. *J Nephrol* 2011, 24:351-358.
26. Jovanovski E, Bateman EA, Bhardwaj J, Fairgrieve C, Mucalo I, Jenkins AL, et al: Effect of Rg3-enriched Korean red ginseng (Panax ginseng) on arterial stiffness and blood pressure in healthy individuals: a randomized controlled trial. *J Am Soc Hypertens* 2014, 8:537-541.
27. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE: Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 2005, 54:8-14.
28. Huang CH, Su SL, Hsieh MC, Cheng WL, Chang CC, Wu HL, et al: Depleted leukocyte mitochondrial DNA copy number in metabolic syndrome. *J Atheroscler Thromb* 2011, 18:867-873. Epub 2011 Aug 2016.
29. Peinado JR, Diaz-Ruiz A, Fruhbeck G, Malagon MM: Mitochondria in metabolic disease: getting clues from proteomic studies. *Proteomics* 2014, 14:452-466.
30. Mozhei OI, Zatolokin PA, Vasilenko MA, Litvinova LS, Kirienkova EV, Mazunin IO: [Evaluating the mitochondrial dna copy number in leukocytes and adipocytes from metabolic syndrome patients: pilot study]. *Mol Biol (Mosk)* 2014, 48:677-681.
31. Litvinova L, Atochin DN, Fattakhov N, Vasilenko M, Zatolokin P,

Kirienkova E: Nitric oxide and mitochondria in metabolic syndrome. *Front Physiol* 2015, 6:20.

32. Chattopadhyay M, Khemka VK, Chatterjee G, Ganguly A, Mukhopadhyay S, Chakrabarti S: Enhanced ROS production and oxidative damage in subcutaneous white adipose tissue mitochondria in obese and type 2 diabetes subjects. *Mol Cell Biochem* 2015, 399:95-103.
33. Sun M, Huang C, Wang C, Zheng J, Zhang P, Xu Y, et al: Ginsenoside Rg3 improves cardiac mitochondrial population quality: mimetic exercise training. *Biochem Biophys Res Commun* 2013, 441:169-174.
34. Brand JS, Rovers MM, Yeap BB, Schneider HJ, Tuomainen TP, Haring R, et al: Testosterone, sex hormone-binding globulin and the metabolic syndrome in men: an individual participant data meta-analysis of observational studies. *PLoS One* 2014, 9(7):e100409.
35. van Bunderen CC, Oosterwerff MM, van Schoor NM, Deeg DJ, Lips P, Drent ML: Serum IGF1, metabolic syndrome, and incident cardiovascular disease in older people: a population-based study. *Eur J Endocrinol* 2013, 168:393-401.
36. Hackett G, Kirby M, Sinclair AJ: Testosterone deficiency, cardiac health, and older men. *Int J Endocrinol* 2014, 2014: :143763.
37. Kapoor D, Goodwin E, Channer KS, Jones TH: Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol* 2006, 154:899-906.
38. Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, Moncada I, et al: Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care* 2011, 34:828-837.
39. Duarte A, Castillo AF, Podesta EJ, Poderoso C: Mitochondrial Fusion and ERK Activity Regulate Steroidogenic Acute Regulatory Protein Localization in Mitochondria. *PLoS One* 2014, 9(6):e100387..
40. Chen WF, Lau WS, Cheung PY, Guo DA, Wong MS: Activation of insulin-like growth factor I receptor-mediated pathway by ginsenoside Rg1. *Br J Pharmacol* 2006, 147:542-551.

41. Rasheed N, Tyagi E, Ahmad A, Siripurapu KB, Lahiri S, Shukla R, et al: Involvement of monoamines and proinflammatory cytokines in mediating the anti-stress effects of Panax quinquefolium. *J Ethnopharmacol* 2008, 117:257-262. Epub 2008 Feb 1018.
42. Rosmond R, Dallman MF, Bjorntorp P: Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* 1998, 83:1853-1859.
43. O'Brien JT, Lloyd A, McKeith I, Gholkar A, Ferrier N: A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. *Am J Psychiatry* 2004, 161:2081-2090.
44. Knoops AJ, Gerritsen L, van der Graaf Y, Mali WP, Geerlings MI: Basal hypothalamic pituitary adrenal axis activity and hippocampal volumes: the SMART-Medea study. *Biol Psychiatry* 2010, 67:1191-1198.biopsych.2010.1101.1025. Epub 2010 Mar 1117.
45. Kim DH, Jung JS, Suh HW, Huh SO, Min SK, Son BK, et al: Inhibition of stress-induced plasma corticosterone levels by ginsenosides in mice: involvement of nitric oxide. *Neuroreport* 1998, 9:2261-2264.

ABSTRACT (IN KOREAN)

대사증후군 환자에서 홍삼이 호르몬 합성과 미토콘드리아 기능에 미치는 영향

<지도교수 **김 춘 배** >

연세대학교 대학원 의학과

정 동혁

1. 연구 배경

대사증후군은 고혈압, 당뇨, 고지혈증뿐 아니라 사망률 증가와 관련된 뇌혈관 및 심혈관질환 발생의 뚜렷한 위험인자이다. 이미 알려진 위험인자인 비만 외에도, 여러 연구들에서 남성호르몬과 성장호르몬의 저하가 대사증후군의 발생 위험인자로 보고되고 있다. 남성호르몬을 포함한 스테로이드 호르몬의 합성이 미토콘드리아와 관련이 있기 때문에 미토콘드리아의 기능 저하와 호르몬 합성 저하 및 대사증후군이 복합적으로 관련이 있음이 보고되고 있다. 최근의 임상 및 동물연구에서 홍삼 복용이 미토콘드리아의 기능 개선과 호르몬의 증가와 관련이 있음이 보고되고 있다. 대사증후군의 지표인 인슐린 저항성과 동맥경화증의 개선 효과가 있음도 보고되고 있다.

2. 연구 목표

이번 연구에서는 대사증후군 환자에서 홍삼의 복용이 대사증후군 지표의 개선에 영향이 있는지 알아보고자 한다. 또한 대사증후군과 관련되어 있는 미토콘드리아의 기능 개선과 성장 호르몬과 남성 호르몬의 변화도 함께 살펴보고자 한다.

3. 연구 방법

대사증후군을 가진 남성 환자 62명을 대상으로 한 무작위배정 대조 임상시험으로써 실험군은 3g의 홍삼을 4주간 복용하고, 위약을 복용한 대조군과 대사증후군 지표의 변화를 비교해 보았다. 또한 혈액에서 미토콘드리아 유전자의 양을 대변하는 copy number와 남성 호르몬과 성장 호르몬, 코티졸과 DHEAs 호르몬의 변화를 비교해 보았다.

4. 결과

실험군에서 4주간의 홍삼 복용 후 대조군과 비교 시, 대사증후군 지표중에서는 이완기 혈압의 감소가 있었으며, 미토콘드리아의 copy number가 증가하였고, 남성호르몬과 성장호르몬의 증가가 관찰되었다.

5. 결론

남성 대사 증후군 환자에서 홍삼의 복용은 혈압의 변화를 포함하여, 미토콘드리아의 기능 향상과 더불어, 대사증후군 개선과 관련된 여러 동화 호르몬의 증가에 도움이 될 수 있다.

핵심되는 말: 홍삼, 미토콘드리아, 호르몬, 대사증후군

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