Korean red ginseng improves metabolic parameters, mitochondrial DNA copy numbers and inflammatory markers in type 2 diabetes mouse

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The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

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June 2014

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June 2014

ACKNOWLEDGEMENTS

First of all, thank you God, you made everything possible. I would take the opportunity to thank my supervisor, Professor Duk Chul Lee, for providing guidance at any time and most valuable suggestions throughout the graduate school days. Despite repeated failure of the experiment, his timely and proper guidance could make this thesis and complete the doctoral dissertation. In particular, I would like to thank Professors, Sang Wha Lee, Jong Rak Choi, Jae-woo Kim and Yong-Jae Lee. Because of their poignant reproach, I was able to move a step further in my paper and commit less mistakes. And without the advice and help from my colleagues, my theory could not become a real one. Finally I am grateful to my family. Without their love and support, I could not have start the PhD map nor finish. This thesis should be dedicated to my parents, my husband, my son and my unborn child.

Another new chapter in my life begins.

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ABSTRACT

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Introduction : Diabetes mellitus is characterized by insulin resistance of target organ and dysfunction of pancreas. Although the underlying whole mechanisms remain unclear, type 2 diabetes mellitus is associated with mitochondrial dysfunction including mitochondrial loss and over-production of oxidants. In addition to the traditional method of strict glucose control, the mechanisms of several traditional herbal medicines which have been known to have glucose lowering effect are based on anti-inflammatory and anti - oxidative action. In Korea, Korean red ginseng is most famous herbal medicines to have anti-diabetic effect. Some studies revealed that ginseng had anti-diabetic efficacy depends on improvement of systemic inflammatory biomarkers but the mechanism of anti-diabetic efficacy is not fully understood. The aim of this study was to investigate the effects of Korean red ginseng supplementation on metabolic parameters including fasting blood glucose concentrations, insulin sensitivity and lipid profiles. We also examined whether regulation of Korean red ginseng is partly mediated through mitochondrial metabolism such as biogenesis and/or decrease of intracellular inflammation levels in an animal model of type 2 diabetes mellitus.

Method : C57BL/KsJ *db/db* mice and C57BL/KsJ *db/+* mice were divided into 3 groups: *db/+* mice with chow diet (n=8, control group), *db/db* mice with high-fat diet (n=8, db/db control group), and *db/db* mice with high-fat diet and

Korean red ginseng administration (n=7, db/db ginseng group) for 12 weeks. After 12 weeks, metabolic parameters including fasting blood glucose concentrations, hemoglobin A_{1C} ($HbA1_c$), insulin and lipid profiles were determined using high-performed liquid chromatography. Mitochondrial DNA (mt DNA) was measured using qPCR. The expression of mitochondrial biogenesis markers including PGC-1α and T-fam and the expression of inflammatory markers such as IL-6, COX-2, and CRP were measured in liver tissues using quantitative real time PCR analysis.

Result : Mean body weight were not significantly different between db/db control group and db/db/ ginseng group. And after 7 weeks, db/db ginseng group showed significantly lower mean fasting blood glucose level than that of db/db control group. Compared with db/db control group, glucose index (fasting glucose, HbA1c, insulin, HOMA-IR) and LDL cholesterol levels are significantly lowered in db/db ginseng group. And the mitochondrial DNA copy numbers were also significantly higher in db/db ginseng group than those in db/db control group. After Korean red ginseng administration, PGC-1α and TFAM were significantly decreased and TFAM was even more decreased than that of control group. Inflammatory protein precursors IL-6, COX-2, and CRP were significantly increased in db/db control group. Korean red ginseng administration made mRNA expression related to IL-6, COX-2 and CRP significantly decreased even more than the control group, so the inflammatory score was calculated and were significantly increased in db/db control group and significantly decreased in db/db ginseng group.

Conclusion : Korean red ginseng improved blood glucose levels and insulin sensitivity and it may be correlated with mitochondrial function and oxidative inflammatory stress. Korean red ginseng could be a useful additive nutraceutical in type 2 diabetes mellitus.

Key words : Korean red ginseng, diabetes, insulin resistance, mitochondria, inflammation, oxidative stress

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I. INTRODUCTION

Diabetes mellitus is characterized by insulin resistance of target organ and dysfunction of pancreas. Although the underlying whole mechanisms of type 2 diabetes mellitus remain unclear, insulin resistance is thought to be correlated with hyperglycemia and increased oxidative stress[1,2]. Hyperglycemia induces overproduction of superoxide and other free radicals and also induces impairment of antioxidant defense mechanism (free radical scavenging systems) by mitochondria in liver, muscle, heart and pancreas. And hyperglycemia-induced cellular oxidative stress and increased mitochondrial free radical production is thought to be a basic mechanism of diabetes initiation, progression and complication development[3,4]. Increasing evidence supports that type 2 diabetes mellitus is correlated with mitochondrial dysfunction including mitochondrial loss and over-production of oxidants[3,5,6].

Mitochondrial dysfunction is the reduction of ATP generation and mitochondrial number in skeletal muscle. And also it is the reduction of ATP generation and mitochondrial stimulus-secretion coupling in the pancreatic beta cell[7-10]. Especially, both decreases in mtDNA copy number and mutations in mitochondrial DNA (mtDNA) have been thought to be linked to the pathogenesis of type 2 diabetes mellitus[2].

And hyperglycemia-induced cellular oxidative stress and increased mitochondrial free radical production is correlated inflammation. The ATP generation from the electron transport chain can produce reactive oxygen species (ROS) as intermediates and electron leakage also be able to make ROS[11]. If ROS react directly with cellular compounds like lipids, proteins, and DNA, intracellular inflammation is occurred and aggravated to cellular damage. High intracellular concentration of glucose induces oxidative stress increase, upregulation of cyclooxygenase (COX2), nitric oxide availability reduction and prostanoid profile alteration[12]. Tumor necrosis factor alpha (TNFα) and IL-6, such as inflammatory cytokines which are increased during the chronic low grade inflammation of type 2 diabetes mellitus, make mitochondrial ROS increment and aggravate intracellular inflammation[13]. On the contrary, mitochondrial ROS provoke the up-regulation of inflammatory cytokines like IL-6 as signal-transducing molecules[14].

And recently C-reactive protein (CRP) has been shown to induce insulin resistance[15, 16]. CRP is a protein found in the blood and it is correlated inflammation. CRP is synthesized by the liver and released by the factors of macrophages and adipocyte[17-19]. And the mechanism of inducing insulin resistance is actively conducted lately[20, 21].

In addition to the traditional method of strict glucose control, recent clinical trials indicate that restoration of reduced mitochondrial function is also effective to prevent the development and progression of diabetes and diabetic complications[22-24]. And the mechanisms of several traditional herbal medicines which have been known to have glucose lowering effect are based on anti-inflammatory and anti-oxidative action[25]. For example, plant extracts such as green tea, *Ajuga iva* or *Cecropia pachystachya* are effective of oxidative stress reduction and tissue damage prevention in diabetes[26-28]. In Korea, Korean red ginseng is most famous herbal medicines to have anti-diabetic effect[29]. Chinese/Korean ginseng, *Panax ginseng*, has been reported to have anti-inflammatory and anti-diabetic activities[30-32]. The active compounds of ginseng are ginsenosides or panaxosides which are a class of steroid glycosides

and triterpene saponins. Ginsenoside can vary widely depending on ginseng species, growth surroundings and age. Ginsenoside are often devided to two groups: the Rb1 group and the Rg1 group. The Rg1 group, especially Rf, Rg1 and Rg2 are abundant in Chinese/Korean ginseng and 38 ginsenosides were found in Korean ginseng. Some studies revealed that ginseng had anti-diabetic efficacy depends on improvement of systemic inflammatory biomarkers but the mechanism of anti-diabetic efficacy is not fully understood[33-36].

The aim of this study was to investigate the effects of Korean red ginseng supplementation on metabolic parameters including fasting blood glucose concentrations, insulin sensitivity and lipid profiles. We also examined whether regulation of Korean red ginseng is partly mediated through mitochondrial metabolism such as biogenesis and/or decrease of intracellular inflammation levels in an animal model of type 2 diabetes mellitus.

II. MATERIALS AND METHODS

1. Materials

A. Animals

The study protocol and all procedures were approved by the Institutional Animal Care and Use Committee at the Yonsei Institute for Life Sciences, Seoul, Korea. C57BL/KsJ *db/db* mice and C57BL/KsJ *db/+* mice were purchased from Joong-Ang Lab (Seoul, Korea). All animals were 4 weeks old male and acclimatized to the laboratory environment for 2 weeks prior to the initiation of experiments. Mice were maintained under specific pathogen-free conditions in a constant room temperature (22±2℃) and humidity (50±10%). Mice were free of access to water and food with an automatic 12-hour light-dark cycles. Mice were fed a high fat diet

comprised of 72% lipids, 28% protein, and <1% carbohydrates. Food intake amount and body weight were recorded daily and glucose concentrations were recorded weekly for 12 weeks.

B. Korean ginseng powder

Korean ginseng powder was provided by Korean Tobacco and Ginseng (Daejon, Korea). Korean ginseng powder was dissolved with 1cc of distilled water and injected into the intra-peritoneal cavity on db/db ginseng group once daily at a dose of 100mg/kg body weight (approximately 0.2 ml in a volume). Db/db control group was received equivalent saline injection. Control group was not received any injection or treatment. No skin irritation or other adverse effects was observed after daily administration.

2. Methods

A. Experimental design

The mice were allowed to acclimatize to the laboratory environment for 2 weeks before experiments. After acclimatization, all mice were divided into three groups; normal control mice with chow diet group (control group, n=8), *db/db* mice with high fat diet group (db/db control group, n=8), and *db/db* mice with high fat diet and Korean ginseng group (db/db ginseng group, n=8). But during experiment, a mouse in db/db ginseng group had been died at experimental day 67. The cause of death is still unknown. So in db/db ginseng group, 7 mice survived at the end of experiment.

B. Measurement of body weight and blood glucose level

Fasting blood glucose was determined in blood samples obtained by pricking the tail of mice and tested using a Glucose Analyzer (Hemocue AB, Angelholm, Sweden) on initial (before treatment) and every week. Mice were 4 hour fasting status when blood sampling and body weight measurement.

C. Tissue sampling and biochemical analysis

After 12 weeks, all 23 mice were sacrificed under light ether anesthesia. Blood samples of fasting plasma glucose, hemoglobinA1c (HbA1c), insulin, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol were obtained by exsanguinations of the heart. These were determined using high performed liquid chromatography (SIMADZU, Kyoto, Japan). Insulin resistance was estimated using the homeostasis model assessment estimate of insulin resistance (HOMA-IR). Liver, pancreas and gastrocnemius muscles were taken and each sample was quickly frozen in liquid nitrogen and kept at -80℃ until analysis. Because of inappropriate storage and too small amount of tissue, only liver tissue was capable of analysis.

D. Quantitative real time PCR analysis

Total RNA was isolated from liver with TRIzol (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instruction. From each sample, total RNA (3 μg) was reverse transcribed into cDNA using a reverse transcription system kit (Thermo Fisher, Middlesex County, Massachusetts, USA). Briefly, quantitative RT-PCR reactions were performed as described in the manufacturer's instructions and analyzed with Power SYBR green Master Mix (Biosystems, Barcelona, Spain) using an ABI 7500

Real-Time PCR system(Biosystems, Barcelona, Spain) with the following cycling parameters: stage 1, 50℃ for 2 minutes; stage 2, 95℃ for 10 minutes; stage 3, 40 cycles for 95℃ for 15 seconds; 60℃ for 1 minutes; and stage 4, 95℃ for 15 seconds; 60℃ for 15 seconds; 95℃ for 15 seconds. Primers (Table 1) were designed using Primer Express software (Biosystems, Barcelona, Spain). Mouse target genes consisted of peroxismal proliferator-activated receptor-γ coactivator -1α (PGC-1α) and mitochondrial transcription factor A (mtFAM or T-fam) for mitochondrial biogenesis markers and interleukine-6 (IL-6), cyclooxygenase-2 (COX-2), and C-reactive protein (CRP) as inflammatory markers (Table 1). The linearity of dissociation curves was analyzed using ABI 7500 software, and the data were analyzed by the comparative method ($2^{-\Delta\Delta Ct}$) using an internal control. Each sample was analyzed in duplicate.

Inflammatory scores to quantify the inflammatory state and to show an additive effect of inflammatory markers of Korean red ginseng were inspired by other studies[37-39]. Inflammatory cores were the sums of mRNA levels of IL-6, COX-2 and CRP.

E. Mitochondrial DNA analysis

Mitochondrial DNA (mt DNA) was measured using qPCR. Briefly, total DNA was extracted with a QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). The ratio of mtDNA to nuclear DNA, which reflects the tissue concentration of mtDNA per cell, was subsequently determined. Targeted genes included nuclear cystic fibrosis (CF) and mitochondrial nicotinamide adenine dinucleotide dehydrogenase-5(ND5). For nuclear DNA quantification, 10ng DNA was used as a template. Mouse specific primers (sequences reported in Table 1) were selected using Primer Express Software (Biosystems, Barcelona, Spain).

Table 1. Primers used for PCR analysis

Gene		Sequence
CF	Sense	5’-TGTTGTGAAGACGAGCTGATGTAAAG-3’
	Antisense	5’-TGCATTAAAAGAGAGCATGTGTTG-3’
ND5	Sense	5’-TGGATGATGGTACGGACGAA-3’
	Antisense	5’-TGCGGTTATAGAGGATTGCTTGT-3’
β-Actin	Sense	5’-GGAAAAGAGCCTCAGGGCAT-3’
	Antisense	5’-GAAGAGCTATGAGCTGCCTGA-3’
PGC1α	Sense	5’-ACTATGAATCAAGCCACTACAGAC-3’
	Antisense	5’-TTCATCCCTCTTGAGCCTTTCG-3’
Tfam	Sense	5’-AAGACCTCGTTCAGCATATAACATT-3’
	Antisense	5’-TTTTCCAAGCCTCATTTACAAGC-3’
IL-6	Sense	5’-TCCTACCCCAACTTCCAATGCTC-3’
	Antisense	5’-TTGGATGGTCTTGGTCCTTAGCC-3’
COX-2	Sense	5’-TGACCCCCAAGGCTCAAATAT-3’
	Antisense	5’-TGAACCCAGGTCCTCGCTTA-3’
CRP	Sense	5’-CCATTTCTACACTGCTCTGAGCAC-3’
	Antisense	5’-CCAAAATATGAGAATGTCGTTAGAGTTC-3’

F. Statistical analysis

Results are presented as mean ± S.D or S.E. Data was compared using ANOVA test and as post hoc analysis, Student’s T test. All analyses were conducted using SAS statistical software, version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). All statistical tests were 2-sided and the threshold for statistical significance was set at P-value <0.05.

III. RESULTS

1. Changes of mean body weight

Mean body weights of each group are increased during experiment (Figure 1). There were no significant differences in mean body weight between the db/db control group (*db/db* mice with high fat diet group) and db/db ginseng group (*db/db* mice with high fat diet and Korean ginseng group) before experimental day 20. But after 57 days, db/db ginseng group showed a little lighter mean body weight than db/db control group almost 2-3g. But error bar of each days were overlapped in almost experimental days. So the mean body weight difference is statistically insignificant (p value = 0.35 ~ 0.71).

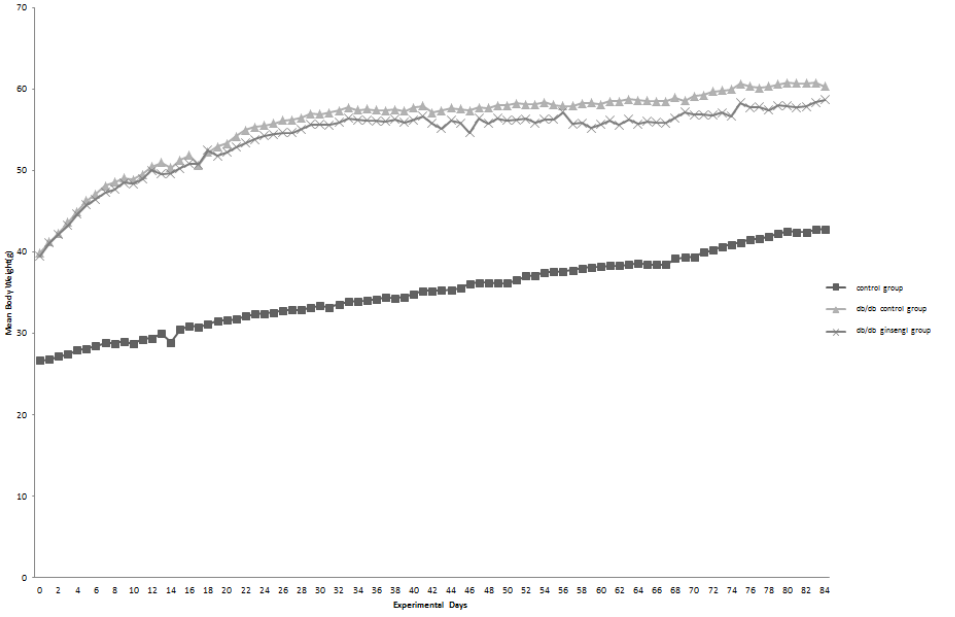

Figure 1. Changes of mean body weight

2. Effects of Korean red ginseng on fasting blood glucose

Mean blood glucose levels of each group are measured weekly in four-hour fasting status after daily intra-peritoneal administration of Korean red ginseng or saline. In db/db ginseng group, Korean ginseng powder was dissolved with 1cc of distilled water and injected into the intra-peritoneal cavity once daily at a dose of 100mg/kg body weight (approximately 0.2 ml in a volume). Db/db control group were received equivalent saline injection. As shown in figure 2, the db/db control group and db/db ginseng group had higher mean blood glucose levels compared with control group and the db/db control group and db/db ginseng group showed no significant difference until 8 weeks. But after 9 weeks, db/db ginseng group showed significantly lower mean fasting blood glucose level than that of db/db control group(P value <0.05).

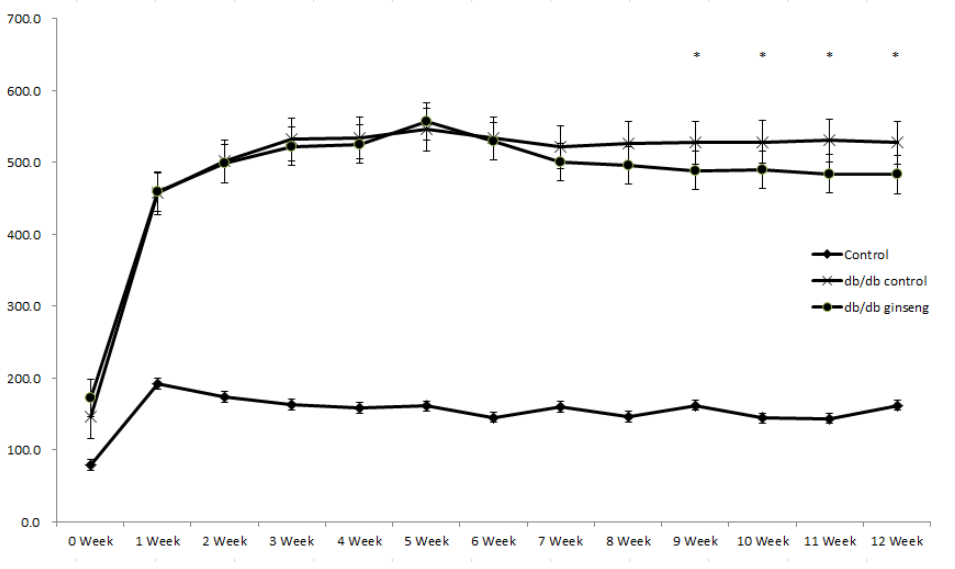

Figure 2. Effects of Korean red ginseng on fasting blood glucose

* P value <0.05 versus db/db control group

3. Effects of Korean red ginseng on metabolic parameters

The changes of metabolic parameters according to Korean red ginseng treatment are shown in the table 2. Compared with db/db control group, glucose index (fasting glucose, HbA1c, insulin, HOMA-IR) and LDL cholesterol levels in blood are significantly lowered in db/db ginseng group. Mean fasting glucose level was significantly decreased to 484.5±47.4mg/dl in Korean red ginseng treatment group compared with 537.5±47.5mg/dl in db/db control group (p value 0.015). And HbA1c, HOMA-IR and insulin levels in db/db ginseng group were also significantly decreased compared with db/db control group (p value 0.027, 0.001, 0.042). LDL cholesterol is significantly decreased (p value 0.04) but other cholesterol parameters like total cholesterol, triglyceride, and HDL cholesterol are not significantly different between db/db ginseng group and db/db control group (p value 0.487, 0.348, 0.318).

Table 2. Effects of Korean red ginseng on metabolic parameters

	Control Group (n=8)	Db/db control Group (n=8)	Db/db ginseng group (n=8)
Fasting glucose (mg/dl)	172.3±35.9	537.5±47.5*	484.5±47.4*†
HbA1c[1] (%)	3.3±0.4	8.4±1.5*	6.9±0.6*†
Insulin (μIU/mL)	10.6±2.7	15.2±6.1*	12.1±5.2*†
HOMA-IR[2]	4.2±1.0	26.9±7.0*	14.5±5.4*†
Total cholesterol (mg/dl)	158.1±22.7	177.1±53.7*	164.7±22.7
Triglyceride (mg/dl)	47.3±13.8	71.9±53.7*	80.1±32.8
HDL cholesterol[3] (mg/dl)	79.2±12.6	83.6±23.4	77.9±10.5
LDL cholesterol[4] (mg/dl)	69.5±11.6	79.4±23.0*	70.1±14.6†

[1] hemoglobinA1c

[2] the homeostasis model assessment estimate of insulin resistance

[3] high-density lipoprotein cholesterol

[4] low density lipoprotein cholesterol

* P value <0.05 versus control group

† P value <0.05 versus db/db control group

4. Effects of Korean red ginseng on mitochondrial DNA contents and proteins related to mitochondrial biogenesis

Compared with the control group, the mitochondrial DNA copy numbers in liver were decreased in db/db control group and db/db ginseng group (Figure 3). But in db/db ginseng group, the mitochondrial DNA copy numbers were significantly higher than those in db/db control group.

We also performed immune-blotting of proteins as peroxisome proliferator activated receptor gamma coactivator-1 alpha (PGC-1α) and mitochondrial transcription factor A (mtTFA or TFAM) associated with mitochondrial biogenesis on the liver among the three groups (Figure 4) using mouse liver tissue. Compared with the control group, the abundance of PGC-1α and TFAM were significantly increased in the other groups and it may be a compensatory mechanism. After Korean red ginseng administration, PGC-1α and TFAM were significantly decreased and TFAM was even more decreased than that of control group.

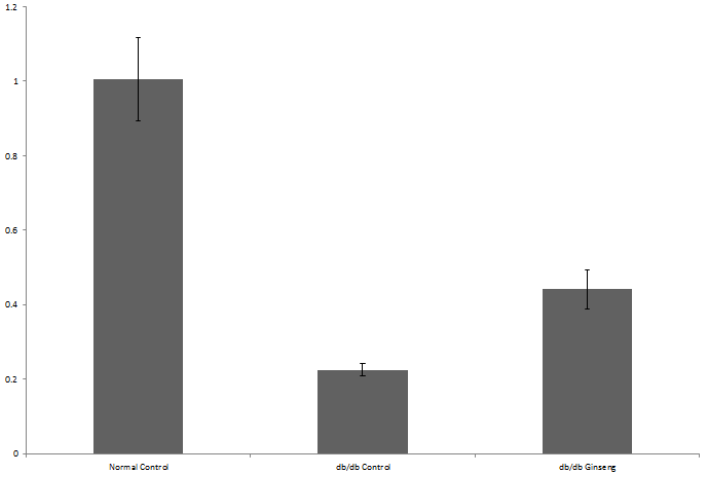

Figure 3. Effects of Korean red ginseng on mitochondrial DNA copy numbers, Data are mean ± S.E.

* P value <0.05 versus control group

† P value <0.05 versus db/db control group

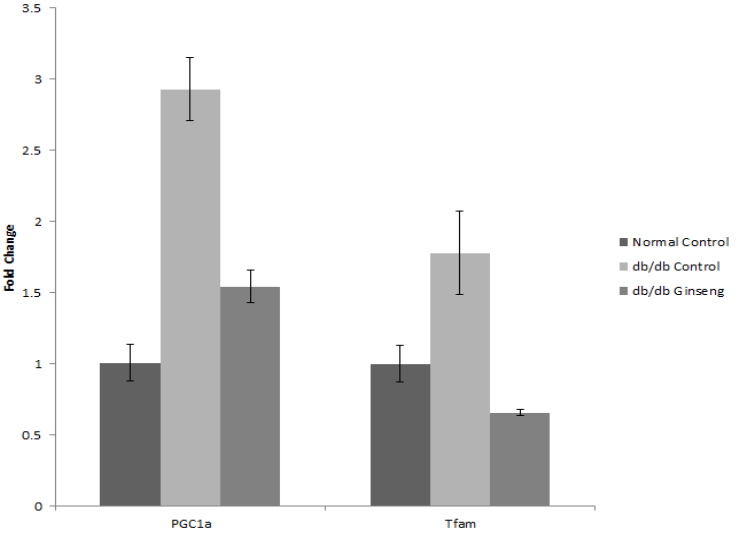

Figure 4. Effects of Korean red ginseng on expression of proteins related to mitochondrial biogenesis, Data are mean ± S.E.

* P value <0.05 versus control group

† P value <0.05 versus db/db control group

5. Effects of Korean red ginseng on inflammatory markers

Compared with the control group, the inflammatory protein precursors IL-6, COX-2, and CRP were significantly increased in db/db control group. Korean red ginseng administration made mRNA expression related to IL-6, COX-2 and CRP significantly decreased even more than the control group (Figure 5).

And an inflammatory score was calculated by summing the levels of mRNA expression of IL-6, COX-2, and CRP. The inflammatory scores were significantly increased in db/db control group and significantly decreased in db/db ginseng group (Figure 6).

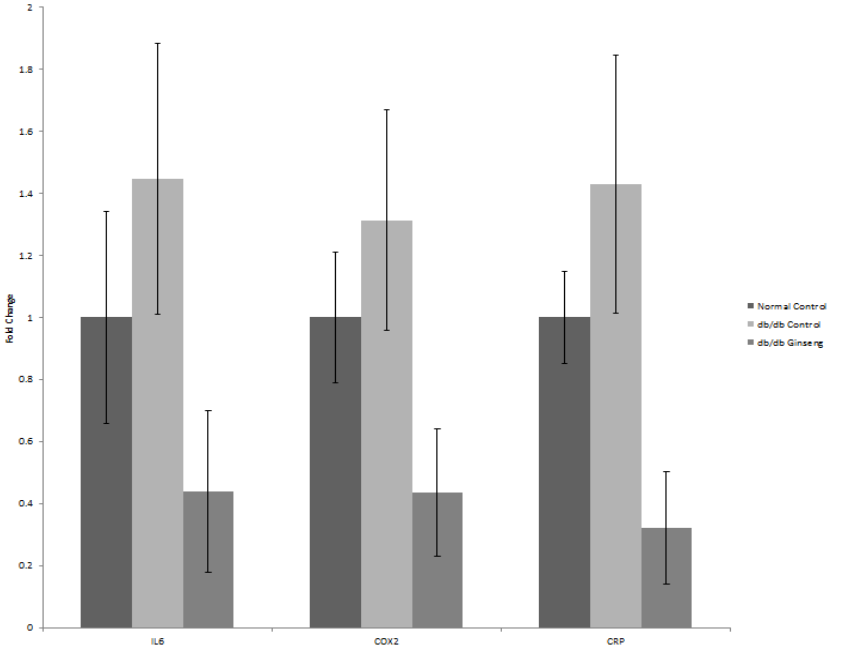

Figure 5. Effects of Korean red ginseng on expression of mRNA related to inflammatory markers, Data are mean ± S.E.

IL-6: interleukin 6, COX-2: cyclooxygenase 2, CRP: C-reactive protein

* P value <0.05 versus control group

† P value <0.05 versus db/db control group

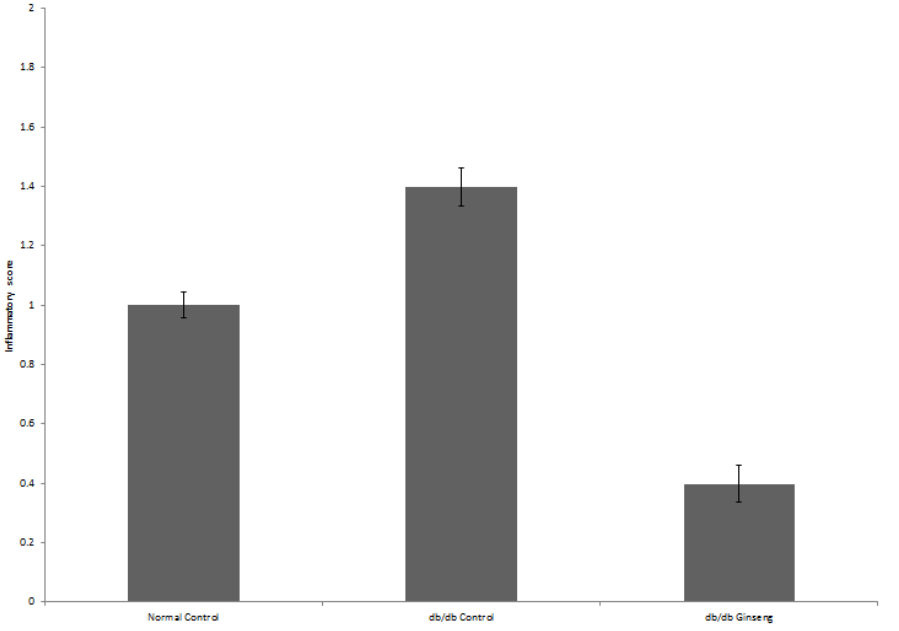

Figure 6. Effects of Korean red ginseng on inflammatory scores

Data are mean ± S.E.

* P value <0.05 versus control group

† P value <0.05 versus db/db control group

IV. DISCUSSION

In our experimental design, C57BL/KsJ mouse was a db/db mouse and it is naturally developing diabetes without high fat diet. It is rudimentary mistakes because of confusion C57BL/KsJ mouse with C57BL/6J mouse[40]. It is thought that high fat diet can do negative influence on ginseng's protective effect of body weight, metabolic parameters like blood glucose, mtDNA copy numbers and inflammatory markers. In other experiments with chow diet, body weight were almost 50g to 66g and blood glucose level was 364.5mg/dL to 624mg/dL at 12 weeks after birth[41,42]. In our experiment, mean body weight was almost 55-57g and blood glucose level was almost 500mg/dL at 12 weeks after birth and 6 weeks after experiment. So, high fat diet was thought to have no significant effect on their body weight but blood glucose level was slightly higher ranger.

High fat diet was given to db/db control group and also to db/db ginseng group. So comparison of both groups was available. And this design may be representative for the normal diet people without diabetes factor, the high fat diet people with diabetic factor, and the high fat diet and ginseng people with diabetic factor.

And because of inappropriate storage and too small amount of tissue, only liver tissue was capable of analysis. It remains regret because gastrocnemius muscles were thought to be the most appropriate tissue to show mitochondrial activity and function.

In this experiment, the effect of Korean red ginseng on type 2 diabetes mellitus is based on improvement of mitochondrial dysfunction and intracellular inflammation in mouse model. After 12 week Korean red ginseng administration, db/db ginseng group showed slightly and not significant decreased body weight gain but significantly decreased mean blood glucose level. HbA1c, insulin and HOMA-IR are significantly improved in db/db ginseng group almost less than 90% of db/db control group. According to the previous study, anti-diabetic effect of ginseng is not associated with body weight changes[43]. Considering other previous experiments, Korean red ginseng showed different anti-diabetic effects depended on treatment duration, amount and the active substance[42,44,45]. After 8 weeks red ginseng treatment of 100mg/kg, antidiabetic effect was not significant in a study[42] but effective that blood glucose level was almost 10% decreased than control group in another study[44]. After 6 weeks treatment of active substance, almost 15% decrease of blood glucose level was found in recent study[45]. Although less effective than previous antidiabetic drug[46], Korean red ginseng showed also anti-diabetic effect in our study like other studies.

In cholesterol analysis, LDL cholesterol was significantly decreased in db/db ginseng group. But total cholesterol, triglyceride and HDL cholesterol were not significantly changed. Some studies about ginseng and its anti-hyperlipidemic efficacies had showed that there had been a significant reduction in total cholesterol and triglyceride levels in ginseng treated animals[47-49]. Another study had showed that HDL cholesterol increase and triglyceride, very low density

lipoprotein cholesterol level decrease in type 2 diabetic Goto-Kakizaki rats on growth-age dependent therapeutic effects[50]. So the differentiation of anti-hyperlipidemic efficacies might be due to variation in both the ratios and concentrations of specific bioactive ginsenosides in ginseng of different growth ages. In this study, Korean red ginseng power was 6-year growth age.

Mitochondrial DNA copy numbers and expression of mitochondrial biogenesis related proteins like PGC1α and T-fam in the liver were also improved in db/db ginseng group. Moreover, T-fam expression is decreased in Korean red ginseng treated group than db/db control group and more than control group. In liver, PGC-1α is an important regulator of the gluconeogenesis and controls tissue-specific gene expression via interaction with liver-enriched transcription factors such as hepatocyte nuclear factor (HNF4α) and other transcription factors[51]. And it is activated in diabetic liver to elevate PGC-1α induced glucose production as it is in the fasted state that contributes to circulating hyperglycemia[52,53]. In this study, PGC1α expression was also elevated in liver of diabetic mouse and decreased in Korean red ginseng treated group. Another mitochondrial biogenesis related proteins, T-fam expression is a key activator of mitochondrial transcription as well as a participant in mitochondrial genome replication where it is essential for mitochondrial DNA expression and maintenance[2]. Protein levels of T-fam had been known to be increased in the liver of diabetic mice[54]. In our study, this was also seen and T-fam was decreased in Korean red ginseng treated diabetic mice. In additional western blot analysis of PGC1-α and T-fam, the effect of Korean red ginseng treatment was not significant. The reason of inconsistency is not clear but improper storage or technical error is suspected.

Mitochondrial DNA copy numbers were also decreased in diabetic mouse liver but markedly increased in liver of Korean red ginseng treatment group. A number of studies had showed reduction of mRNA for mitochondrial genes, decrease of mitochondrial DNA and lower protein expression of respiratory chain subunits in mitochondria in diabetes mellitus[55]. In a mouse study about ginsenoside Rd and stroke, ginsenoside protected mitochondria from reperfusion

injury as indicated by lowered mitochondrial hydrogen peroxide production and hyperpolarized mitochondrial membrane potential[56]. And in other study, mitochondrial DNA copy number and complex protein levels were increased in Sprague-Dawley (SD) rat cardiac muscle after ginsenoside Rg3 administration[57]. But any previous study did not describe the effect of Korean red ginseng on mitochondrial DNA copy numbers in diabetic mouse liver.

And mRNA related to intracellular inflammatory markers like IL6, COX2 and CRP in liver of Korean red ginseng treated diabetic mouse are decreased more than control group, too. A lot of previous studies had shown that inflammatory markers were elevated in diabetes like TNF-α, IL-6, Cox 2 and CRP[37-39,58,59]. A study had found that ginseng had altered CRP on diabetic rats and concluded ginseng might have improved diabetes and its complications by alleviation of inflammation[60]. Another study also had found that ginsenoside Rh1 ameliorated TNF-α and IL-6 in high fat diet induced obese mouse by inhibiting adipocyte differentiation[61]. A study about diabetic retinopathy, Panax notoginseng decreased the expression of inflammatory factors including IL-6, TNF-α, nuclear factor kappaB (NF-κB), and other inflammatory markers in retinal vasculature of diabetic rats[62]. In ICR mouse liver, ginsenoside Rd had inhibited the expressions of nitric oxide synthase (iNOS), COX-2 and NF-κB activity tested at 4 hours after intraperitoneal injection[63]. In this study, expression of mRNA related to inflammatory markers like IL-6, COX2, and CRP were decreased in liver of Korean red ginseng treated group. In additional study, mRNA expression related to TNF-α did not show significant changes between db/db control group and db/db ginseng group and the reason is still unclear.

In an aspect of inflammation, the calculated inflammation scores by summing the levels of mRNA expression of IL-6, COX-2 and CRP were significantly decreased in db/db ginseng group. TNF-α, IL-6, COX 2 and CRP are known as inflammatory markers but also as oxidative stress markers and they are elevated in type 2 diabetes mellitus. Considering all this results, Korean red ginseng improves metabolic parameters, mitochondrial DNA copy numbers and inflammatory markers in type 2 diabetes mellitus. But the correlation between

mitochondrial function and inflammatory marker is still unknown. Further evaluations and studies are necessary.

V. CONCLUSION

In this study, Korean red ginseng had an effect on weight gain and reduction of fasting blood glucose concentration in db/db mice. Other diabetic parameters like HbA1c, insulin, HOMA-IR and LDL cholesterol were also decreased in Korean red ginseng treated group. And this improvement might be correlated to increase of mitochondrial DNA copy numbers and improvement of compensatory increased proteins related to mitochondrial biogenesis. Also decrease of inflammatory markers like IL-6, COX 2 and CRP might be correlated to anti-diabetic effect. But the cause-effect relationship is unclear on this study. Further experiment is necessary but Korean red ginseng could be a useful additive nutraceutical in type 2 diabetes mellitus.

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ABSTRACT(IN KOREAN)

홍삼이 제2형 당뇨 쥐의 대사 지표, 미토콘드리아 유전자 및 염증 지표에 미치는 영향

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당뇨는 인슐린 저항성과 췌장의 기능 부전에 의해 발생하는 질환이다. 비록 발병 기전이 아직 충분히 밝혀지지 않고 있으나 미토콘드리아의 기능 부전, 특히 미토콘드리아의 수적 감소와 산화 스트레스의 과형성과 제2형 당뇨가 관련되어 있다고 알려져 있다. 기존의 당뇨치료제와 흔히 병용되는 여러 전통적인 보조 식품들도 기존 약제만큼은 아니지만 어느 정도의 혈당 강하 효과가 입증된 바 있으며 이는 항염증작용 및 항산화작용에 기초하고 있음이 밝혀지고 있다. 우리 나라에서는 홍삼이 대표적인 당뇨 보조 식품으로 이용되고 있으며 염증 지표에 영향을 미치고 있음이 밝혀진 바 있으나 명확한 기전은 아직 충분히 연구된 바가 없다. 따라서 본 연구는 홍삼의 혈당, 인슐린 민감성, 지질 등 대사 지표에 미치는 영향을 살펴보고 미토콘드리아와 세포 내 염증반응에 미치는 영향을 제2형 당뇨병 동물 모델을 통해 밝혀보고자 하였다.

실험에는 C57BL/KsJ db/db 쥐와 대조군으로 C57BL/KsJ db/+ 쥐를 사용하였으며 C57BL/KsJ db/db 쥐는 고지방식이를 투여한 양성대조군(8마리)와 고지방식이 및 홍삼을 투여한 실험군(8마리)으로 나누었다. 대조군(7마리)은 정상 식이를 투여하였다. 12주간 실험하면서 체중과 혈당을 측정하였고 실험 완료 후 혈당, 당화혈색소, 혈중 인슐린과 지질을 측정하였다. 쥐의 간 조직으로부터 미토콘드리아 유전자와 유전자 발현의 생합성

지표인 peroxismal proliferator-activated receptor-gamma coactivator-1 alpha(PGC-1 alpha) 및 mitochondrial transcription factor A(mtFAM 또는 T-fam)을 측정하였으며 염증 지표인 인터류킨-6, 사이클로옥시게나아제-2, C-염증성 단백질도 측정하였다.

그 결과 홍삼을 투여한 군에서 양성대조군에 비해 경미하게나마 체중이 적게 증가하였으나 큰 차이는 보이지 않았다. 혈당은 유의하게 감소하였다. 당화혈색소, 인슐린 및 HOMA-IR 수치도 더 낮게 나타났으며 저밀도 콜레스테롤 수치도 의미있게 실험군에서 낮게 나타났다. 미토콘드리아 유전자는 홍삼을 투여한 군에서 더 많이 나타났으며 유전자 생합성 지표들은 양성대조군에서는 보상적으로 증가하였고 홍삼을 투여한 군에서는 감소하는 것으로 나타났다. 염증 지표들도 양성대조군에서는 증가하였으나 홍삼을 투여한 군에서는 현저히 감소하였다.

따라서 홍삼의 혈당과 당화혈색소, 인슐린 민감성을 호전시키는 기능이 본 연구에서 나타났으며 미토콘드리아 유전자 수와 그 발현에도 영향을 미치고 세포 내 염증 반응에도 영향을 미치고 있음을 알 수 있었다.

핵심되는 말 : 홍삼, 당뇨, 인슐린 민감성, 미토콘드리아, 염증 반응, 산화 스트레스