Hematologic and Serological Investigation of Effect on Gyeongokgo in Healthy Individuals : a Randomized, Subject-assessor-blind, Placebo-controlled, Single-center Pilot Study

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There are no published data on Gyeongokgo (GOK) safety or efficacy despite being commonly use. The Gyeongokgo (GOK) is commonly used in traditional Korean medicine to promote a health qi and blood, but their objective data was not sufficient in clinical field. To investigate the safety and efficacy of GOK with hematologic and serologic testing and the change of the quality of life in healthy individuals. Randomized, subject-assessor-blind, placebo-controlled, single-center pilot study Participants and Interventions 29 healthy volunteer subjects were randomly placed into the GOK group (n = 20) or placebo control group (n = 9) and instructed to take one treatment packet (GOK or placebo) twice daily for 4 weeks. Subjects were assessed using the Fatigue Severity Scale (FSS) and Short Form 36 Health Survey (SF-36) and underwent hematologic and serologic tests and body composition analysis. The FSS total score (p = 0.093) and SF-36 general health index (p = 0.002) were improved following treatment in the GOK group. Post-treatment thyroid-stimulating hormone levels were increased in the GOK group compared with pre-treatment levels (p = 0.0042). C-reactive protein levels decreased (p = 0.0256) in the GOK group compared with that the placebo group. In time-series tests, GOK did not affect post-prandial serum triglycerides, glucose, insulin, or C-peptide levels. Notably, elevations in serum fasting triglycerides at 2- (p = 0.0333) and 4-hours (p = 0.0414) post-prandial were lower than those in the placebo group. GOK reduced fatigue levels and did not significantly affect laboratory test results performed to measure safety, serum glucose, and lipid profiles. Post-meal triglyceride levels were effectively reduced with treatment.

keywords : Fatigue Severity Scale, Short Form 36, Quality of life, Safety, Gyeongokgo, Traditional Korean Medicine

Introduction

Gyeongokgo (GOK) is a prescription recorded in the Donguibogam that consists of four ingredients: ginseng (Panax ginseng CA Meyer, root), poria (Poria cocos [Schw.] Wolf), Rehmannia glutinosa (Rehmannia glutinosa var. purpurea), and honey (Apis indica Radoszkowski). Clinically, GOK is widely used in patients who have been weakened by surgery or have reduced immune function and as a health restorative by students, office workers, and the elderly. Several experimental studies have reported its effects on preventing osteoporosis[1)] and improving atopic symptoms[2)], as well as its anti-inflammatory[3)] and anti-cancer effects[4)]. To date, the only studies on GOK have been conducted on restricted subjects or variables, including reports of its beneficial effects on athletes[5,6)]. There are no published data on GOK safety or mechanism of action. In South Korea, Western medicine and Traditional Korean medicine are dichotomized, and patients often take both kinds of medicine simultaneously. This means there is a particular need for basic data on the pharmacologic activity and safety of traditional Korean medicines. We conducted the randomized clinical trial including 29 healthy 20-year-old to 40-year-old subjects using hematologic and serologic tests, urinalysis, body analysis and questionnaires, before and after 4 weeks of GOK or placebo treatment. The aim of this study was to acquire objective data about the hematologic and serological test and questionnaires of this representative restorative used in traditional Korean medicine.

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Materials and Methods

1. Study subjects

This study was a 4-week-long, single-center, subject-assessor-blind, placebo-controlled, parallel, randomized clinical trial. Before starting the study, approval was granted by the Catholic Kwandong University International St. Mary's Hospital Institutional Review Board(IRB) of the under project number IS14TISF0016. This study was also registered as KCT0002652 in Clinical Research inform action Service(CRIS). Subjects were physically and mentally healthy males and females aged 20 to 40 who had seen one of the clinical trial flyers posted in the hospital between March and April 2015 and had willfully consented to participate(Fig. 1). The following exclusion criteria were applied: 1) underlying diseases including hypertension, hepatitis, or tuberculosis; 2) a history of endocrinologic or hormone disorders; 3) a history of hepato-pancreato-biliary or renal disorders; 4) a history of impaired blood glucose regulation (i.e., impaired fasting glucose, impaired glucose tolerance, or diabetes); 5) steroid or hormonal drug use less than 1 month before the start of the study; 6) pregnancy or breastfeeding; and 7) any abnormal findings that might possibly have impacted the study results. The researchers first confirmed any matters that contravened the clinical trial conditions via a phone call or visit to each subject. Following this, a total of 32 subjects (16 male and 16 female) underwent screening including hematologic tests, urinalysis, electrocardiography, and a chest x-ray. The 29 subjects who passed the screening were randomly allocated by the random number table to a GOK treatment group of 20 subjects (9 males and 11 females), or a placebo control group of 9 subjects (4 males and 5 females). The probability of random allocation to the GOK group was set at 2:1 to encourage participation. Later, the subjects were gathered at a pre-designated location; provided with identical meals; and subjected to a questionnaire, hematologic tests, urinalysis, and body composition analysis. Subsequently, the subjects were asked to complete a daily medication diary to assess compliance, use of other medicines, and alcohol consumption. At the end of the 4-week treatment, the subjects were again gathered, provided with identical meals, and subjected to the same questionnaire and tests as at the first visit.

2. GOK and placebo preparation

GOK was manufactured according to methods recorded in the Donguibogam[7] under the management and supervision of a specialist in traditional Korean pharmacology at the external manufacturer of traditional medicines associated with our hospital. After pulverizing 200 g poria and 100 g ginseng, these were thoroughly mixed with 1056 g Rehmannia glutinosa juice and 704 g honey. This mixture was poured into an earthenware jar, and the opening was sealed with five layers of oiled paper and one layer of silk fabric. The pot was heated for 72 hours using the bain-marie method in a copper pot over a mulberry wood fire and then cooled for 24 hours in cold well water. It was then heated for another 24 hours using the same method, and cooled for 24 hours before being made into its final, solid form and packed into a small earthenware jar. Finally, 1100 g GOK mixture was added to 2600 mL distilled water and heated for 10 minutes in a slow boiler used to manufacture herbal medicines. The 3600-mL volume of GOK liquid obtained was divided into 30 packets of 120 mL each, and the packets were sealed.

The placebo was prepared by adding 4000 mL distilled water to 800 g black rice powder and boiling for 30 minutes while mixing thoroughly. Disaccharide (300 g) was added, and the mixture was reheated while stirring thoroughly for another 5 minutes. The resulting placebo was divided into 30 120-mL packets that were then sealed.

3. Administration method

Subjects in each group drank one individually sealed 120-mL packet of liquid GOK (GOK group) or liquid placebo (control group) twice a day after breakfast and dinner, for 4 weeks.

5. Outcome measurements

1) Questionnaires

The Fatigue Severity Scale (FSS) is an instrument for assessing fatigue that was developed by Krupp et al[8] in 1989. It consists of nine items each scored on a 7-point Likert scale. It can easily be used in medical consultations and has shown strong validity and reliability in various fatigue-related disorders[9,10].

The 36-Item Short Form Survey (SF-36) is a representative instrument for assessing quality of life (QOL) comprised of 36 questions, divided into the three domains of functional status, well-being, and overall evaluation of health[10]. Study subject QOL was assessed based on FSS and SF-36 scores before and after the study period. Both questionnaires can be viewed in the Appendix. We used the Korean versions of the assessments.

2) Laboratory tests for hematologic and immunologic

responses

Blood samples were obtained from subjects' antecubital veins after a 12-hour overnight fast, centrifuged, and stored at -80℃. Blood samples were analyzed with an automated blood cell counter (Sysmex XN (XN) modular system, Sysmex, Kobe, Japan), Beckman Coulter AU5800 chemistry analyzers (Beckman Coulter Inc., Brea, CA, USA), and Unicel DXI 800 chemiluminescent enzyme immunoassays (Beckman Coulter Inc.). Glycosylated hemoglobin (HbA1C) levels were measured by high-performance liquid chromatography using a HLC-723G8 analyzer (Tosoh Co., Tokyo, Japan). Routine urinalysis was performed on a US-3100Rplus analyzer (Eiken Chemical Co. Ltd., Tokyo, Japan) in chronological order, and urine sediments were subjected to automated morphologic examination using a laser-based fluorescent flow cytometer, UF-1000i (Sysmex Co., Hyogo, Japan). For cytokine analysis, plasma samples were collected using EDTA, centrifuged, and stored at -8 0℃. Plasma cytokine levels including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, interferon (IFN)-r, IL-2, and IL-10 were determined using a Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corp., Billerica, MA, USA) following the manufacturer's kit-specific protocols. All samples were assayed in triplicate, and the mean absorbances were calculated from standard curves.

3) Body composition tests

The participants' body compositions were measured using the Inbody 770 analyzer (Inbody Co., Ltd., Seoul, Korea) after vital sign stabilization.

4) Adverse event monitoring

Adverse events during the trial were assessed through safety assessments and laboratory tests. All adverse reactions that occurred throughout the intervention and follow-up periods were evaluated as World Health Organization Adverse Reaction Terminology, and classified in 1-5 grades according to the Common Terminology Criteria for adverse Events (version 4.03).

5) Measurement of compliance and

The subject who was less than 70% with compliance were dropped out.

6) Assessment of blinding

There was no assessment on blinding success.

7) Statistical analysis

Statistical analyses were performed by a statistician who was blinded to the subjects' status. We used a paired t-test or Wilcoxon signed-rank test for changes within the groups and an independent two-sample t-test or Mann-Whitney U test (Wilcoxon rank sum test) for inter-group differences following treatment. All statistical analyses were performed using SAS v9.1.3 (SAS Institute Inc., Cary, NC, USA). P values less than 0.05 were considered statistically significant.

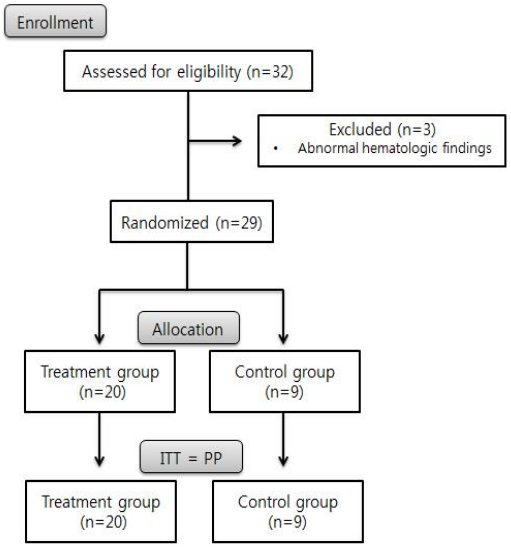

Fig. 1. Study flow chart (ITT, intention to treat; PP, per protocol).

Results

Of the 32 subjects originally enrolled in this study, 3 were excluded due to abnormal hematologic findings during screening, and the remaining 29 subjects were included in the final analysis. There were no significant differences in general characteristics between the two groups(Table 1).

Table 1. The General Characteristic of the study

Parameters	Treatment	Control	Total	P-value
Age (year)	31.25±5.3	30.00±4.47	30.86±4.96	NS
Male/ Female	9/11	4/5	13/16	NS
Height (cm)	166.00±12.91	169.22±5.38	167.00±11.12	NS
Weight (kg)	63.75±12.28	62.96±9.03	63.50±11.21	NS
BMI (kg/m^2)	22.62±2.32	21.92±2.05	22.40±2.23	NS
Smoking positive/Negative	6/14	3/6	9/20	NS
Drinking Positive/Negative	17/3	8/1	25/4	NS

The FSS questionnaire results showed no significant difference in scores between the GOK and control groups following the treatment period. However, after the treatment period, the GOK group did show a statistically significant decrease in overall score, as well as for the following specific items: 1) "I lose motivation when I am fatigued," 2) "I become tired when I exercise," 3) "I become tired easily," 4) "I engage in less physical activity because of fatigue," and 5) "I often experience problems due to fatigue"(Table 2).

Table 2. Fatigue severity scale results after the duration of the clinical trial

Variables	Treatment			Total			P-value 3
	Before	Difference	P-value 1	Before	Difference	P-value 2	
Total	32.35±9.30	-6.6±10.2	0.0093	38.78±5.59	-1±7.57	0.7021	0.1533

P-value 1 : Wilcoxon signed rank test for the change within the treatment group following treatment with GOK. P-value 2 : Wilcoxon signed rank test for the change within the control group following treatment with the placebo. P-value 3 : Mann-Whitney U test (Wilcoxon rank sum test) for inter-group differences in the change following treatment

Table 3. The 36-Item Short Form Survey Results after the Duration of clinical trial

Variables	Treatment			Total			P-value 3
	Before	Difference	P-value 1	Before	Difference	P-value 2	
PF	52.17 ± 5.7	0.95 ± 2.68	0.1797†	51.17 ± 6.71	0.23 ± 2.45	0.7824	0.738†
RP	53.58 ± 4.01	1.1 ± 3.96	0.2268	45.73 ± 10.48	3.26 ± 6.27	0.3125†	0.7379†
BP	49.91 ± 9.01	2.86 ± 9.94	0.2137	46.72 ± 12.3	2.39 ± 5.87	0.2568	0.8964
GH	46.83 ± 8.24	3.01 ± 3.76	0.0020	42.19 ± 8.29	2.24 ± 3.27	0.0731	0.6026
VT	48.65 ± 10.67	3.76 ± 8.69	0.0683	45.83 ± 7.17	3.81 ± 6.19	0.1017	0.9862
SF	36.66 ± 4	-1.38 ± 4.29	0.0996†	36.84 ± 3.87	-4.26 ± 6.53	0.0864	0.2307†
RE	50.64 ± 8.21	1.75 ± 7.42	0.3115†	47.24 ± 8.65	0.42 ± 5.98	0.8375	0.4984†
MH	47.49 ± 9.62	3.79 ± 9.29	0.0839	42.19 ± 5.43	4.06 ± 7.34	0.1358	0.9403
PCS	51.85 ± 5.52	1.39 ± 3.84	0.1220	48.23 ± 10.73	1.71 ± 3.3	0.1584	0.8299
MCS	44.28 ± 8.03	2.44 ± 7.28	0.1501	41.19 ± 4.13	1.2 ± 6.62	0.6012	0.6663

PF, physical functioning; RP, role limitations due to physical health; BP, bodily pain; GH, general health perceptions; VT, vitality; SF, social functioning; RE, role limitations due to emotional problems; MH, general mental health; PCS, summary measures of physical; MCS, summary measures of mental. P-value 1 : Wilcoxon signed rank test† for the change within the treatment group following treatment with GOK. P-value 2 : Wilcoxon signed rank test† for the change within the control group following treatment with the placebo. P-value 3 : Mann-Whitney U test† (Wilcoxon rank sum test) for inter-group differences in the change following treatment.

Table 4. The 36-Item Short Form Survey Results after the Duration of clinical trial

Variables	Treatment			Total			P-value 3
	Before	Difference	P-value 1	Before	Difference	P-value 2	
Hematology							
WBC (x10^3㎕)	6.97±2.41	-0.74±2.23	0.0490†	6.2±1.72	-0.33±0.83	0.2676	0.4785
RBC (x10^6㎕)	4.74±0.52	0.03±0.17	0.4888	4.82±0.56	-0.08±0.17	0.2066	0.1321
Hemoglobin(g/dL)	14.5±1.67	0.05±0.54	0.6855	14.63±1.69	-0.31±0.57	0.1382	0.1133
Hematocrit(%)	42.94±4.45	-0.04±1.68	0.9149	43±4.2	-1.06±1.59	0.0821	0.1386
Platelet count (x10^3㎕)	245.7±48.38	9±22.54	0.0583	285±119.01	6.33±25.9	0.4841	0.7803
ANC (absolute neutrophil count)	4357.95±2269.54	-646.95±2291.66	0.6291†	3304.44±947.81	-485.56±706.76	0.0732	0.8335†
Biochemistry							
Protein(g/dL)	7.49±0.33	-0.02±0.37	0.7810	7.38±0.35	-0.04±0.38	0.7377	0.8711
Albumin(g/dL)	4.78±0.22	-0.14±0.2	0.0234	4.7±0.23	-0.17±0.21	0.0415	0.5880†
Total bilirubin(mg/dL)	0.79±0.36	0.01±0.36	0.6926	0.8±0.31	0±0.42	1.0000	0.9475
AST(U/L)	21.1±4.87	-0.5±8.51	0.7905†	21.44±4.5	-0.22±7.21	0.9286	0.7787†
ALT(U/L)	17.1±8.01	-0.2±6.25	1.0000†	18±7.68	-0.89±10.28	0.8018	1.0000†
γ-GTP(U/L)	22.5±18	0.1±5.28	1.0000†	18.22±10.41	1.67±5.92	1.0000†	0.7410†
ALP(U/L)	61.35±17.53	-1.35±6.29	0.5045	60.67±13.27	-1.67±6.02	0.4304	0.8999
BUN(mg/dL)	12.78±2.59	0.1±2.99	0.8233	10.5±3	-0.13±2.78	0.8890	0.8474
Creatinine(mg/dL)	0.64±0.14	0.06±0.06	0.0063†	0.62±0.11	0.03±0.05	0.2500†	0.3076†
HbA1c(%)	5.23±0.22	0±0.08	1.0000†	5.17±0.23	-0.04±0.07	0.2188†	0.2039†
Total Cholesterol(mg/dL)	188.8±28.85	4.65±24.09	0.3210	193.22±16.15	-5.67±20.84	0.4384	0.2772
Triglyceride(mg/dL)	101.85±77.19	-2.45±36.41	1.0000†	68.67±32.04	17±30.29	0.5078†	0.2390†
HDL(mg/dL)	58.85±14.82	1.8±5.12	0.0984	66.56±7.52	-1.78±7.12	0.4753	0.1348
LDL(mg/dL)	119.1±18.76	3.8±18.8	0.3023	120.67±15.41	-4.44±14.06	0.3708	0.2515
TC/HDL	3.37±0.89	-0.05±0.37	0.5672	2.94±0.43	-0.02±0.27	0.8488	0.8236
CRP(mg/L)	0.97±1.18	-0.5±1.22	0.0768†	0.39±0.33	0.33±0.69	0.2891†	0.0256†
Free T4(ng/dL)	0.89±0.09	0.05±0.09	0.3323†	0.89±0.1	0.09±0.09	0.0141	0.2670†
TSH(uIU/mL)	1.69±0.79	0.35±0.48	0.0042†	1.82±0.8	0.77±0.94	0.0389	0.4158†
Urinalysis							
Specific Gravity	1.02±0.01	0±0.01	0.1434	1.03±0	0±0.01	0.2624	0.0582
pH	5.88±0.56	-0.18±0.61	0.2188†	6.17±0.66	-0.17±0.97	0.6195	0.8399†

WBC, white blood cell; RBC, red blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma(γ)-glutamyl transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; HbA1c, Hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, c-reactive protein; Free T4, free thyroxine (T4); TSH, thyroid stimulating hormone. P-value 1 : Wilcoxon signed rank test† for the change within the treatment group following treatment with GOK. P-value 2 : Wilcoxon signed rank test† for the change within the control group following treatment with the placebo. P-value 3 : Mann-Whitney U test† (Wilcoxon rank sum test) for inter-group differences in the change following treatment.

The SF-36 was administered to assess QOL, and both intra- and inter-group analyses were performed. Ten different items were evaluated, consisting of the eight individual items: PF (physical functioning), RP (role

limitations – physical), BP (bodily pain), GH (general health), VT (vitality), SF (social functioning), RE (role limits – emotional), and MH (mental health), in addition to the physical component summary (PCS = PF + RP + BP + GH) and mental component summary (MCS = VT + SF+ RE + MH). Apart from general health, which significantly improved after GOK treatment, there were no significant differences(Table 3).

Table 5. The laboratory findings for serum glucose, TG, C-peptide, and insulin level after meal after the duration of the clinical trial

Variables		Before			After			P-value		
		Fasting	PP2hr	PP4hr	Fasting	PP2hr	PP4hr	p-value1	p-value2	p-value3
Glucose	Treatment (n=20)	99.4±6.81	99.85±14.7	97.75±14.89	95.9±8.77	104.3±12	91.5±8.36	0.0812	0.1643	0.0961
	Control (n=9)	99.22±5.83	102.67±15.36	90.89±9.85	93.78±6.24	97.67±11.2	87.22±12.36	0.0970	0.1887	0.2891†
	p-value2	0.4989†	0.6415	0.3530†	0.9440†	0.1718	0.2825	0.5760	0.0783	0.7087†
Triglyceride	Treatment (n=20)	101.85±77.19	125.95±87.43	131.4±105.27	99.4±65.31	111.3±84.17	105.65±87.17	0.8238†	0.0391	0.0056
	Control (n=9)	68.67±32.04	85.44±64.84	84.11±51.83	85.67±58.34	95.78±66.97	87.89±73.71	0.5078†	0.2130	0.6906
	p-value2	0.2134†	0.2304†	0.2303†	0.4852†	0.8335†	0.4429†	0.2390†	0.0333	0.0414
C-peptide	Treatment (n=20)	1.53±0.41	4.75±1.79	3.57±1.67	1.89±0.92	4.75±1.62	2.32±0.74	0.2632†	0.9950	0.0032
	Control (n=9)	1.39±0.53	4.85±2.5	3.02±1.77	1.81±0.92	4.1±1.52	2.35±1.03	0.0391†	0.1828	0.1396
	p-value2	0.4570	0.9044	0.4853†	0.7615†	0.3232	0.6081†	0.8335†	0.2892	0.3526
Insulin	Treatment (n=20)	5.87±2.64	26.53±19.12	17.2±11.94	9.11±5.49	24.36±13.2	8.67±3.2	0.0118†	0.8238†	0.0026†
	Control (n=9)	6.69±2.81	28.98±17.25	13.46±9.88	8.37±4.11	17.3±13.34	8.83±5.81	0.2572	0.0115	0.0972
	p-value2	0.4552	0.5604†	0.3658†	0.6744†	0.0731†	0.2674†	0.6576†	0.0498†	0.4164†

All values are expressed as mean±S.D. and median(Q1, Q3). P-value 1 were calculated for difference (after-before) within group at each time using Wilcoxon signed test †. P-value 2 were calculated for variables between treatment and control group at each time using an Mann-Whitney U test (Wilcoxon rank sum test)†

Table 6. Serum cytokine levels after the duration of the clinical trial

Variables	Treatment			Total			P-value 3
	Before	After	P-value 1	Before	After	P-value 2	
TNF-a	2.88±2.18	2.53±1.26	1.0000†	2.55±1.37	3.67±3.45	0.2891†	0.2674†
IL-1β	3.29±0.33	3.26±0.29	0.4959	3.21±0.22	3.22±0.23	0.8602	0.5955
IL-6	5.27±4.15	3.98±2.43	0.1435†	3.45±1.01	3.2±0.23	1.0000†	0.2862†
IFN-r	8.85±15.34	5.14±0.55	0.5034†	4.97±0.48	4.97±0.42	1.0000†	0.4409†
IL-2	3.23±4.53	2.39±0.57	0.8036†	1.92±0.62	2.33±0.93	0.0226	0.1879†
IL-10	10.38±30.94	3.38±7.52	0.2379†	1.08±0.31	1.34±0.5	0.1466	0.1244†

P-value 1 : Wilcoxon signed rank test† for the change within the treatment group following treatment with GOK. P-value 2 : Wilcoxon signed rank test† for the change within the control group following treatment with the placebo. P-value 3 : Mann-Whitney U test† (Wilcoxon rank sum test) for inter-group differences in the change following treatment.

Table 7. Body composition findings after the duration of the clinical trial

Variables	Treatment			Total			P-value 3
	Before	After	P-value 1	Before	After	P-value 2	
Total body mass	63.75±12.28	63.66±12.42	1.0000†	62.96±9.03	63.09±9.42	0.6565	0.5657
Body fat mass	16.61±3.45	16.55±3.56	0.7036	16.32±3.37	16.71±3.41	0.1267	0.1175
Fat-free mass	47.15±11.08	47.11±11.17	0.8265	46.63±7.92	46.38±7.98	0.2709	0.4316

P-value 1 : Wilcoxon signed rank test† for the change within the treatment group following treatment with GOK. P-value 2 : Wilcoxon signed rank test† for the change within the control group following treatment with the placebo. P-value 3 : Mann-Whitney U test† (Wilcoxon rank sum test) for inter-group differences in the change following treatment.

Table 4 summarizes the inter- and intra-group differences in hematologic and serologic findings following treatment. There was no statistical difference between the two groups except that the GOK group had lower C-reactive protein (CRP) levels after treatment (P = 0.0256). In the intra-group comparison, the GOK group showed significant decreases in white blood cell and albumin levels (P = 0.049 and P = 0.023, respectively), and significant increases in creatinine and thyroid-stimulating hormone (TSH) levels (P = 0.006, and P = 0.004, respectively). The control group showed a significant decrease in albumin (P = 0.042) and a significant increase in free thyroxine (T4) and TSH (P = 0.014 and P = 0.039, respectively). However, in the individual data, five participants (four and one in the GOK and control groups, respectively) showed values higher than the reference ranges in liver function tests for aspartate aminotransferase (AST), alanine aminotransferase (ALT), or gamma-glutamyl transferase (GGT).

Regarding post-prandial laboratory findings for serum glucose, triglycerides (TG), C-peptide, and insulin levels following the treatment period, the GOK group showed a significant decrease in TG at 2- and 4-hours post-prandial. There were also significant inter- and intra-group differences regarding the change at each measurement

time point from before to after the trial. Among the five subjects who had a reduction in TG of at least 50 mg/dl, all five had a decrease in TG 4 hours after eating, three had a decrease at 2 hours, and one had a decrease at fasting (Table 5).

Additionally, we investigated the regulatory effect of GOK on inflammatory-related cytokine levels but found no significant differences within or between groups (Table 6). The same was true for the body composition analysis for total body, body fat, and fat-free masses (Table 7).

Finally, There was no adverse events during the trial. Every subjects was more than 70% with compliance.

Discussion

The FSS questionnaire results for the GOK group revealed significant decreases in scores for the following items after taking GOK: "I lose motivation when I am fatigued," "I become tired when I exercise," "I become tired easily," "I engage in less physical activity because of fatigue," and "I often experience problems due to fatigue." In the SF-36, there was a significant increase in general health scores in the GOK group. Collectively, these findings suggest that GOK improved subjective fatigue, which is in line with previous reports that GOK ingestion can have beneficial effects on certain physical functions[5,6]. In addition, an analysis of GOK composition revealed that it contains various amino acids and minerals with antioxidant functions, and the authors suggested that this is the mechanism of action by which it relieves fatigue[11]. There was no notable difference in questionnaire results between the two groups, even though there was a significant difference after treatment in the GOK group.

Oral GOK administration did not significantly affect the laboratory findings. There were no significant intra- or inter-group differences regarding glucose metabolism. There are two likely explanations for this. First, the degree of perceived change was not great because the subjects in both groups were healthy adults with normal insulin tolerance before treatment. Second, the saccharide and black rice in the placebo are nutritious ingredients commonly used in traditional Korean medicine to nourish the body.

Interestingly, we observed GOK-related improvements in post-prandial TG levels. This was similar to the findings of a previous mouse study, in which serum total cholesterol and TG levels were decreased, while high-density lipoprotein levels were increased following treatment with GOK and modified GOK[12].

CRP, a major inflammatory markers, was significantly lowered in the GOK group after the treatment period. Elevated CRP and high fasting TG are common hematologic findings in obese individuals, indicating that obesity is closely connected with both lipid metabolism abnormalities and vascular health[13]. Previous research revealed that GOK reduces inflammatory cytokine secretion and effectively decreases the range of infarction in mice with middle cerebral artery (MCA) occlusion[14]. Considering that elevated CRP can be used as an important indicator of obesity[15], GOK might exert an effect on vascular health, which is in turn related to obesity and lipid metabolism. Furthermore, CRP can be used as an indicator of fatigue[16], and the results of our study are consistent with a report describing a correlation between decreasing CRP and indicators of actual QOL[17-19]. Also, our GOK group exhibited a significant increase in TSH. This is consistent with a report stating that, although not statistically significant, the TSH concentration was higher in a group of GOK-treated mice with induced growth disorder[20]. Given the results of a study on individuals with normal thyroid function, in which people with lower normal-range TSH values felt more fatigue[21], our study suggests a possible mechanism for reduced fatigue after taking GOK. However, a biological false positive have to be considered in the case of CRP and TSH.

Five participants (four in the GOK group and one in the control group) exhibited levels of AST, ALT, GGT, or total bilirubin that were above the standard range after the treatment period. We found that these participants had above average alcohol intake during the study. Hence, they may have experienced adverse reactions unrelated to GOK.

Numerous traditional Korean medicines including GOK have been shown to affect levels of various cytokines[22-25], and our earlier study demonstrated that GOK significantly reduced TNF-α and IL-1β levels compared to a control in induced MCA infarction mice[15]. We anticipated that GOK treatment would influence blood cytokine levels in humans, but possibly because the subjects in this study were healthy individuals with baseline cytokine levels within normal ranges, there were no statistically significant changes. Conversely, subjects in the previous studies had blood cytokine levels that were already altered by various pathologic disease states, which might have predisposed them to a greater response to GOK.

Many people worry that taking a restorative will make them put on weight, and because traditional Korean

medicines are not zero-calorie, we measured subjects' total body, body fat, and fat-free masses. There was no significant increase in total body weight for either group; indeed, a slight decrease in body weight was observed, although this was not statistically significant.

Our study had several limitations. First, the subjects' lifestyle habits were not controlled during the trial period. There have been differences in daily habits such as diet and exercise, but also, because most of the subjects were young office workers, alcohol consumption and excessive work were frequent. The second limitation was the choice of placebo. The black rice and saccharide may have impacted glucose load and imparted generic restorative benefits to the control group subjects. Third, we only included young, healthy adults in whom any measurable effects were below the level of evidence required for clinical application. Finally, traditional Korean medicine prescriptions should be made according to the patient's medical symptoms, a practice that was not incorporated into the study design and might have reduced the precision of the results. Future studies should include further investigation into CRP and TSH levels and identify objective indices that can be applied to explain the clinical effects of GOK and investigate its mechanism(s) of action.

Conclusion

Hematologic and serologic test results and QOL questionnaires revealed that in healthy 20- to 40-year-old adults, a 4-week GOK regimen did not cause any hepatotoxicity or nephrotoxicity; had a beneficial effect on some items of perceived QOL; and was associated with elevated TSH, reduced CRP, and reduced fasting TG levels.

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Appendix 1. FSS (Fatigue Severity Scale)

		전혀 그렇지 않다 ←					매우 그렇다 →	
1	피로하면 의욕이 없어진다. My motivation is lower when I am fatigued	1	2	3	4	5	6	7
2	운동을 하면 피곤해진다. Exercise brings on my fatigue	1	2	3	4	5	6	7
3	쉽게 피곤해진다. I am easily fatigued	1	2	3	4	5	6	7
4	피로 때문에 신체 활동이 감소된다. Fatigue interferes with my physical functioning	1	2	3	4	5	6	7
5	피로로 인해 종종 문제가 생긴다. Fatigue causes frequent problems for me	1	2	3	4	5	6	7
6	피로 때문에 지속적인 신체 활동이 어렵다. My fatigue prevents sustained physical functioning	1	2	3	4	5	6	7
7	피로 때문에 업무나 책임을 다 하지 못 한다 Fatigue interferes with carrying out certain duties and responsibilities	1	2	3	4	5	6	7
8	내가 겪고 있는 가장 힘든 문제를 세 가지 뽑는다면 그 중에 피로가 포함된다. Fatigue in among my most disabling symptoms	1	2	3	4	5	6	7
9	피로 때문에 직장·가정·사회 활동에 지장을 받는다 Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

Appendix 2. SF-36 (36 Item Short Form Survey)

1	당신의 평소 건강상태는 어떻습니까? In general, would you say your health is					
	최고로 좋다 Excellent	매우 좋다 Very good	좋다 Good	조금 나쁘다 Fair	나쁘다 Poor	
2	작년과 비교했을 때 현재 당신의 건강 상태는 어떻습니까? Compared to one year ago, how would you rate your health in general now?					
	훨씬 좋다 Much better now than one year ago	조금 좋다 Somewhat better now than one year ago	거의 비슷하다 About the same	조금 나쁘다 Somewhat worse than one year ago	훨씬 나쁘다 Much worse than one year ago	
3	다음은 당신의 평상시 활동에 관한 질문입니다. 다음과 같은 활동을 할 때 지장이 있습니까? 지장이 있다면 어느 정도의 제한을 받습니까? The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?					
			지장이 많다 Yes, Limited a lot	지장이 약간 있다 Yes, Limited a little	지장이 전혀 없다 No, Not limited at all	
	1)	달리기, 무거운짐 들기, 격렬한 운동 등 격렬한 활동 Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports				
	2)	탁자 옮기기, 집안 청소, 한두시간 걷기 등 다소 힘든 활동 Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf				
	3)	조금 무거운 시장 바구니를 들거나 운반하기 Lifting or carrying groceries				
	4)	계단으로 여러 층을 걸어 올라가기 Climbing several flight of stairs				
	5)	계단으로 한 층을 걸어 올라가기 Climbing one flight of stairs				
	6)	허리를 굽히거나 무릎을 꿇는 것 Bending, kneeling, or stooping				
	7)	1km 정도 걷기 Walking more than a mile				
	8)	200-300m 정도 걷기 (동네 한바퀴 산책) Walking several blocks				
	9)	100m 걷기 (집 주변 걷기) Walking a block				
	10)	혼자서 목욕을 하거나 옷을 갈아 입는 것 Bathing or dressing yourself				
4	지난 한 달간 당신의 신체적인 건강 상태 때문에 일상 활동을 하는데에 다음과 같은 어려움이 있었습니까? During the past 4 weeks, have you had any of the following problems with work or other regular daily activities as a result of your physical health?					
			항상 그랬다	대부분 그랬다	때때로 그랬다	드물게 그랬다	전혀 없었다
	1)	일하는 시간을 줄여야만 했다 Cut down the amount of you spent on work or other activities					
	2)	원하는 만큼의 일을 하지 못했다 Accomplished less than you would like					
	3)	일이나 일상 활동 중에서 할 수 없는 것이 있었다 Were limited in the kind of work or other activities					
	4)	일이나 일상 활동 중에 어려움이 있었다. Had difficulty performing the work or other activities.					

5 지난 한 달간 당신의 정서적인 문제 때문에 일상 활동을 하는데에 다음과 같은 어려움이 있었습니까?
During the past 4 weeks, have you had any of the following problems with work or other regular daily activities as a result of any emotional problems(such as feeling depressed or anxious)?

		항상 그랬다	대부분 그랬다	때때로 그랬다	드물게 그랬다	전혀 없었다
1)	일하는 시간을 줄여야만 했다. Cut down the amount of you spent on work or other activities					
2)	원하는 만큼의 일을 하지 못했다. Accomplished less than you would like					
3)	일이나 일상 활동 중에 평소처럼 집중하지 못하였다. Didn`t do work or other activities as carefully as usual					

6 지난 한달 동안 당신의 신체적인 건강 혹은 정서적인 문제로 당신의 사회생활 (가족, 친구, 이웃, 동료 등)에 어려움이 있었습니까?
Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

전혀 없었다 Not at all	약간 있었다 Slightly	보통 이다 Moderately	꽤 많이 있었다 Severe	대단히 많이 있었다 Very Severe

7 지난 한달 동안 몸에 통증이 얼마나 많이 있었습니까?
How much bodily pain have you had during the past 4 weeks

전혀 없었다 None	아주 가벼운 통증이 있었다 Very mild	가벼운 통증이 있었다 Mild	보통의 통증이 있었다 Moderate	심한 통증이 있었다 Severe	격심한 통증이 있었다 Very Severe

8 지난 한달 동안 당신은 몸의 통증 때문에 정상적인 일상생활을 하는데에 얼마나 지장이 있었습니까?
During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

전혀 없었다 Not at all	약간 있었다 A little bit	보통 이다 Moderately	꽤 많이 있었다 Quite a bit	대단히 많이 있었다 Extremely

9 다음 질문은 당신이 지난 한 달간 어떻게 느끼고 어떻게 지냈는지에 관한 것입니다. 각 문항에 관하여 가장 알맞은 대답에 하나씩 표시하세요.
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling

		항상 그랬다	대부분 그랬다	때때로 그랬다	드물게 그랬다	전혀 없었다
1)	나는 의욕이 넘치고 원기 왕성했다 Did you feel full of pep?					
2)	나는 초조하고 신경질적 이었다 Have you been a very nervous person?					
3)	나는 기분이 저조하고 의기소침하여 즐거운 일이 없었다 Have you felt so down in the dumps that nothing could cheer you up?					
4)	나는 차분하고 평온하였다 Have you felt calm and peaceful?					
5)	나는 활력이 넘쳤다 Did you have a lot of energy?					
6)	나는 마음이 상하고 우울했다 Have you felt downhearted and blue?					
7)	나는 매우 지쳤었다 Did you feel worn out?					
8)	나는 행복했었다 Have you been a happy person?					
9)	나는 피곤했었다 Did you feel tired?					

10 지난 한달 동안 당신의 신체적인 건강 혹은 정서적인 문제로 인하여 사회활동에 얼마나 자주 지장이 있었습니까?
During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

전혀 없었다 None of the time	약간 있었다 A little bit of the time	보통 이다 Some of the time	꽤 많이 있었다 Most of the time	대단히 많이 있었다 All of the time

11 다음 질문에 관하여 당신은 어디에 해당하는지 답하십시오.
How true or false is each of the following statements for you?

		확실히 그렇다 Definitely true	대체로 그렇다 Mostly true	잘 모르겠다 Don`t know	대체로 아니다 Mostly false	전혀 아니다 Definitely false
1)	나는 다른 사람들보다 병에 쉽게 걸리는 것 같다 I seem to get sick a little easier than other people					
2)	나는 내가 아는 다른 사람들만큼 건강하다 I am as healthy as anybody I know					
3)	나의 건강은 점점 나빠질 것이라고 생각한다 I expect my health to get worse					
4)	나의 건강상태는 매우 좋다 My health is excellent					