The effects of Chitosan oligosaccharide supplementation on glucose control in subjects with pre-diabetes

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A Dissertation

Submitted to the Department of Science for Aging

and the Graduate School of Yonsei University

in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

Hee Jun Kim

January 2014

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January 2014

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ABSTRACT

The effects of chitosan oligosaccharide supplementation on glucose control in subjects with pre-diabetes

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We aimed to evaluate the effect of chitosan oligosaccharide supplementation on glucose control in subjects with pre-diabetes. This study was a randomized, double-blind, placebo-controlled clinical trial. Subjects with pre-diabetes were randomly assigned to the chitosan oligosaccharide intervention group or the placebo group for 12-weeks. We assessed the serum levels of glucose, insulin, and C-peptide by 2-hour value in the 75g oral glucose tolerance test (OGTT), HbA1c, pro-inflammatory cytokines, and plasma adiponectin at baseline and after the 12 -week intervention. The treatment group showed a significant decrease in serum

glucose level at 30 min (p=0.013) and at 60 min (p=0.028). The change of serum glucose level at 60 min was significant in the treatment group compared with the placebo group (p=0.030). Also, the plasma level of hemoglobin A1c (HbA1c) (p=0.023), the pro-inflammatory cytokines (IL-6 and TNF-α) and the plasma adiponection were reduced in the chitosan oligosaccharide intervention group after the 12-week treatment. However, the placebo group did not show any significant changes in these biomarkers. In conclusion, the chitosan oligosaccharide supplementation may be effective for controlling serum glucose levels and postprandial glycemic responses in pre-diabetes.

Key words: Double-blind/placebo-controlled study, Chitosan oligosaccharide, Glucose control, Impaired fasting glucose, Impaired glucose tolerance

Abbreviation

Apo A1	: Apolipoprotein A1
Apo B	: Apolipoprotein B
AUC	: Area Under the Curve
BMI	: Body Mass Index
BMR	: Basal Metabolic Rate
CVD	: Cardiovascular Disease
EDTA	: Ethylenediaminetetracetate
FFQ	: Food Frequency Questionnaire
FPG	: Fasting Plasma Glucose
HDL	: High Density Lipoprotein
HOMA-IR	: Homeostasis Model Assessment-Insulin Resistance
IFG	: Impaired Fasting Glucose
IGT	: Impaired Glucose in Tolerance
IL-6	: Serum Interleukin-6
OGTT	: Oral Glucose Tolerance Test
TCI	: Total Calorie Intake
TEE	: Total Energy Expenditure
TNF-α	: Tumor Necrosis Factor–α

1. INTRODUCTION

As economies develop, trends in dietary patterns gradually change to richer food and this change in dietary patterns results in an increased prevalence of chronic diseases, such as obesity, diabetes and cardiovascular diseases. According to the 2011 Korean National Health and Nutrition Examination Survey, the prevalence of diabetes and impaired fasting glucose (IFG) were 10.5% and 19.3% in Korean adults (≥ 30 years old) respectively [1]. Currently, in 2013, 347 million people worldwide have diabetes, which is predicted to be the seventh leading cause of death by 2030 [2]. The worldwide prevalence of diabetes among adults aged 20-79 years was 6.4% in 2010 and is expected to increase to 7.7% by 2030 [3]. Diabetes arising from reduced organ function leading to insulin resistance or lack of insulin, is a highly prevalent and chronic metabolic disease [4, 5]. According to the results of epidemiological and clinical studies, hyperglycemia is a primary cause of diabetic complications such as cardiovascular disease (CVD), coronary heart disease (CHD), nephropathy and retinopathy [6, 7]. Therefore, glucose control is vital in preventing diabetic complications and improving quality of life [8-10]. Recently, many people have become more concerned about health and formed preferences for healthy food. Thus, research and development is underway on functional foods containing biologically- active compounds that provide a clinically positive effect for the prevention, management, and treatment

of chronic diseases. Because of the increased interest in living a healthy life-style, studies have been carried out to reduce the risk of various diseases by extracting the active components from functional food ingredients and natural materials [11-13].

Chitosan is a deacetylated polymer of *N*-acetyl glucosamine. Chitosan oligosaccharide is a natural product obtained by the enzymatic digestion of chitosan made up of β-(1, 4)-D-glucosamine units [14-16]. Chitosan oligosaccharide is a water-soluble, low-molecular-weight chitosan and it is easily absorbed by gastric acid hydrolysis. Chitosan oligosaccharide has been reported to be beneficial in reducing blood cholesterol and blood pressure, increasing immunity, and enhancing antitumor properties [17-21]. In addition, consumption of chitosan oligosaccharide reduced the postprandial blood glucose level in subjects with a normal blood glucose level [22]. We have previously reported that chitosan oligosaccharide significantly reduced postprandial blood glucose levels through the inhibition of carbohydrate hydrolysis enzymes (α-glucosidase) in animal and *in vitro* models [23]. Because few studies have examined the effects of chitosan oligosaccharide on blood glucose control in pre-diabetes patients, the current study evaluated whether chitosan oligosaccharide has an effect on the blood glucose concentration in Korean subjects with pre-diabetes conditions.

2. BACKGROUND

2.1 Diabetes Mellitus

2.1.1 Diabetes Mellitus epidemics

Diabetes Mellitus is an global health problem. In the USA, the prevalence of diabetes has doubled from 4% to 8% during the past 40 years [24, 25] and the worldwide diabetes prevalence among adults aged 20-79 years is 6.4% in 2010 and will be increasing to 7.7% by 2030 [3]. In other study, the world prevalence of diabetes among adults (≥ 20 years) was estimated to be 2.8%, affecting 171 million adults, in 2000 and will increase to 4.4%, affecting 366 million, by 2030 and the number of people with diabetes in the world is expected to approximately double between 2000 and 2030 [26]. And then the prevalence of diabetes in Korea has rapidly increased in the past 40 years from 1.5% to 9.9% for diabetes [27]. If the current trend continued, the number of people with diabetes is likely to increase rapidly 3.51 million by the year 2010 (7.08% of the estimated total population), 4.55 million (8.97%) by 2020, and 5.45 million (10.85%) by 2030 (Figure 1) [28]. The rapid increase in the prevalence of diabetes was related to environmental factors and genetic predisposition. Especially in Asia, Rapid economic developments have improved the availability of a nutritional transition [25]. Asian have also higher body fat content and are at higher risk for diabetes

[29]. Several studies have reported the importance of early-phase insulin secretory defects in Asian [25, 30, 31], and people of Asia have a strong genetic susceptibility to diabetes, which are characterized by early β-cell failure. Therefore, preventive action should begin urgently, and it is necessary for the lifestyle changes [25].

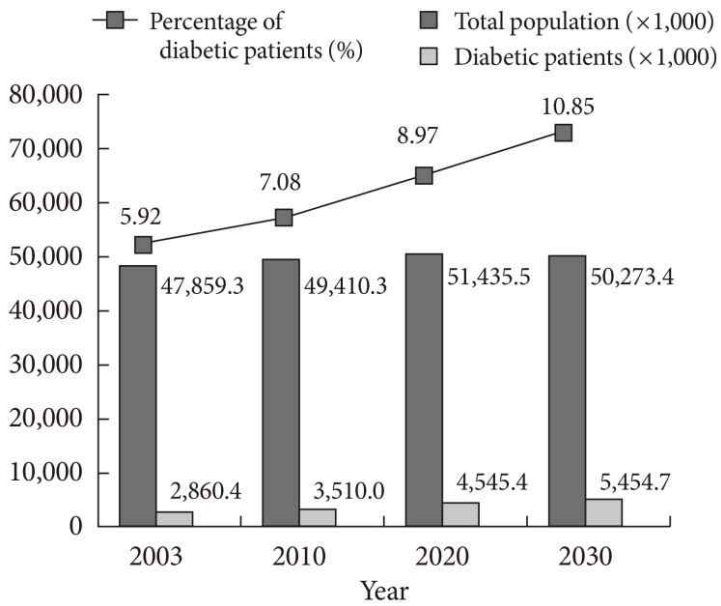

Fig.1. Diabetic population estimates from 2003 to 2030 in Korea.
Diabetes Epidemics in Korea: Reappraise Nationwide Survey of Diabetes "Diabetes in Korea 2007". Diabetes Metab J. 2013 August;37(4):233-239.

2.1.2 Diabetes Mellitus diagnosis

Normal range of blood glucose is defined when fasting blood glucose concentration is 70-100 mg/dl or postprandial blood glucose concentration is under the 140 mg/dl. It is diagnosed as diabetes when fasting blood glucose concentration is over 126 mg/dl (Table 1) [7].

Table 1. Criteria for the diagnosis of diabetes

HbA1C ≥ 6.5%	The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
or	
FPG ≥126 mg/dL (7.0 mmol/L)	Fasting is defined as no caloric intake for at least 8 h.
or	
2-h plasma glucose ≥200 mg/dL (11.1 mmol/L)	During an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.*
or	
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L)	

* In the absence of unequivocal hyperglycemia, result should be confirmed by repeat test.
American Diabetes Association. Standards of medical care in diabetes--2012. Diabetes Care. 2012; 35 Suppl 1:S11-63

Pre-diabetes means that blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes (impaired fasting glucose 100-125 mg/dl, impaired glucose tolerance 140-199 mg/dl, and HbA1c 5.7-6.4%) (Table 2) [7, 32]. Pre-diabetes is not defined as diabetes but it has significant relationship in the progress of diabetes. Not all increased fasting blood glucose don't lead to diabetes, but hyperglycemia is one of the important factors in metabolic syndrome, diabetes and cardiovascular disease [33-39]. Impaired fasting glucose (IFG) occurs in uncontrolled glucose homeostasis due to the dysfunction of insulin secretion [40-44]. The fasting plasma glucose (FPG) and the 2-h plasma glucose predict diabetes and the impaired glucose in tolerance (IGT) is associated with cardiovascular disease (CVD) events and CVD mortality [41].

Table 2. Categories of increased risk for diabetes (prediabetes)*

Impaired fasting glucose (IFG)	FPG ≥100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L)
or	
Impaired glucose tolerance (IGT)	2-h plasma glucose in the 75g OGTT 140 mg/dL (7.8 mmol/L) to 199 mg/Dl (11.0 mmol/L)
or	
HbA1C ≥ 6.5%	5.7 – 6.4 %*

* For all there tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at higher ends of the range.
American Diabetes Association. Standards of medical care in diabetes--2012. Diabetes Care. 2012; 35 Suppl 1:S11-63

2.2 Chitosan oligosaccharides

2.2.1 Structure of chitosan oligosaccharides

Chitin, which is the starting material of chitosan, is a natural polymer materials distributed widely in marine invertebrates, insects, fungi, and yeast [15, 45, 46]. Chitin is composed of a high-molecular weight linear polymer of *N*-acetyl-D-glucosamine units (GlcNAc) and insoluble in water. Chitosan, deacetylated polymer of *N*-acetyl-D-glucosamine, is made from enzymatic hydrolysis or chemical hydrolysis such as treating shrimp and other crustacean shells with the alkali sodium hydroxide (Figure 2) [15-17, 47, 48]. Chitosan has reactive amino groups which are responsible for complex formation between metal ions and the polymer chain [48]. Chitosan goes to enzyme process and turns into chitosan oligosaccharide which has a small degree of polymerization (DP) of saccharides between 2 to 10 [16, 47]. Chitosan oligosaccharide is low molecular and water soluble chitosan, it is easy to be absorbed by gastric acid hydrolysis [17, 49].

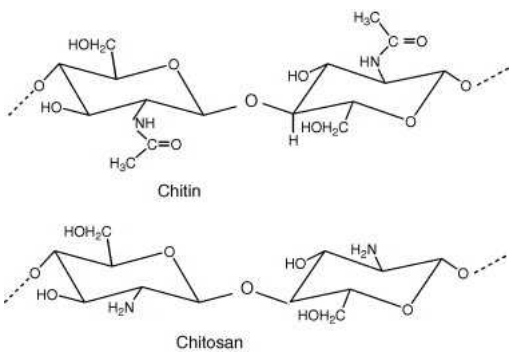

Fig.2. Chemical structures of chitin and chitosan.
Use of chitosan for the removal of metal ion contaminants and proteins from water.
Food Chemistry Volume 104, Issue 3. 2007. 989 - 996.

2.2.2 Effects of chitosan oligosaccharides

Chitosan is prepared by the alkaline deacetylation of chitin. Biological activities of chitosan, such as its antitumor effects, cholesterol-lowering effects and antibacterial effects are well known [16-18, 50]. Chitosan as an animal dietary fiber has a weak positive charge and a significant viscosity in vitro. And then chitosan in combination with bile acids which has a negative charge is excreted. In this process, the synthesis of bile acid is needed, cholesterol in body is used for the synthesis of bile acid. Therefore, chitosan intake increases the secretion of bile acid and decreases serum cholesterol level [18]. Recently, bioavailability of chitosan oligosaccharide by means of functional ingredient has been studied. Although chitin and chitosan are known to have very interesting functional properties, their high molecular weights and highly viscous nature may restrict their uses *in vivo* systems. Chitosan oligosaccharide as well as chitin and chitosan possess many bio-functional properties. Chitosan oligosaccharide has a low viscosity, small molecular weights and short-chain lengths, which makes it easily soluble in water [16]. Therefore, Chitosan oligosaccharide has more effective biological activity *in vivo*. Chitosan oligosaccharide has been reported to be beneficial in reducing blood cholesterol and blood pressure, increasing immunity and antioxidative activity, enhancing antitumor properties, and accelerating calcium and iron absorption *in vivo* [17-21, 51, 52]. However, few reports are

available on the antidiabetic effects of chitosan oligosaccharide. Chitosan oligosaccharide prevented diabetes via inhibition of carbohydrate hydrolysis enzymes in animal and *in vitro* models [23, 53]. Especially, Low molecular weight chitosan in a pre-diabetic stage prevents the progression of slowly progressive diabetes in mice [49]. Consumption of chitosan oligosaccharide has been reported to reduce postprandial blood glucose level in subjects with normal blood glucose level [22]. Few studies have reported the effect of chitosan oligosaccharide on blood glucose control in pre-diabetes or type 2 diabetes patients [54].

2.2.2.1. Antimicrobial effects of chitosan oligosaccharides

Antimicrobial activity of chitosan and its derivatives as chitosan oligosaccharide has been recognized and is considered to be one of the most important properties [55, 56]. In general, positively charged nature of chitosan and chitosan oligosaccharide molecules facilitates their binding with bacterial cell wall and leads to the inhibition of bacterial cell growth. This is because positively charged amino group at C-2 position of the glucosamine monomer interacts with negatively charged carboxylic acid group of the macromolecules of bacterial cell surface and forms polyelectrolyte complexes [57, 58]. The structural modification of chitosan oligosaccharide by introducing positively charged groups can improve the antibacterial activity ensuring better interaction with negative charged groups

on bacterial and fungi cell wall. In addition to positively charged characteristics of chitosan oligosaccharide, charge distribution of bacterial and fungi cell wall seems to play a considerable role for observed antibacterial and antifungal activities of chitosan oligosaccharide and their derivatives [17].

2.2.2.2. Immuno-enhancing and antitumor effects of chitosan oligosaccharides

In the studies of an antitumor agent, chitin and chitosan oligosaccharide inhibit the growth of tumor cells by immune-enhancing effects [16, 59]. Results of some related studies suggest that, the observed antitumor activities were not due to direct killing of tumor cells, but might be due to increased production of lymphokines, leading to manifestation of antitumor effect through proliferation of cytolytic T-lymphocytes [51, 60]. The antitumor activities observed in chitosan oligosaccharide are dependent on their structural characteristics such as degree of deacetylation (DD) and molecular weight [17]. Studies on the antitumor activity of chitosan and chitosan oligosaccharide revealed that partially deacetylated chitin and carboxymethyl chitin with an adequate degree of substitution were effective toward controlling various tumor cells [61].

2.2.2.3. Antioxidant effects of chitosan oligosaccharides

Both chitosan and chitosan oligosaccharide showed antioxidant effects. In our recent study, the chitosan oligosaccharide has antioxidative activity *in vitro* and *in vivo* [62, 63]. Chitosan oligosaccharide could significantly reduce malondialdehyde (MDA) concentrations and elevate superoxide dismutase (SOD) and catalase (CAT) activities, the latter being the major antioxidant enzymes in the body, indicating that chitosan oligosaccharide regulated the antioxidant enzyme activities and reduced lipid peroxidation [63]. Chitosan oligosaccharides have free-radical scavenging effects in a cellular system. They also stimulate an increase in intracellular Glutathione (GSH) levels. Based on the results, they concluded that chitosan oligosaccharides have free-radical scavenging effects, acting in both indirect and direct ways to inhibit and prevent biological molecular damage by free radicals in living cells [64]. Chitosan and chitosan oligosaccharides can be used as a scavenger to control radical-induced damage to cellular systems and promise further applications in the future [18, 64, 65].

2.2.2.4. Hypocholesterolemic effects of chitosan oligosaccharides

Chitosan and chitosan oligosaccharide applications lead to control of blood cholesterol level [22, 66]. Especially, chitosan oligosaccharide is capable of decreasing cholesterol level in the liver. Unlike high molecular weight chitosan, chitosan oligosaccharide application does not lead to enhancement compensatory

cholesterol synthesis, and decrease in essential fatty acids [17, 66]. Despite few researches which were carried out to search for the ability of chitosan and chitosan oligosaccharide to bind with bile salts and lipids, up-to-date their exact mechanism on lowering blood cholesterol level remained unclear [17, 66, 67]. Several hypotheses have been suggested to explain the possible actions of chitosan oligosaccharide in reducing blood cholesterol levels. One hypothesis suggests that, ionic binding of chitosan oligosaccharide with bile salts and bile acids may inhibit micelle formation during lipid digestion in the digestive track [68]. Another hypothesis suggests that chitosan and chitosan oligosaccharide can directly trap lipids and fatty acids [69].

2.2.2.5. Antidiabetic effects of chitosan oligosaccharides

Chitosan oligosaccharides possess various biological activities and can be used in the treatment of diabetes mellitus. Chitosan oligosaccharide had an antidiabetic effect by playing importance roles in β-cells through promoting proliferation, increasing insulin release [70, 71]. Chitosan oligosaccharide treatment was found to prevent diabetic alterations in heart tissues in diabetic rats. This protective effect against myocardial structural alteration may be caused by the glucose-lowering and TG-lowering effects of the chitosan oligosaccharides. Chitosan oligosaccharides increased glucose tolerance and insulin secretion, and then may be useful for preventing diabetic cardiomyopathy [50]. On the other

hand, the therapeutic effects of the chitosan oligosaccharide on type 2 diabetes might be related to its ability of increasing liver glycogen synthesis and GLUT-4 gene expression in soleus muscle and adipose to improve insulin resistance [70].

3. SUBJECTS AND METHODS

3.1. Study subjects and chitosan oligosaccharide

Study participants were included in age between 20 and 75 year old and recruited from outpatient clinics at Yonsei University Severance hospital (Seoul, Korea) and advertisement in local newspapers. After the glucose screening test, subjects with impaired fasting glucose (IFG; 100 mg/dl ≤ fasting blood glucose ≤ 125 mg/dl) or impaired glucose tolerance (IGT; 140 mg/dl ≤ 2-h OGTT) were enrolled in this study. Subjects were excluded if they had any diagnosis of heart failure, myocardial infarction, stroke, renal disease, liver disease, dyslipidemia, chronic gastrointestinal disorder, chronic alcoholism and acute or chronic inflammatory disease; were pregnant, breast feeding, or intending to become pregnant during the time of study; took corticosteroid or glucose-lowering medications for the past 1 month; had a hypersensitive reaction to medications. 60 subjects with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) were enrolled in this study with their written consent forms for the study, and the protocol was approved by the Institutional Review Board of Yonsei University. This trial is registered under the trial registration code: clinicaltrials.gov: NCTO1496820. Study participants were assigned randomly to the test or placebo group. Nine subjects were dropped out from the randomized participants. Among

the dropped out subjects, 1 subject had headache, 1 subject felt dizziness, 5 subjects gave up for personal reasons and 2 subjects were out of contact. The test group (n=25) consumed chitosan oligosaccharide (6 capsules/day for 12-weeks) before each meal. The chitosan oligosaccharide capsule contained 100% enzymatically digested low molecular weight chitosan oligosaccharide (1500 mg/day) that was prepared as described in the next paragraph. And the placebo group (n=26) consumed roasted barley meal powder (6 capsules/day for 12-weeks) before each meal. Chitosan oligosaccharide was prepared by dissolving 5.0 % (w/v) chitosan in 1.8 % (v/v) ascorbic acid, 1.2% (v/v) succinic acid and it was titrated to pH 4.5~5.5. Then 3~5 g of refined chitosanase was added to the 50 g of low molecular chitosan and was shaken at 120 rpm and was incubated at 50~60 °C for 15 hours. The product was filtered by 0.5 μm filter and was sterilized at 121 °C for 15 min. The resulting product (chitosan oligosaccharide) was composed of monomer to tetramer (82.7 %) and of more than pentamer (17.3 %) by MALDI-TOF and HPLC analysis. Chitosan oligosaccharide and placebo capsules were provided by Kunpoong Bio Co., Ltd. (Gangseo-gu, Seoul, Korea).

3.2. Materials and methods

3.2.1. Assessment of food intake and physical activity

This study was a randomized, double-blind, placebo-controlled intervention trial. The study subjects were maintained their usual diet and physical activity during the 12-week intervention period. Dietary intake was assessed using a semi-quantitative food frequency questionnaire (FFQ) and 24-hour recall method [72]. Nutrient intake was determined and calculated based on the 3-day food records using the Computer Aided Nutritional Analysis Program (CAN-pro 2.0, Korean Nutrition Society, Seoul, Korea). Total energy expenditure (TEE) (kcal/day) was calculated based on the activity patterns of the study participants, such as basal metabolic rate (BMR), 24-h physical activity [73], and specific dynamic actions of food. The BMR for each subject was calculated with the Harris-Benedict equation [74].

3.2.2. Anthropometric parameters, blood pressure, and blood collection

Body weight and height were measured without clothes and shoes in the morning. Body mass index (BMI) was calculated by kilograms per square meter (kg/m^2). Percentage of body fat was measured by a TBF-105 body fat analyzer

(Tanita Co, Tokyo, Japan). Waist and hip circumferences were measured to the nearest 0.1cm using a plastic measuring tape. Blood pressure (BP) was measured 2 or 3 times in the left arm of seated subjects with an automatic BP monitor (TM-2654; A&D, Tokyo, Japan) after a 20-minute rest. After a 12-hour fasting period, venous blood specimens were collected in ethylenediaminetetraacetate (EDTA) - treated and plain tubes and centrifuged to obtain plasma and serum. The collected plasma or serum samples were stored at −70°C until used in further analysis.

3.2.3. Serum lipid profiles

Total serum cholesterol and Triglycerides (TG) were measured using commercially available kits on a Hitachi model 7150 autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of high density lipoprotein cholesterol (HDL-C) left in the supernatant was measured by an enzymatic method. Low density lipoprotein cholesterol (LDL-C) was indirectly calculated using the Friedewald formula for subjects with serum TG concentrations < 400 mg/dl (4.52mol/L) [Friedewald formula ; LDL-cholesterol = total cholesterol – HDL-cholesterol - (triglyceride × 0.2)]. Serum apolipoprotein A1 and B were determined by turbidometry at 340 nm using a specific anti serum (Roche, Basel, Swizerland).

3.2.4. Serum fasting glucose, insulin, C-peptide, hemoglobin A1c (HbA1c), HOMA-IR

Fasting serum glucose was measured by a glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Irvine, CA, USA). Serum insulin was measured by radioimmunoassay with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Serum C-peptide was measured by immunoradiometric assay with C-peptide IRMA kit (Immunotech, Czech). HbA1c was measured by Immunoturbidimetric analyzer using turbidimeter. Insulin resistance (IR) was calculated from the homeostasis model assessment-insulin resistance (HOMA-IR) (11) using the following equation : HOMA-IR = [fasting insulin(μIU/mL) × fasting glucose(mmol/L)] / 22.5.

3.2.5. Serum interleukin-6 (IL-6), tumor necrosis factor–α (TNF-α), and plasma adiponectin

Serum interleukin-6 (IL-6), and tumor necrosis factor–α (TNF-α) concentrations were measured using the Bio-Plex™ Reagent Kit (Bio-Rad Laboratories, Hercules, CA, USA). Plasma adiponectin concentration was measured by an enzyme immunoassay (Human adiponectin ELISA kit, B-Bridge International Inc., Mountain View, CA)

3.3. Statistical analysis

Statistical analysis was performed with Win SPSS Package 18.0 (Statistical Package for the Social Science, SPSS Inc., Chicago, IL, USA). A paired t-test with the Wilcoxon signed rank test was evaluated the effect within each group both before and after intervention. Independent t- test with the Mann-Whitney U-test was evaluated to compare net change between the test and placebo groups. Results were expressed as mean ± SE. A value of $p<0.05$ was considered statistically significant.

4. RESULT

4.1. Background characteristics of subjects

A total of 60 subjects were enrolled the intervention study and 9 subjects dropped out for personal reasons. 51 subjects completed the study and were consumed chitosan oligosaccharide supplementation or placebo capsules for 12-week. Compliance was high (85%) and no adverse reactions were noted. If the study capsules were consumed more than 80% compliance was considered good.

The baseline characteristics for the 51 subjects are shown in Table 3. The participants' mean age was 57.88 ± 1.78 years in the placebo group and 54.40 ± 2.02 years in the treatment group. When the placebo and treatment parameter of the subjects were compared, there was no significant difference between the groups in baseline characteristics, such as sex, age, height, BMI (p=0.516), blood pressure (systolic BP : p=0.910, diastolic BP : p=0.429) (p-value not shown) and were significant baseline to end-of-study changes in weight and body fat within placebo group.

Table 3. Baseline clinical characteristics of study participants

	Placebo (n=26)	**P[a]**	**Chitosan oligosaccharide (n=25)**	**P[a]**	**p[b]**
Sex (male / female) (n)	19/7		17/8		0.694
Age (year)	57.88 ± 1.78		54.40 ± 2.02		0.227
Height (cm)	164.42 ± 1.31		165.32 ± 1.34		0.584
Weight (kg)					
Before	**64.49 ± 1.96**	**0.048***	66.68 ± 1.79	0.075	
After	**64.97 ± 0.92**		67.11 ± 1.80		
Change	0.48 ± 0.23		0.43 ± 0.22		0.784
BMI (kg/m^2)					
Before	23.82 ± 0.63	0.053	24.38 ± 0.56	0.056	
After	23.98 ± 0.61		24.54 ± 0.56		
Change	0.17 ± 0.09		0.16 ± 0.08		0.857
Body fat (%)					
Before	**23.24 ± 1.71**	**0.001****	26.30 ± 1.50	0.957	
After	**24.95 ± 1.52**		26.25 ± 1.57		
Change	**1.70 ± 0.44**		**-0.05 ± 0.28**		**0.005****
Blood pressure (mmHg)					
Systolic BP					
Before	125.56 ± 2.08	0.737	126.80 ± 2.58	0.367	
After	125.23 ± 1.67		125.90 ± 2.06		
Change	-0.33 ± 1.59		-0.90 ± 1.97		0.644
Diastolic BP					
Before	78.04 ± 1.61	0.324	79.86 ± 1.93	0.332	
After	79.08 ± 1.42		78.66 ± 1.89		
Change	1.04 ± 1.01		-1.20 ± 1.56		0.117

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p[a]-values derived from paired t-test with the Wilcoxon signed rank test.

p[b]-values derived from independent t-test with the Mann-Whitney U-test.

4.2. Serum lipid profiles

After the 12-week intervention, triglyceride, total cholesterol, and LDL-cholesterol were not significantly changed in both placebo and treatment groups. However, HDL-cholesterol was significantly increased in the placebo group ($p=0.004$), while there was no significant differences in the treatment group (Table 4). The change in serum level of HDL-cholesterol in the placebo group tended to be different from that in the treatment group ($p=0.060$).

Other serum lipid profiles including the concentrations Apo A was significantly increased within both placebo group (162.77 ± 5.36 vs. 170.42 ± 5.04 mg/dL; $p=0.032$) and treatment group (149.32 ± 3.46 vs. 155.04 ± 3.96 mg/dL; $p=0.019$), but there was no significant differences between groups. Apo B did not show any significant changes in both placebo and treatment groups.

Table 4. Serum lipid profiles at baseline and after the 12-week intervention

	Placebo (n=26)	**P[a]**	**Chitosan oligosaccharide (n=25)**	**P[a]**	**P[b]**
TG (mg/dL)					
Before	143.23 ± 18.95	0.148	151.80 ± 30.86	0.657	
After	126.15 ± 13.05		177.28 ± 37.96		
Change	-17.08 ± 16.42		25.48 ± 33.58		0.107
T-cholesterol (mg/dL)					
Before	195.50 ± 9.04	0.360	194.36 ± 6.19	0.843	
After	195.58 ± 7.04		195.72 ± 6.08		
Change	0.08 ± 5.19		1.36 ± 3.56		0.559
HDL- cholesterol (mg/dL)					
Before	**54.42 ± 2.29**	**0.004****	51.36 ± 1.69	0.120	
After	**58.12 ± 2.54**		53.04 ± 2.25		
Change	3.69 ± 1.50		1.68 ± 1.50		0.060
LDL- cholesterol (mg/dL)					
Before	115.17 ± 7.92	0.563	118.88 ± 5.41	0.330	
After	113.63 ± 6.75		114.91 ± 5.46		
Change	-1.54 ± 5.36		-3.97 ± 3.29		0.369
Apo-AI (mg/dL)					
Before	**162.77 ± 5.36**	**0.032***	**149.32 ± 3.46**	**0.019***	
After	**170.42 ± 5.04**		**155.04 ± 3.96**		
Change	8.92 ± 2.97		6.22 ± 2.46		0.363
Apo-B (mg/dL)					
Before	104.65 ± 5.81	0.628	103.08 ± 5.05	0.966	
After	104.42 ± 4.69		102.76 ± 4.81		
Change	-0.64 ± 3.22		-0.78 ± 2.34		0.606

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p[a]-values derived from paired t-test with the Wilcoxon signed rank test.

p[b]-values derived from independent t-test with the Mann-Whitney U-test.

4.3. Dietary food intake and total energy expenditure

After the 12-week intervention, total energy expenditure (TEE), total calorie intake (TCI) and percent energy intake from carbohydrate, protein, and fat had no significant effect in treatment group (Table 5). However, total energy expenditure (TEE), total calorie intake (TCI), and percent energy intake from protein in the placebo group were significantly changed after the intervention. Only the change of percent energy intake from protein was significantly different between the placebo group (0.37 ± 0.15%) ($p=0.017$) and the treatment group (-0.12 ± 0.12%).

Table 5. Daily food intake and total energy expenditure at baseline and after the 12-week intervention

	Placebo (n=26)	**p[a]**	**Chitosan oligosaccharide (n=25)**	**p[a]**	**p[b]**
TEE (kcal)					
Before	**2175.47 ± 49.21**	**0.014***	2281.15 ± 53.25	0.326	
After	**2145.68 ± 50.10**		2268.75 ± 51.83		
Change	-29.8 ± 10.07		-12.4 ± 18.31		0.309
Estimates of daily nutrient intakes					
TCI (kcal/d)					
Before	**2159.66 ± 52.27**	**0.004****	2270.28 ± 51.85	0.174	
After	**2212.39 ± 53.23**		2292.46 ± 50.24		
Change	52.74 ± 19.62		22.17 ± 14.56		0.418
Carbohydrate (%)					
Before	61.88 ± 0.20	0.849	61.97 ± 0.21	0.420	
After	61.78 ± 0.20		61.71 ± 0.17		
Change	-0.09 ± 0.27		-0.26 ± 0.24		0.658
Protein (%)					
Before	**16.05 ± 0.10**	**0.022***	16.39 ± 0.10	0.264	
After	**16.42 ± 0.13**		16.27 ± 0.11		
Change	**0.37 ± 0.15**		**-0.12 ± 0.12**		**0.017***
Fat (%)					
Before	22.26 ± 0.21	0.328	22.21 ± 0.20	0.936	
After	22.03 ± 0.20		22.25 ± 0.17		
Change	-0.23 ± 0.26		0.04 ± 0.26		0.462
Cholesterol (mg)					
Before	222.55 ± 15.66	0.849	194.40 ± 14.21	0.211	
After	212.98 ± 16.95		205.05 ± 17.12		
Change	-9.56 ± 17.32		10.66 ± 19.03		0.309

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p[a]-values derived from paired t-test with the Wilcoxon signed rank test.

p[b]-values derived from independent t-test with the Mann-Whitney U-test.

TEE : Total energy expenditure (kcal), TCI : Total calorie intake (kcal)

4.4. Laboratory biomarker profiles

Biomarkers of safety assessed by white blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit, platelet, creatinine and blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT) and albumin. Hematocrit was significantly decreased in the treatment group (46.02 ± 0.86 vs. 42.26 ± 0.91 %; p=0.002) and GOT was significantly increased in the treatment group (22.88 ± 1.11 vs. 25.00 ± 1.39 IU/L; p=0.035). Creatinine was significantly increased in the placebo group (0.90 ± 0.04 vs. 0.93 ± 0.04 mg/dL; p=0.007) and significantly different between the placebo and the treatment group (0.03 ± 0.01 vs. -0.02 ± 0.02 mg/dL; p=0.016). Biochemical markers of safety were shown in significant differences were included in the normal range (data not shown).

4.5. Serum glucose concentration

After the 12-week dietary intervention, the treatment group showed a significant decrease in serum level of blood glucose at 30 min ($p=0.013$) and at 60 min ($p=0.028$) (Table 6) and the placebo group did not show significant changes in serum level of blood glucose at 0 (fasting), 30, 60 and 120 min (Figure 3) and area under the curve of glucose (glucose AUC). The change (Δ) in serum level of blood glucose at 60 min in dietary chitosan oligosaccharide treatment group was significantly different from in the placebo group ($p=0.030$). Area under the curve of glucose (glucose AUC) tended to decrease in the treatment group ($p=0.061$).

Table 6. Level of glucose at baseline and after the 12-week intervention

	Placebo (n=26)	P^a	Chitosan oligosaccharide (n=25)	P^a	P^b
Glucose (mg/dL)					
0 min					
Before	118.58 ± 3.23	0.637	115.36 ± 3.18	0.927	
After	119.42 ± 3.15		114.12 ± 2.56		
⊿ Change	0.85 ± 2.27		-1.24 ± 1.86		0.692
30 min					
Before	197.81 ± 8.80	0.170	**196.52 ± 7.15**	**0.013***	
After	183.88 ± 6.96		**178.56 ± 6.90**		
⊿ Change	-13.92 ± 8.08		-17.96 ± 6.92		0.534
60 min					
Before	214.81 ± 13.89	0.316	**222.00 ± 10.79**	**0.028***	
After	221.96 ± 12.31		**202.72 ± 12.50**		
⊿ Change	**7.15 ± 9.62**		**-19.28 ± 8.22**		**0.030***
120 min					
Before	172.65 ± 11.79	0.629	165.96 ± 12.33	0.700	
After	175.27 ± 10.97		167.88 ± 13.39		
⊿ Change	2.62 ± 8.29		1.92 ± 11.08		0.888
Glucose_AUC					
Before	376.02 ± 19.49	0.751	376.60 ± 16.92	0.061	
After	375.93 ± 16.48		353.82 ± 18.21		
⊿ Change	-0.08 ± 13.13		-22.79 ± 12.30		0.200

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p^a-values derived from paired t-test with the Wilcoxon signed rank test.

p^b-values derived from independent t-test with the Mann-Whitney U-test.

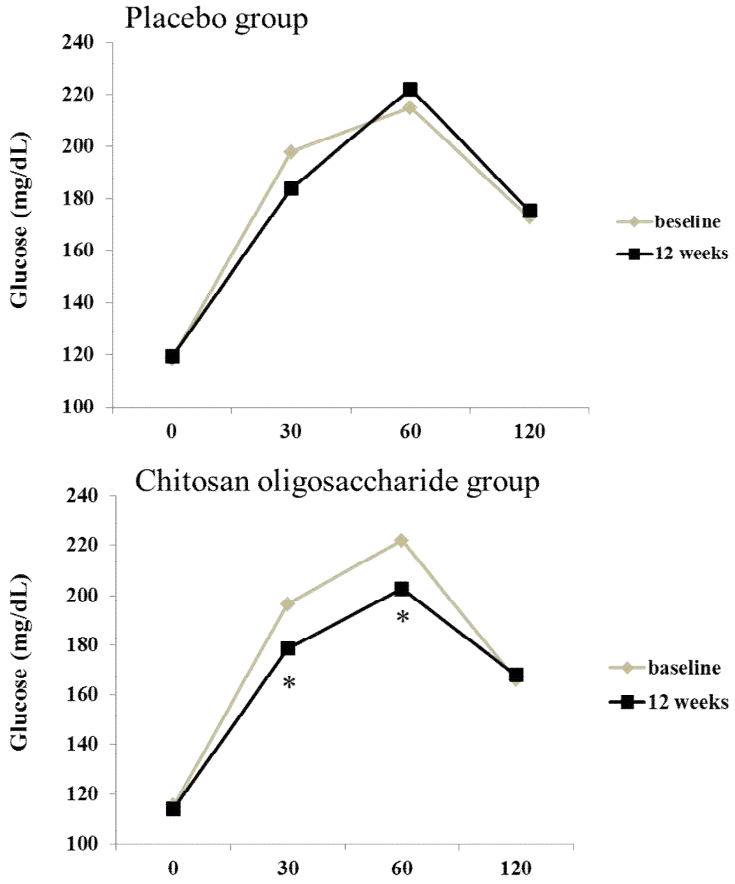

Fig.3. Serum glucose during oral glucose tolerance test (OGTT) at baseline and after the 12-week intervention. Mean ± S.E. *P<0.05 compared to baseline within the group.

†P<0.05 compared with the changed value of placebo group. Paired t-test with the Wilcoxon signed rank test. P-values derived from independent t-test with the Mann-Whitney U-test.

4.6. HOMA-IR, HbA1C, Insulin and C-peptide concentration

Effect of chitosan oligosaccharide supplementation on HbA1c during the 12-week study was shown in Figure 4. HbA1c in the treatment group was significantly decreased after 12-week chitosan oligosaccharide supplementation (6.36 ± 0.19 vs. 6.11 ± 0.11; p=0.023). The change (⊿) in HbA1c was statistically different between the treatment group (-0.26 ± 0.15 %) and the placebo group (0.09 ± 0.06 %) (p=0.021). HOMA-IR did not significantly change in both placebo and treatment groups (Table 7).

Serum insulin concentration did not significantly change before and after dietary chitosan oligosaccharide treatment. 30 min-serum insulin level had a tendency to decrease in placebo group (p=0.050) (Table 8). The change (⊿) in serum level of insulin at 0 (fasting), 60 and 120 min did not significantly change in both groups. Area under the curve of insulin (insulin AUC) was no significant difference in each groups and between the placebo and the treatment groups.

Serum C-peptide concentration at 120 min in the placebo group was significantly increased after 12-week intervention (9.16 ± 0.80 vs. 9.89 ± 0.76; p=0.046). The change (⊿) in serum level of C-peptide at 120 min in the treatment group tended to be different from that in the placebo group (p=0.069) (Table 9). Area under the curve of C-peptide (C-peptide AUC) was no significant difference in each groups and between two groups.

Table 7. Glucose-related biomarkers at baseline and after the 12-week intervention

	Placebo (n=26)	P^a	Chitosan oligosaccharide (n=25)	P^a	P^b
HbA1c (%)					
Before	6.19 ± 0.12	0.238	**6.36 ± 0.19**	**0.023***	
After	6.28 ± 0.13		**6.11 ± 0.11**		
Change	**0.09 ± 0.06**		**-0.26 ± 0.15**		**0.021***
HOMA-IR					
Before	1.80 ± 0.16	0.989	1.73 ± 0.11	0.206	
After	1.79 ± 0.17		1.61 ± 0.13		
Change	0.00 ± 0.14		0.11 ± 0.09		0.509

Means±S.E.
*P<0.05, **p<0.01 compared with baseline in each group.
p^a-values derived from paired t-test with the Wilcoxon signed rank test.
p^b-values derived from independent t-test with the Mann-Whitney U-test.
HOMA-IR = [fasting insulin(μIU/mL) × fasting glucose(mmol/L)] / 22.5

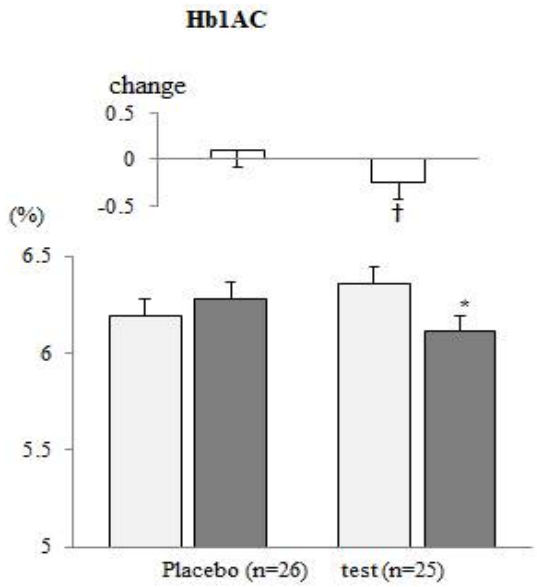

Fig.4. Effect of 12-week chitosan oligosaccharide supplementation on HbA1C.
Mean ± S.E. *P<0.05 compared to baseline within the group.
†P<0.05 compared with the changed value of placebo group. Paired t-test with the Wilcoxon signed rank test.
P-values derived from independent t-test with the Mann-Whitney U-test.

Table 8. Glucose-related biomarkers at baseline and after the 12-week intervention

	Placebo (n=26)	P^a	Chitosan oligosaccharide (n=25)	P^a	P^b
Insulin (uU/mL)					
0 min					
Before	6.66 ± 0.54	0.780	6.66 ± 0.41	0.225	
After	6.64 ± 0.59		6.25 ± 0.47		
⊿ Change	-0.02 ± 0.47		-0.40 ± 0.32		0.401
30 min					
Before	**43.07 ± 10.41**	**0.050**	39.47 ± 7.31	0.319	
After	**33.25 ± 6.68**		31.56 ± 4.39		
⊿ Change	-9.82 ± 6.03		-7.91 ± 4.10		0.418
60 min					
Before	58.83 ± 9.61	0.485	52.67 ± 6.11	0.476	
After	51.77 ± 7.60		47.59 ± 6.62		
⊿ Change	-7.07 ± 7.23		-5.08 ± 6.04		0.713
120 min					
Before	36.29 ± 4.73	0.124	41.14 ± 6.17	0.778	
After	43.48 ± 6.55		46.79 ± 7.92		
⊿ Change	7.18 ± 4.14		5.65 ± 5.88		0.468
Insulin_AUC					
Before	85.48 ± 11.59	0.722	81.48 ± 9.57	0.638	
After	78.86 ± 10.18		76.45 ± 9.81		
⊿ Change	-6.62 ± 7.04		-5.04 ± 6.61		0.851

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p^a-values derived from paired t-test with the Wilcoxon signed rank test.

p^b-values derived from independent t-test with the Mann-Whitney U-test.

Table 9. Glucose-related biomarkers at baseline and after the 12-week intervention

	Placebo (n=26)	P^a	Chitosan oligosaccharide (n=25)	P^a	P^b
C-peptide (ng/mL)					
0 min					
Before	2.20 ± 0.24	0.666	1.97 ± 0.13	0.134	
After	2.17 ± 0.26		1.87 ± 0.14		
⊿ Change	-0.03 ± 0.14		-0.10 ± 0.09		0.251
30 min					
Before	6.02 ± 0.82	0.238	5.67 ± 0.58	0.476	
After	5.51 ± 0.63		5.39 ± 0.47		
⊿ Change	-0.51 ± 0.38		-0.27 ± 0.34		0.763
60 min					
Before	8.93 ± 0.72	0.909	8.89 ± 0.50	0.201	
After	8.74 ± 0.63		8.16 ± 0.56		
⊿ Change	-0.18 ± 0.49		-0.74 ± 0.52		0.361
120 min					
Before	**9.16 ± 0.80**	**0.046***	9.31 ± 0.70	0.420	
After	**9.89 ± 0.76**		8.98 ± 0.83		
⊿ Change	**0.75 ± 0.43**		**-0.33 ± 0.69**		**0.069**
C-peptide_AUC					
Before	14.84 ± 1.23	0.839	14.65 ± 0.92	0.353	
After	14.81 ± 1.11		13.77 ± 0.93		
⊿ Change	-0.04 ± 0.54		-0.88 ± 0.71		0.365

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p^a-values derived from paired t-test with the Wilcoxon signed rank test.

p^b-values derived from independent t-test with the Mann-Whitney U-test.

4.7. Pro-inflammatory cytokine and plasma adiponectin concentration

After the 12-week dietary intervention, the treatment group showed a significant decrease in the serum IL-6 level ($p=0.002$) (Figure 5). Serum TNF-α level tends to decrease in the treatment group ($p=0.077$). No significant differences were found in the change level of IL-6 and TNF-α between the placebo and the treatment groups (Table 10).

Plasma adiponectin concentration was significantly increased in the treatment group after the 12-week intervention ($p=0.013$) (Figure 6).

Table 10. Pro-inflammatory cytokine and plasma adiponectin at baseline and after the 12-week intervention

	Placebo (n=26)	**P[a]**	**Chitosan oligosaccharide (n=25)**	**P[a]**	**P[b]**
TNF-α (pg/mL)					
Before	10.02 ± 1.15	0.107	15.65 ± 3.64	0.077	
After	8.29 ± 0.62		10.85 ± 1.06		
Change	1.74 ± 0.99		4.80 ± 2.87		0.953
IL-6 (pg/mL)					
Before	3.56 ± 0.43	0.573	**4.74 ± 0.88**	**0.002****	
After	3.52 ± 0.49		**3.01 ± 0.30**		
Change	0.04 ± 0.44		1.73 ± 0.78		0.109
Adeponectin (ug/mL)					
Before	7.07 ± 0.70	0.174	**7.40 ± 0.66**	**0.013***	
After	7.37 ± 0.68		**7.93 ± 0.67**		
Change	-0.30 ± 0.17		-0.53 ± 0.18		0.254

Means±S.E.

*P<0.05, **p<0.01 compared with baseline in each group.

p[a]-values derived from paired t-test with the Wilcoxon signed rank test.

p[b]-values derived from independent t-test with the Mann-Whitney U-test.

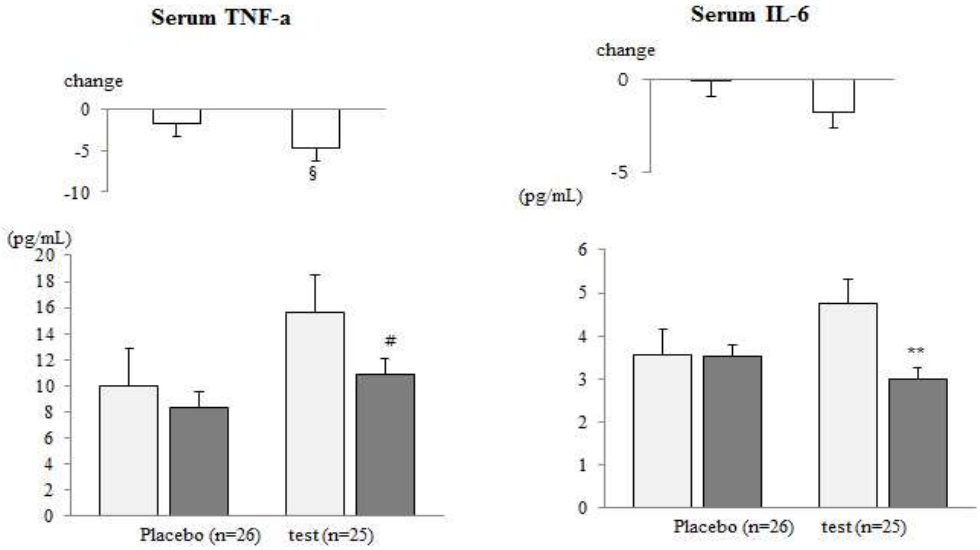

Fig.5. Effect of 12-week chitosan oligosaccharide supplementation on serum TNF-α and IL-6.
Mean ± S.E. **P<0.01, *P<0.05 , $^{\#}$P<0.10 compared to baseline within the group.
†P<0.05, §P<0.10 compared with the changed value of placebo group.
Paired t-test with the Wilcoxon signed rank test. P-values derived from independent t-test with the Mann-Whitney U-test.

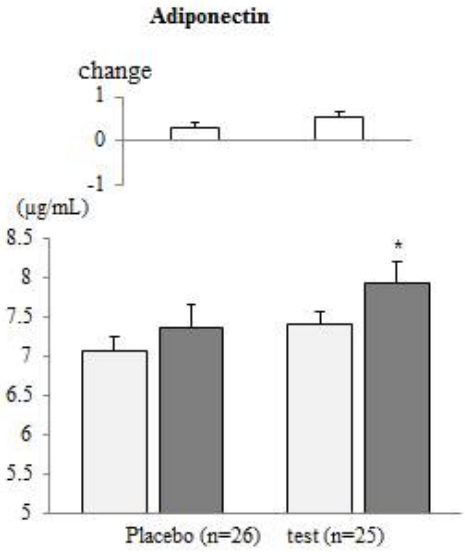

Fig.6. Effect of 12-week chitosan oligosaccharide supplementation on adiponectin
Mean ± S.E. *P<0.05 compared to baseline within the group.
Paired t-test with the Wilcoxon signed rank test. P-values derived from independent t-test with the Mann-Whitney U-test.

5. DISCUSSION

This randomized, double-blind, placebo-controlled study evaluated the effect of chitosan oligosaccharide on glucose control among subjects with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

The prevalence of type 2 diabetes is increasing worldwide. Due to the high blood glucose, an estimated 4.8 million people died in 2012. According to the WHO project, diabetes deaths will double between 2005 and 2030. More than 471 billion USD were spent on healthcare for diabetes in 2012 [75, 76]. Therefore, prevention and control of diabetes are critical issue. People with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) have a higher risk of type 2 diabetes. Thus, pre-diabetes should be managed for the prevention of diabetes.

Chitosan oligosaccharide is made from chemical or enzymatic hydrolyzed chitosan and it has effects on blood pressure and cholesterol control, and anti-inflammation [17]. Wang D et al. observed that chitosan oligosaccharide has beneficial lipid-regulating effects in high-fat-diet-fed rats, which decreased triglyceride (TG), very low density lipoprotein (VLDL) and increased high density lipoprotein (HDL) [77]. In addition, several studies have reported the effect of chitosan oligosaccharide on glucose control. In particular, low molecular weight chitosan oligosaccharide was shown to have the strong effect for blood glucose management in normal and pre-diabetic individuals. We have previously

reported that low molecular weight chitosan oligosaccharide effectively reduced glucose level in SD Rat model by inhibition of carbohydrate hydrolysis enzymes and by more easier glucose absorption into muscle and fat cells in the cellular level [23]. However, there are few intervention studies about chitosan oligosaccharide and glucose control in humans. Ju et al. observed that after 8 weeks of chitosan oligosaccharide treatment in type 2 diabetes rats, chitosan oligosaccharide significantly reduced fasting blood glucose, fasting insulin, increased the insulin sensitivity index and improved oral glucose tolerance [70]. Kim et al. observed that in Koreans with normal blood glucose level, consuming 500 mg chitosan oligosaccharide reduced the level of postprandial blood glucose [22]. In this study, we investigated the effect of chitosan oligosaccharide on serum blood glucose control. Subjects consuming chitosan oligosaccharide 1500 mg per day (6 capsules per day) significantly reduced postprandial serum blood glucose level in 30 min and 60 min over the 12-week intervention period. The 60 min serum blood glucose level in treatment group especially significantly decreased while it was increased in placebo group.

HbA1c reflects long-term glycemic level and is less affected by current health status [78, 79]. Thus, it is one of the useful methods to diagnose diabetes. According to several studies, which reported that the dietary chitosan oligosaccharide had interestingly a tendency to decrease on HbA1c in human [77]. Kim et al. showed the effect of chitosan oligosaccharide on HbA1c in diabetes

rats. In treatment group, which was fed with chitosan oligosaccharide, HbA1c was decreased (4.9 ± 0.2 %) compared to the chitosan oligosaccharide non-treated group (5.8 ± 0.2 %) [80]. In our study, HbA1c was significantly reduced in chitosan oligosaccharide consuming group after the 12-week, while it was increased in placebo group. These results have a practical meaning because it showed the long-term effect of dietary chitosan oligosaccharide on glycemic control.

Insulin is produced by β-cells of the pancreas, which stimulates glucose uptake into insulin responsive tissues and thus increases insulin sensitivity. Liu B et al. studied that the chitosan oligosaccharides (100 mg/L) had direct and significant effect on pancreatic β-cells increasing insulin release from islet cells in diabetes rats. It is important to note that chitosan oligosaccharides have been shown to promote the proliferation of β-cells and recovery of damaged β-cells functions [71].

The C-peptide is a 31 amino acid peptide which connects insulin's A-chain to its B-chain in the proinsulin and the half-life of C-peptide is longer than that of insulin. Such that serum insulin levels will fall more rapidly than those of the C-peptide [81, 82]. Thus, C-peptide is reliable indicator of insulin-secretory capacity. Kim et al. reported that C-peptide level was higher in the chitosan oligosaccharide treatment group (770 ± 197.6 pmol/I) than in the non-treated group (611 ± 132.6 pmol/I) in diabetes rats [80].

However, serum insulin and C-peptide level was not significantly changed by consuming chitosan oligosaccharide in the present study. The difference with other studies on serum insulin and C-peptide concentrations could be attributed to difference in subject characteristics. Previous studies were conducted with diabetes rats, whereas this intervention study was conducted with pre-diabetes subjects.

IL-6 and TNF-α are pro-inflammatory cytokines produced by adipose tissue and their levels are raised in diabetes [83, 84]. Increased IL-6 and TNF-α level significantly correlates with BMI, blood pressure, fasting insulin levels, insulin resistance [84-87]. Yoon et al. showed chitosan oligosaccharide has been associated with the anti-inflammatory regulation of IL-6 and TNF-α gene expression in RAW 264.7 cells. Thus, chitosan oligosaccharide was effect of the down-regulation of IL-6 and TNF-α [52]. Kim et al. reported that TNF-α level was slightly decreased after chitosan oligosaccharide intake in elderly adults [88]. In our study, we observed that serum IL-6 level was significantly decreased by consuming chitosan oligosaccharide and serum TNF-α level tended to decrease by consuming chitosan oligosaccharide.

Adiponectin is a peptide hormone which is synthesized and secreted from adipose tissue and thus important in glucose and lipid metabolism [89, 90]. High levels of adiponectin are strongly associated with increased insulin sensitivity and reduced risk of type 2 diabetes, whereas low adiponectin concentrations are

shown in patients with insulin resistance and type 2 diabetes [91, 92].

Furthermore, several studies observed the lower plasma adiponectin and higher pro-inflammatory cytokines in type 2 diabetes. Hotta et al. reported that plasma adiponectin concentration in the diabetic patients were lower than that in the non-diabetic subjects (6.6 ± 0.4 vs. 7.9 ± 0.5 mg/ml in men, 7.6 ± 0.7 vs. 11.7 ± 1.0 mg/ml in women; $P<0.001$) [93]. Adiponecin has a key role in the connection between insulin resistance and a subsequent development of type 2 diabetes [94]. Kumar et al. reported that oligosaccharide treatment group was significantly higher in gene expression of adiponectin in ob/ob mice [95]. According to other previous studies, chitosan feeding significantly decreased plasma IL-6 and TNF-α, and increased plasma adiponectin levels in diabetic rats [96]. In present study, we observed that plasma adiponectin concentration was significantly increased by consuming chitosan oligosaccharide.

The limitations of this study include the small number of participants and the lack of testing for a dose-response relationship or subject of gender between the chitosan oligosaccharide supplement and glucose control. Thus, further large-scale research in which several test doses and subject of gender are examined is required to obtain detailed and accurate results.

Despite these limitations, this study shows that chitosan oligosaccharide supplement group improves serum glucose level, HbA1c, pro-inflammatory cytokines, such as IL-6, TNF-α, and serum adiponectin compared to a placebo

group with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).

In conclusion, we have shown that the chitosan oligosaccharide supplementation helps to control the postprandial glycemic response in pre-diabetic population. Further studies are still required to re-evaluate the level of insulin and C-peptide, and larger clinical studies with diabetes patients are needed to confirm the effect of chitosan oligosaccharide whether directly control of blood glucose or indirectly control of insulin secretion.

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국 문 요 약

전당뇨 대상자에서 키토산 올리고당 보충제의 섭취가 혈당조절에 미치는 효과 연구

건강에 대한 관심이 높아짐에 따라 식품의 기능성 성분과 천연물질로부터 유효성분을 추출하여 다양한 질병의 위험을 감소시키기 위한 연구와 개발이 이루어지고 있다. 이 중 지질대사 개선 소재로 알려져 있는 키토산의 항당뇨 활성에 대한 연구가 진행되고 있었으나 인체 내 흡수율이 높지 않아, 최근에는 흡수율이 높은 가수분해 저분자 화합물인 키토산 올리고당의 혈당조절효과에 대한 연구가 활발히 진행되고 있다.

본 연구에서는 전당뇨 대상자에게서 키토산 올리고당 보충제의 섭취가 혈당조절에 미치는 효과를 보고자 하였다. 공복혈당장애, 내당능장애가 있는 전당뇨 대상자 60명을 대상으로 무작위 배정, 이중맹검, 플라시보-대조군 인체시험을 실시하여, 각 군별로 키토산 올리고당 캡슐 또는 플라시보 캡슐을 12주간 섭취하도록 한 후, 12주 전후 혈당, 혈당반응면적, 인슐린, C-peptide, HbA1c, pro-inflammatory cytokine, plasma adiponectin 등을 측정하여 섭취 전후의 유의적인 변화가 있는지 확인하고, 대조군과 실험군 간의 유의적인 차이가 있는 지 검정하였다. 그 결과

시험군은 혈청에서 분석한 30분대 혈당이 초기 196.52 ± 7.15 mg/dL 에서 12주 후 178.56 ± 6.90 mg/dL (p=0.013) 로, 60분대 혈당이 초기 222.00 ± 10.79 mg/dL 에서 12주 후 202.72 ± 12.50 mg/dL (p=0.028) 로 유의적으로 감소하였다. 또한 시험군에서 혈당반응면적(AUC)은 초기 376.60 ± 16.92 mg/dL 에서 12주 후 353.82 ± 18.21 mg/dL 로 감소하는 경향이 있었다. 혈당지표의 군간 변화값 비교에서 시험군의 60분대 혈당이 초기값 대비 −19.28 ± 8.22 mg/dL 감소하여 대조군의 60분대 혈당 변화값과 유의적인 차이(p=0.030) 가 있었다. 혈당 관련 지표인 당화혈색소 분석 결과 시험군에서 초기 6.36 ± 0.19 % 에서 12주 후 6.11 ± 0.11 % 로 유의적으로 감소 (p=0.023) 하였고, 당화혈색소 군간 변화값 비교시 시험군에서 초기값 대비 −0.26 ± 0.15 % 감소하여 대조군 변화값과 유의적인 차이 (p=0.021) 가 있었다. C-peptide 군간 변화값 비교시, 시험군에서 120분대 C-peptide가 초기값 대비 −0.33 ± 0.69 ng/mL 이었고, 대조군은 0.75 ± 0.43 ng/mL 로 군간 유의적인 경향이 있었다. 염증지표 중 IL-6는 시험군에서 각각 초기 4.74 ± 0.88 mg/dL 에서 12주 후 3.01 ± 0.30 mg/dL (p=0.002) 로, adiponection은 초기 7.40 ± 0.66 mg/dL 에서 12주 후 7.93 ± 0.67 mg/dL (p=0.013) 로 유의적으로 감소하였다

결론적으로 본 연구에서 전당뇨 대상자들에게 12주동안 1일 3회 (6

캡슐, 1500mg) 의 키토산 올리고당 보충제를 섭취시켰을 때 혈당 및 식후혈당관련 지표가 개선되는 것을 볼 수 있었다. 앞으로의 연구에서 인슐린과 C-peptide 수준의 재평가가 요구되며 당뇨병 환자들을 대상으로 키토산 올리고당의 효과에 대한 후속 임상연구가 진행되어야 할 것이다.

핵심 되는 말 : 키토산 올리고당, 무작위배정/이중맹검, Placebo 대조 인체시험, 공복혈당 및 내당능장애자, 초기 당뇨병