The effect of Oligonol intake on cortisol and related cytokines in healthy young men

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Abstract

This study investigated the effects of Oligonol intake on cortisol, interleukin (IL)-1β, and IL-6 concentrations in the serum at rest and after physical exercise loading. Nineteen healthy sedentary male volunteers participated in this study. The physical characteristics of the subjects were: a mean height of 174.2 ± 2.7 cm, a mean weight of 74.8 ± 3.6 kg and a mean age of 22.8 ± 1.3 years. Each subject received 0.5 L water with Oligonol (100 mg/day) (n = 10) or a placebo (n = 9) daily for four weeks. The body composition, the white blood cell (WBC) and differential counts as well as the serum cortisol, IL-1β, and IL-6 concentrations were measured before and after Oligonol intake. The cortisol concentration and serum levels of IL-1β and IL-6 after Oligonol intake were significantly decreased compared to before treatment ($P < 0.01$, respectively). In addition, the rate of increase of these factors after exercise was decreased compared to the placebo group. There was no change in the WBC and differential cell counts. These results suggest that oral Oligonol intake for four weeks had a significant effect on inhibition of inflammatory markers in healthy young men.

Key Words: Oligonol, cortisol, interleukin -1β, interleukin -6

Introduction

The plants, vegetables, herbs and spices used in traditional medicine have been widely studied for their prophylactic and chemopreventive effects on human disease; in addition, they have been used for drug discovery and development [1-2]. Oligonol is a novel compound produced from the oligomerization of polyphenol. It is an optimized phenolic product containing catechin-type monomers and oligomers (dimer, trimer, and tetramer) of proanthocyanidin that are easily absorbed [3]. Oligonol is composed of 50% oligomers whereas a typical polyphenol polymer contains less than 10%. Thus, polyphenol polymers are not as efficiently bioactive or easily absorbed as Oligonol because of their high molecular weight.

Extracts or other purified preparations of phenolic rich foods have antioxidant, antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, vasodilatory, and neuroprotective properties [4-7]. Nagakawa *et al.* [8] examined the effects of proanthocyanidin-rich extracts in rats subjected to renal ischemia-reperfusion. Their results suggested that Oligonol might play a role in modulating the cerebral and renal ischemia associated with oxidative stress. It has been shown that Oligonol exhibits significant protection against b-amyloid- and high glucose-induced cytotoxicity in rat pheochromocytoma PC12 cells and in the porcine proximal tubule cell line LLC-PK1, respectively [9,10].

In spite of the findings of recent studies on Oligonol, except for the study reported by Fujii and colleagues [11], there has been no study demonstrating the anti-inflammatory and anti-oxidative effects of Oligonol in humans. Thus, the purpose of the present study was to examine the effects of Oligonol intake for four weeks on cortisol and related cytokines, such as interleukin (IL)-6 and IL-1β, in healthy male subjects.

Exercise-induced stress was evaluated in this study. Exercise has acute and chronic effects on the systemic immunity and inflammatory response. It causes changes in stress hormones and cytokine concentrations. Following prolonged running at high intensity, the concentration of serum cortisol has been shown to be significantly elevated above control levels for several hours; this has been related to many of the cell trafficking changes that occur during recovery. Exercise that causes muscle cell injury can result in sequential release of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6 [12,13]. The inflammatory cytokines help regulate the rapid migration of neutrophils, and then later monocytes, into the areas of injured muscle cells and other metabolically active tissues to initiate repair [14].

Subjects and Methods

Subjects

Following approval of the experimental protocol from the University of Soonchunhyang Research Committee and obtaining written informed consent, normotensive participants were enrolled in the study. Nineteen male volunteers, 22.1 years of age, participated in this study. The subjects were randomly assigned to one of two groups: the Oligonol group (n = 10) received Oligonol whereas the control group (n = 9) received a placebo similar in appearance.

All of the subjects were informed of the aims, risks and benefits of participating in this investigation, both verbally and in writing, prior to signing an informed consent document. The study was carried out in accordance with the Helsinki Declaration of 1975. All subjects refrained from specific medications or diets for the period of the study and they restricted alcohol intake and smoking before the day of the test. The physical characteristics of the subjects are shown in Table 1. There were no significant differences in age, height, weight, and % fat and body mass index (BMI), VO_{2max}.

The maximum oxygen consumption and heart rate were measured with a pulse sensor on the chest and an expired air gas analyzer (COSMED; Quark Pulmonary Function Testing Lung Volumes Module 2 ergo, Rome, Italy) during treadmill Bruce protocol test. The criteria for determining VO_{2max} were that the respiratory exchange ratio was 1.1, VO_{2max} leveled off despite increasing workload, and heart rate reached the age-predicted maximal value. All of the subjects carried out 60 min of running at 75% intensity based on the VO_{2max} data.

The oral Oligonol supplement

Oligonol was supplied by Amino Up Chemical Company (Sapporo, Japan), and produced by oligomerization of polyphenols rich in lychees. The dose of Oligonol was determined according to the study of Fujii and colleagues [11] that demonstrated that Oligonol is safe after repeated intake of doses lower than 200 mg/day. All subjects had 0.5 L water with oral OL intake (100 mg/2 times/day) for four weeks. The Oligonol was powdered and heated to 98~100℃ in reverse osmotic purified water. The Oligonol was then filtered and combined in reverse osmotic water to maintain the physiological activity of the Oligonol. The composition of the Oligonol is shown in the Table 2.

WBC analysis

Whole peripheral blood was collected from the antecubital vein with the subject at rest. The white blood cell count (WBC) and differential counts were analyzed using a LH750 Beckman Coulter, USA.

Analysis of cortisol concentration

Cortisol was determined with the COAT-A-COUNT cortisol/DPC by Radioimmunoassay (RIA, Competitive method), and analyzed using the 1470 Wizard (PerkinElmer, Finland)

Analysis of IL-6 and IL-1β by ELISA

For the analysis of the serum IL-1β (R&D system, USA) and IL-6 (R&D systems, San Diego, CA, USA), $100 \mu\ell$ and $150 \mu\ell$, respectively, of serum was analyzed using kits. The concentrations of IL-6 and IL-1β were measured by an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, Sunnyvale, CA, USA) at 490 nm. The lower limit of detection was 3.13 pg/ml. The values below this limit were assumed to be zero for the statistical analysis. The inter- and intra-assay coefficients of variance were below 10%.

Statistical analysis

Descriptive statistics are expressed as the means ± SD (standard deviation) using SPSS/Windows 11.0. Repeated two way analysis of variance was used to compare values, and contrast method was used to compare values within each group. The level of significance was set at $P < 0.05$.

Results

WBC and subtypes

As shown in Table 3, there were no significant differences in the WBC and subtypes between before and after 4 weeks Oligonol intake in each group.

Table 1. Physical characteristics of the subject

Groups	Age (yrs)	Height (cm)	Weight (kg)	% Fat	BMI	VO_{2max} (ml/kg/min)
Control-G	23.1 ± 1.1	174.6 ± 1.9	72.9 ± 4.7	18.3 ± 4.1	24.3 ± 2.9	48.5 ± 5.2
OL-Sup-G	22.5 ± 1.4	173.8 ± 3.2	76.8 ± 2.3	17.9 ± 4.1	25.1 ± 3.0	51.3 ± 3.3

The values are the mean ± SD.
Control-G (placebo-supplemented subjects group, n = 9) and OL-Sup -G (Oligonol-supplemented subjects group, n = 10)

Table 2. The composition of the Oligonol

Carbohydrate	93.6-98.6
Protein	1.4-2.2
Lipid	0.0-1.4
Total Polyphenol	More than 70
Monomers	More than 10
Sodium	Not more than 200 mg/100 g
Potassium	Not more than 100 mg/100 g

Unit (%)

Table 3. Comparison of the WBC and subtypes before and after Oligonol intake for 4 weeks

| Variables (10^3/L)	Before 4 weeks		After	
	Control-G (n = 9)	OL-Sup-G (n = 10)	Control-G (n = 9)	OL-Sup-G (n = 10)
WBC	6.45 ± 0.63	5.83 ± 0.84	6.50 ± 0.71	5.95 ± 0.61
Neutrophil	3.21 ± 2.07	2.94 ± 0.79	3.36 ± 1.65	2.73 ± 0.82
Lymphocyte	2.54 ± 2.31	2.17 ± 1.19	2.42 ± 1.93	2.52 ± 1.13
Monocyte	0.52 ± 0.09	0.60 ± 0.08	0.57 ± 0.11	0.62 ± 0.05
Eosinophil	0.12 ± 0.07	0.09 ± 0.05	0.10 ± 0.06	0.05 ± 0.04
Basophil	0.06 ± 0.05	0.03 ± 0.02	0.05 ± 0.04	0.01 ± 0.03

The values are the mean ± SD.
Control-G (placebo-supplemented subjects group) and OL-Sup-G (Oligonol-supplemented subjects group)
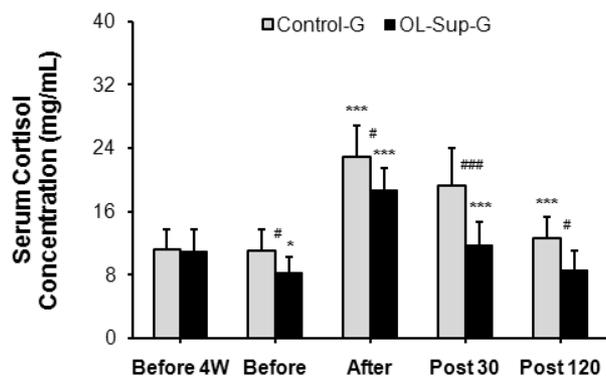
Fig. 1. Effect of Oligonol intake and physical loading on the serum cortisol concentration level in the Control-G (placebo-supplemented subjects group) and OL-Sup-G (Oligonol-supplemented subjects group). Before 4W: before Oligonol intake; Before: resting condition, after 4weeks Oligonol intake; After: immediately after the physical loading VO$_{2max}$ 75% for 60 min; Post 30: recovery after of 30 min; Post 120: recovery after of 120 min. The values are the mean ± SD. ### $P < 0.001$ and # $P < 0.05$ indicates a significant difference between groups. *** $P < 0.001$ and * $P < 0.05$ indicates a significant difference in the each group at Before, After, Post 30 and Post 120.
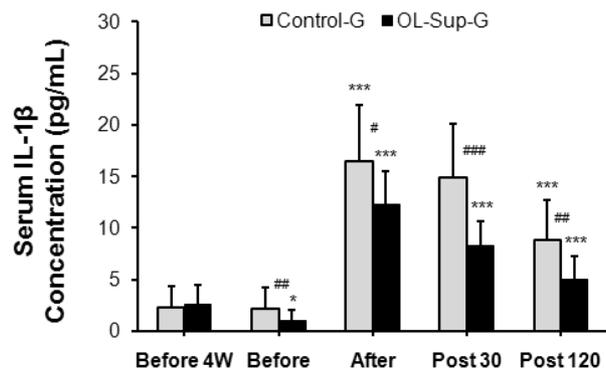
Fig. 2. Effect of Oligonol intake and physical loading on serum IL-1β protein level in the Control-G (placebo-supplemented subjects group) and OL-Sup-G (Oligonol-supplemented subjects group). Before 4W: before Oligonol intake; Before: resting condition, after 4weeks Oligonol intake; After: immediately after the physical loading VO$_{2max}$ 75% for 60 min; Post 30: recovery after of 30 min; Post 120: recovery after of 120 min. The values are mean ± SD. ### $P < 0.001$, ## $P < 0.01$ and # $P < 0.05$ indicates a significant difference between groups. *** $P < 0.001$ and * $P < 0.05$ indicates a significant difference in the each group at Before 4W, Before, After, Post 30 and Post 120.
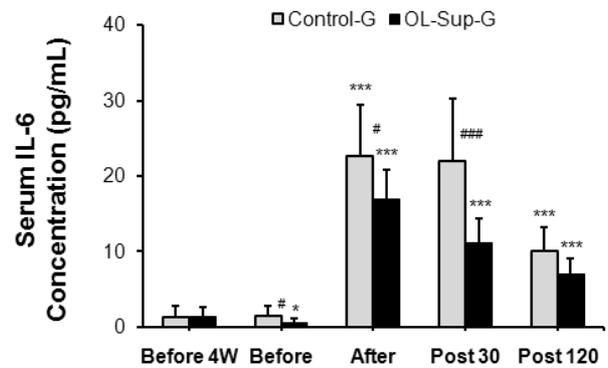
Fig. 3. Effect of Oligonol intake and physical loading on serum IL-6 protein level in the Control-G (placebo-supplemented subjects group) and OL-Sup-G (Oligonol-supplemented subjects group). Before 4W: before Oligonol intake; Before: resting condition, after 4weeks Oligonol intake; After: immediately after the physical loading VO$_{2max}$ 75% for 60 min; Post 30: recovery after of 30 min; Post 120: recovery after of 120 min. The values are mean ± SD. ### $P < 0.001$ and # $P < 0.05$ indicates a significant difference between groups. *** $P < 0.001$ and * $P < 0.05$ indicates a significant difference in the each group at Before, After, Post 30 and Post 120.

Cortisol, IL-1β and IL-6 concentration

The serum cortisol concentrations of the groups (all subjects) were significantly different in comparisons between the before (resting condition) and the after (during the physical loading for 60 min) values (*** $P < 0.001$, Fig. 1). After Oligonol intake, the serum cortisol concentration of the OL-Sup-G was significantly reduced compared to the before intake values (Fig. 1).

The IL-1β concentrations of the groups (all subjects) were significantly different between the before (resting condition) and the after (during the physical loading for 60 min) values (*** $P < 0.001$, Fig. 2). However, resting IL-1β level of only OL-Sup-G was significantly decreased compared to the before Oligonol intake ($P < 0.05$, Fig. 2).

The IL-6 concentrations of the groups (all subjects) were significantly different between the before (resting condition) and the after (during the physical loading for 60 min) values (*** $P < 0.001$, Fig. 3). However, resting IL-6 level of only OL-Sup-G was significantly decreased compared to the before Oligonol intake ($P < 0.05$, Fig. 3).

Discussion

Oligonol is a compound containing catechin-type monomers and oligomers derived from the lychee fruit. Several recent studies have demonstrated the antioxidative and anti-inflammatory potential of Oligonol [1,15-18]. In the present study, the anti-inflammatory effects of Oligonol intake were studied. The results of this study showed that the serum IL-1β, IL-6, and cortisol were significantly reduced as a result of four weeks of Oligonol oral supplementation. In addition, acute exercise caused a small perturbation of blood inflammatory cytokine levels compared with control group. This is the first study to investigate

and demonstrate the anti-inflammatory effects of Oligonol in men.

The circulating numbers and functional capacities of leukocytes may demonstrate the ability of immune response and be decreased by repeated bouts of intense, prolonged exercise by the increased levels of stress hormones during exercise. In our results, there was no alteration of WBC and differential counts at resting condition despite the decrease of cortisol. Further research is needed to identify the effect of Oligonol intake on immunity system.

An acute session of physical activity is accompanied by responses that are remarkably similar in many respects to those induced by infection, sepsis, or trauma [19]; there is a substantial increase in the number of circulating leukocytes and inflammatory cytokines that are known to influence leukocyte function. Hormonal changes also occur in response to exercise, including increases in the plasma concentration of several hormones [e.g., epinephrine (adrenaline), cortisol, growth hormone, and prolactin] that are known to have immunomodulatory effects [20]. Increase in the number of circulating leukocytes and inflammatory cytokines after exercise is associated with prolonged and high intensity endurance exercise. The results of this study showed a decrease in the levels of cortisol and cytokines, after Oigonol supplementation, which suggests an anti-inflammatory effect of Oligonol.

A number of cytokines and hormones change in response to exposure to inflammation and stress. It has been well documented that proinflammatory cytokines including IL-1β, IL-6 and IFN-ɣ are increased in response to stress [21-22]. Metz et al. [21] showed that IL-1β mRNA in the kidney and pituitary of the common carp was significantly increased after exposure to acute stress for 24-h. In addition, O'Conner et al. [22] reported that the IL-1β protein of the hypothalamus, pituitary and spleen of rats was significantly increased by an acute severe stress even though the IL-6 was unchanged.

Among the cytokines, IL-1β plays a crucial role in the cytokine network and T cell activation during the immune response [23]. IL-1β is produced by the activation of phagocytes and other antigen-presenting cells under conditions of immune stimulation by various microorganisms [24-25]. Therefore, IL-1β has been reported to mediate inflammation as well as function as a histamine releasing factor [26]; furthermore, it increases with exercise [27-28]. IL-6 is classified as an anti-inflammatory cytokine [29] and is known to inhibit TNF-α by the stimulation of endotoxin; IL-6 plays a crucial role in the host immune response, acute protein synthesis and maintenance of homeostasis.

Communication among the immune-derived signals with the hypothalamus-pituitary-adrenal (HPA) axis occurs via IL-1β and to a lesser extent with IL-6 and TNF-α. These cytokines have been shown to influence neuroendocrine activity in mammals, resulting in increased activity of the adrenocorticotropic hormone (ACTH) and cortisol during infection, inflammation and stress. Among these, IL-1β mediates its effects through the cell surface receptor type I (IL-1RI), which forms a receptor complex with the recruitment of the IL-1R accessory protein.

As mentioned above, the effects of Oligonol intake on inflammatory cytokine production is not well documented in humans. In the present study, the results showed that oral Oligonol intake for four weeks had a significant effect on inhibition of inflammatory markers and cortisol in healthy young men.

Acknowledgments

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