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Original article

Chlorella ingestion suppresses resistin gene expression in peripheral blood cells of borderline diabetics

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SUMMARY

Background & aims: Type 2 diabetes can lead to arteriosclerosis, renal damage, retinopathy, and several other serious complications. To prevent or delay the progression of this disease, considerable attention has been paid to improve exercise and dietary habits. Chlorella ingestion can reportedly reduce high blood glucose and cholesterol levels in mice and humans, although no studies have critically evaluated the effects of Chlorella on human borderline diabetics. Thus, we conducted a randomized, double-blind placebo-controlled test with volunteer borderline diabetics.

Methods: We recruited 57 subjects and randomly divided into a Chlorella ingestion group (n = 28) and a Placebo ingestion group (n = 29). Blood samples were collected every 4 weeks for laboratory tests. Gene expression analyses using peripheral blood cell RNA were performed before and 12 weeks after the trial.

Results: A total of 252 genes showed changed expression levels between these two groups. Six of these were type 2 diabetes-associated genes, including resistin, an insulin resistance inducer that exhibited markedly reduced expression with Chlorella ingestion (P = 0.01). Resistin mRNA expression significantly correlated with changes in HbA1c and TNF-α and IL-6 levels, all of which are strongly associated with glucose metabolism and/or inflammation.

Conclusions: Chlorella ingestion may be useful in preventing or ameliorating the course of type 2 diabetes development. In addition, gene expression analysis may be a means to investigate the effects of foods and supplements in humans.

1. Introduction

Type 2 diabetes incidence is increasing worldwide. This disease can lead to the development of various complications, such as arteriosclerosis, renal failure [1,2], and retinopathy, all of which affect the quality of life of patients [3]. Several borderline (pre-diabetic) status patients, who want to avoid these adverse complications [4], engage in exercise and change their dietary habits to reduce the risk of type 2 diabetes [5,6]. In addition, their needs for appropriate supplement intake are high. Chlorella ingestion can reportedly improve glucose intolerance and dyslipidemia [7–9].

Chlorella is a unicellular green alga that can be ingested as a powder or a hot water extract. This food contains high concentrations of natural-origin essential amino acids, vitamins, and minerals. The effects of ingesting Chlorella have been investigated in animal models, such as obese model mice (ob/ob mice) that showed significant reductions in serum total cholesterol levels and upregulated insulin concentrations with concomitant increases in adiponectin concentrations [7] or diabetic rat models that showed favorable effects on high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) cholesterol concentrations [9]. In humans with possible “lifestyle-related diseases,” Chlorella ingestion resulted in improved body fat percentages, serum total cholesterol concentrations, and fasting blood

Abbreviations: HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; FOSHU, food for specified health uses; GA, glycosylated albumin; 1,5-AG, 1,5-anhydroglucitol; Apo-B, cholesterol and apolipoprotein B; IL, Interleukin; TNF, -6,tumor necrosis factor; PAI-1, -αadiponectin and plasminogen activator inhibitor-1; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; QRT-PCR, quantitative reverse-transcription polymerase chain reaction; BMI, body mass index; ADIPOR1, adiponectin receptor 1; AMPK, activated protein kinase; LDLR, LDL receptor; PER1, period homolog 1; PPARD, peroxime proliferator-activated receptor delta; ALMS1, alstrom syndrome 1; AUTS2, autism susceptibility candidate 2.

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glucose concentrations [8]. Another report showed improvements in insulin resistance at the gene level for diabetic rats [9].

However, few studies have systematically investigated the effects of Chlorella ingestion in humans. In this study, we report on our results of using Chlorella ingestion based on a randomized, double-blind, parallel, placebo-controlled trial for its blood-borne effects. The subjects of our study were borderline diabetics. If Chlorella intake would be able to suppress progression of glucose intolerance, pre-diabetic persons might gain beneficial quality of life. Chlorella used in this study was pulverized *Chlorella pyrenoidosa* (Sun Chlorella strain). We performed comprehensive gene expression analyses using peripheral blood cells along with records of physiological changes. Peripheral blood RNA reflects the transcriptome activities of circulating immune cells and can be used as a barometer of health conditions, such as fatigue [10], obesity [11], and type 2 diabetes [12]. In addition, only a few studies have reported on these effects associated with foods [13,14]. We used blood cell RNA assays to determine any subtle effects that may have occurred during a long trial period.

Our results showed that Chlorella ingestion affected resistin gene expression, an indicator of ameliorating type 2 diabetes, and that this effect paralleled reductions in HbA1c, IL-6, and TNF-α levels. These results strongly suggested that inflammation was also affected for ameliorating this condition. Furthermore, resistin gene expression negatively correlated with the adiponectin receptor gene expression levels, which again reflected favorable effects of Chlorella ingestion on ameliorating type 2 diabetes development.

2. Methods

2.1. Subjects

The subjects were adult Japanese men aged 40–54 years and who had borderline diabetes as defined by their hemoglobin A1c (HbA1c). Their HbA1c (NGSP values) concentrations were between 6.0% and 6.5%. We excluded subjects who had any history of serious diseases of the metabolic and/or endocrine systems, Food for Specified Health Uses (FOSHU), and any allergy to foods and/or drugs. We also excluded those who had received any medications. Date of approval of the ethics committee is June 17, 2010. We carried out recruitment and the follow-up of the patient in a period from June 18, 2010 to December 18, 2010. Subjects were randomly assigned following simple randomization procedures to 1 of 2 treatment groups (30/group): a Chlorella group that ingested bulk Chlorella (8.0 g/day) and a Placebo group that ingested a lactose formulation (8.0 g/day). Subjects were asked to participate in this test for 12 weeks. The placebo was matched to the study food for taste, color, and size. The subjects were monitored for 4 weeks following these administrations. The Chlorella powder used was "Sun Chlorella A" tablets (Sun Chlorella, Corp., Kyoto, Japan). Overall subject compliance with food ingestion was estimated by food diaries.

Blood, including HbA1c levels, and urine analyses were performed at 0, 4, 8, and 12 weeks during the test period and at 4 weeks after the test period. We collected blood under non-fasting conditions. The subjects in these groups were not significantly different with regard to their demographic and clinical backgrounds.

This study conformed to the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Studies. Protection of human rights of the subjects was always considered, and our study protocol was approved by the Institutional Review Board of the Chiyoda Paramedical Care Clinic. Written informed consent was obtained from all participants. This study is registered in the UMIN Clinical Trials Registry with the identifier UMIN000009130.

2.2. Clinical laboratory tests

Serum was obtained by centrifuging coagulated blood samples. HbA1c concentrations were determined by a latex aggregation method. Serum interleukin (IL)-6, rumor necrosis factor (TNF)-α, and adiponectin levels were determined by enzyme-linked immunosorbent assay (ELISA). Total homocysteine levels were determined using high-performance liquid chromatography (HPLC).

2.3. Total RNA extraction from blood

A peripheral blood sample was collected in a PAXgene blood RNA tube (BD, Franklin Lakes, NJ, USA), left at room temperature for 2 h after sufficient mixing and subsequently stored at −20 °C. The stored tube was used for total RNA extraction using a PAXgene Blood miRNA Kit (QIAGEN, Valencia, CA, USA).

The amount of extracted total RNA was determined using a NanoDrop 1000 (Thermo Fisher Scientific Inc., Waltham, MA). RNA degradation was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

2.4. Gene expression microarray analysis

Microarray analyses were performed with SurePrint G3 human GE 8 × 60K microarrays (Design ID; 028004, Agilent Technologies). Cyanine 3-labeled cRNA was prepared from 100 ng of total RNA using a Low Input Quick Amp Labeling Kit (Agilent Technologies) according to the manufacturer's protocol. Microarrays were scanned using a G2505C DNA Microarray Scanner (Agilent Technologies). The images were converted to numerical values by Feature Extraction Software ver. 10.7.3.1 (Agilent Technologies).

Data analysis was performed using GeneSpring GX v11.5 (Agilent Technologies), with which inter-array normalization was performed using the 75th percentile, and inter-gene normalization was performed on the basis of the median value of all samples. There are total of 42,405 probes on Agilent Human GE 8 × 60K Microarray (Design ID: 028004) without control probes. Signals were categorized as either present, marginal, or absent based on the signal of each probe after conversion to a numerical value for

Table 1
Subjects' clinical characteristics.[a]

Variable	Chlorella group	Placebo group
N	28	29
Age (years)	46.4 ± 3.6	46.9 ± 3.9
BMI (kg/m2)	26.5 ± 0.8	25.6 ± 0.6
Abdominal girth (cm)	93.1 ± 2.03	89.2 ± 1.7
Triglyceride (mg/dL)	148.5 ± 19.8	179.1 ± 22.9
Blood pressure (max; mmHg)	124.0 ± 2.4	123.5 ± 2.3
Blood pressure (mini; mmHg)	82.0 ± 1.9	81.6 ± 2.1
Total cholesterol (mg/dL)	220.3 ± 6.9	221.0 ± 6.5
HDL cholesterol[b] (mg/dL)	56.7 ± 3.3	55.5 ± 2.5
LDL cholesterol[b] (mg/dL)	130.3 ± 7.1	128.5 ± 5.7
Apo-B[b] (mg/dL)	103.3 ± 3.2	106.2 ± 3.2
Blood glucose (mg/dL)	97.6 ± 1.9	95.2 ± 1.4
1.5-AG[b] (%)	18.9 ± 1.1	21.5 ± 1.4
HbA1c[b](%)	6.0 ± 0.0	6.1 ± 0.0
GA[b] (%)	14.2 ± 0.2	14.3 ± 0.2
IL-6[b] (pg/mL)	1.6 ± 0.1	1.5 ± 0.2
CRP[b] (μg/mL)	119.4 ± 24.8	117.3 ± 23.2
Adiponectin (μg/mL)	8.7 ± 0.7	8.4 ± 0.5
TNF-α[b] (pg/mL)	0.7 ± 0.1	0.6 ± 0.1

[a] Values are means ± S.E.M (Chlorella vs. Placebo) (*t*-test: #$P \leq 0.05$).
[b] Apo-B, Apolipoprotein B; 1.5-AG, 1.5-Anhydro-D-glucitol; HbA1c, Hemoglobin A1c (NGSP); GA, Glycoalbumin; HDL cholesterol, High density lipoprotein cholesterol; LDL cholesterol, Low density lipoprotein cholesterol; IL-6, Interleukin-6; CRP, C-reactive protein; TNF-α, Tumor necrosis factor-alpha.