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*Original article*

# Effects of ingesting food containing auraptene on lipid and glucose metabolism

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## Abstract

***Objectives*:** Hassaku (*Citrus hassaku*) contains higher levels of auraptene in its rind compared to other citrus fruits. Auraptene has been reported to potentially have various health benefits in both *in vitro* and *in vivo* studies. We conducted an exploratory study of the effects of auraptene on humans at risk of metabolic syndrome using fractions of oil squeezed from Hassaku fruits, which contain high concentrations of auraptene.

***Methods*:** The participants were 14 adult men with an abdominal circumference of 85 cm or more and low-density lipoprotein-cholesterol (LDL-C) of 140 mg/dL or more. The participants were randomly divided into 2 groups of 7 each. The test food was Hassaku oil extract in the form of soft capsules, which was administered as auraptene (10 or 20 mg/day, respectively) for 12 consecutive weeks. The participants underwent clinical examinations, physical examinations, blood and urine tests before ingestion, and at 6 weeks and 12 weeks after ingestion.

***Results*:** In the 10 mg/day group (n = 7), a significant decrease from pre-ingestion to 12 weeks after ingestion was observed as follows: Total cholesterol: 233.7 ± 34.7 mg/dL → 218.3 ± 28.4 mg/dL (p = 0.038, t-test), Insulin: 13.17 ± 4.41 → 9.76 ± 3.71 μU/mL (p = 0.022, t-test). The 20 mg/day group (n = 7) also showed the same tendency of variation, but it was not significant.

***Conclusions*:** In the 10mg/day group, the ingestion of high-auraptene Hassaku oil extract improved lipid and glucose metabolism, which suggested the contribution to the effectiveness in prophylaxis and prevention of progression of metabolic syndrome and arteriosclerosis, and suppression of glycative stress.

***KEY WORDS*:** auraptene, *Citrus hassaku*, lipid metabolism, total cholesterol, insulin resistance

## Introduction

Hassaku (*Citrus hassaku*), which has been widely consumed in Japan for a long time, contains higher levels of auraptene (a type of coumarin, ***Fig. 1***) in its rind compared to other citrus fruits [1)]. Auraptene has been reported to have various beneficial effects such as maintenance of cognitive function [2)], anticancer effects [3)], improvement of muscular endurance [4)], heat stroke prevention [5)], anti-Helicobacter pylori effects [6)], and improvement of non-alcoholic fatty liver disease (NAFLD) [7)]. In terms of lipid and glucose metabolism related to anti-metabolic syndrome, auraptene has been reported to activate the peroxisome proliferator-activated receptors (PPAR)-α and PPAR-γ. Auraptene increased adiponectin by acting on PPAR-γ in mouse precursor adipocyte 3T3-L1 and decreased monocyte chemoattractant protein (MCP)-1 [8)].

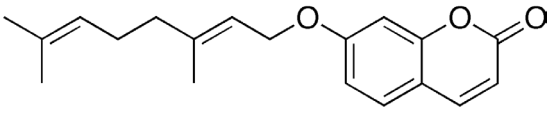

***Fig. 1.*** **Auraptene.**

In the hepatocyte cell line HepG2, auraptene increased the enzymes (acyl-CoA oxidase, carnitine palmitoyltransferase 1A, and acyl-CoA synthetase) of the target gene of PPAR-α, and accelerated the uptake of fatty acid to hepatocyte cells [9)]. Auraptene was administered to diabetic and obese model mice (KK-$A^y$) fed with a high-fat diet. As a result, a decrease in the levels of triglycerides and free fatty acids in the blood,

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inhibition of the accumulation of triglycerides in the liver and the skeletal muscles, an increase in mRNA expression levels of adiponectin in adipocytes, and improvement of hyperglycemia and abnormal glucose tolerance induced by the high-fat diet were observed [10]. Meanwhile, in the case of administration of auraptene to Otsuka Long-Evans Tokushima fatty (OLETF) rats, a type 2 diabetes model animal, the weight of visceral white adipose tissue and the levels of liver triglycerides dose-dependently decreased [11].

On the other hand, not only do the reactive dicarbonyls methylglyoxal, 3-deoxyglucosone, and glyoxal increase in the serum of diabetic patients along with blood glucose levels due to abnormalities in glucose metabolism, but also the peroxidation of fatty acids can lead to the production of numerous dicarbonyls including malondialdehyde and 4-hydroxynonenal, suggesting that abnormalities in lipid metabolism may also be involved in the enhancement of glycative stress [12, 13].

Based on these prior studies, we conducted a 2-dose clinical trial of auraptene as a pilot study.

## Methods

### Participants

Participants were paid volunteers selected from 39 participants (enrolled in e-Kencom corporation, Tokyo, Japan) who underwent screening prior to the start of test food ingestion. The exclusion criteria were set to exclude persons in the state such as taking medication for an illness and regularly consuming healthy foods containing auraptene. In accordance with the inclusion criteria, 14 men aged between 40 and 64 with an abdominal circumference of 85cm or more and low-density lipoprotein-cholesterol (LDL-C) of 140 mg/dL or more were selected. The allocation manager (TTC Co., Ltd., Tokyo), who was not directly involved in this study, randomly assigned 14 participants to a 10 mg/day group and a 20 mg/day group so that there would be no inter-group imbalances of LDL-C (calculated), body weight, and body mass index (BMI). The allocation table was sealed by the allocation manager and was kept sealed until it was opened. After analysis sets and data were fixed, a person in charge of allocation opened the allocation table and disclosed the information. Since this study was conducted as an exploratory dose-finding study, 6 participants per group needed to be ensured, so taking dropouts into account, the number of participants in this study was set to be 7 per group.

### Test Food

The auraptene was formulated using Hassaku oil extract (Product name: Rapten, Karada Lab, Inc., Kyoto, Japan). The concentration of auraptene in this product is specified to be at least 80%. The test food consisted of 2 doses of auraptene, 10 mg/day and 20 mg/day, in the form of soft capsules that are indistinguishable by taste, flavor, color, and other features. Each of the soft capsules were designed such that 3 capsules would provide daily intake per day. The nutritional constituents of the test food are shown in ***Table 1***.

***Table 1. Test food nutritional constituents (Administration amount per day: per 3 capsules).***

| | Auraptene 10 mg/day | Auraptene 20 mg/day |
|---|---|---|
| Energy | 8.9 kcal | 8.9 kcal |
| Protein | 0.3 g | 0.3 g |
| Lipid | 0.8 g | 0.8 g |
| Carbohydrate | 0.1 g | 0.1 g |
| Sodium | 0.002 g | 0.002 g |

### Trial Design

This study was conducted as a randomized, double-blind, parallel-group study of 2 dose levels with no placebo control. The participants were instructed to consume 3 capsules of the test food (10 mg/day or 20 mg/day) basically after meal once a day for 12 weeks, and underwent physical measurement and examination, blood and urine tests, medical interviews, and lifestyle surveys. All tests were conducted 3 times in total, prior to administration and at 6 and 12 weeks after administration. The participants were prohibited from drinking alcohol and had to finish meals by 10:00 p.m. on the day before their clinic visit, after which, they were instructed to fast, except for drinking water, until the completion of the testing.

The primary endpoints were high molecular weight adiponectin, total adiponectin, and high molecular weight/total adiponectin ratio. Secondary endpoints were LDL-C, high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), non-HDL cholesterol (non-HDL-C), LDL-C/HDL-C ratio (L/H ratio), arteriosclerosis index, triglycerides, free fatty acids, fasting blood glucose, HbA1c, insulin (immunoreactive insulin), and HOMA-IR (homeostatic model assessment for insulin resistance). LDL-C was calculated using the Friedewald equation as a calculation method, and in the case where triglycerides is 400 mg/dL or more, the LDL-C direct method was used. But all results were measured by the calculation method.

Additional safety endpoints included body weight, BMI, abdominal circumference, hip circumference, waist/hip ratio, systolic blood pressure, diastolic blood pressure, pulse rate, white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin consentration), platelet count, white blood cell differential, total bilirubin, alanine aminotransferase (ALT [glutamic pyruvate transaminase; GPT]), aspartate aminotransferase (AST [glutamic oxaloacetic transaminase; GOT]), LDH (lactate dehydrogenase), γ-GTP (glutamyl transpeptidase), ALP (alkaline phosphatase), CPK (creatine phosphokinase), total protein, albumin, A/G ratio, uric acid, urea nitrogen, creatinine, Na, K, Cl, Ca, Fe, C-reactive protein (CRP), high-sensitivity CRP, and urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone body, protein, glucose, occult blood, white blood cell, and nitrites). The medical interviews were conducted by a physician.

The participants recorded their ingestion status of the test food (time of ingestion), changes in their body conditions (adverse event survey), presence or absence of changes in

lifestyles, administration status of medication and supplements, a simplified dietary survey, and exercise status in each diary. Separately, the participants also recorded dietary contents and step counts (by pedometer) for 3 weekdays prior to each examination.

The duration of ingestion was from May 9 to August 6, 2011. Medical history interviews by the physician, body measurements, blood sampling, and urine sampling were conducted at Nihonbashi Cardiology Clinic (Tokyo).

### *Safety of auraptene*

Regarding the safety of auraptene, there are experiences with eating foods (marmalade, candied fruit, etc.) using the peel of Hassaku. Acute and subacute toxicity studies of oral administration of auraptene in rats were conducted. Acute administration of auraptene in doses of 125 ~ 2,000 mg/kg body weight had no mortality or clinical signs in a period of two days. To evaluate subacute toxicity, auraptene was administrated for 28 days by oral gavage in doses of 125 and 250 mg/kg, with no observed hematological, histopathological, or biochemical modifications[14]. In a clinical trial, no significant changes were observed in any of the blood biochemical parameters during the trial due to intake of the test juice containing 6.0 mg of auraptene per day, and no adverse effects that could be attributed to the test juice were observed[2].

### *Testing Method*

Physical measurements included height, weight, blood pressure, pulse rate, abdominal circumference, and hip circumference, as well as body fat percentage by bioelectrical impedance analysis. Blood and urine tests were performed at the Japan Institute for the Control of Aging, Nikken Seil Co., Ltd. (Shizuoka, Japan).

### *Statistical Analysis*

Measurements were presented as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft Corporation). The amount of variation from pre-ingestion to the 12th week of the ingestion was compared between groups using a 2-sample t-test. Also, measurement values of pre-ingestion and post-ingestion for each group were assessed using a 1-sample t-test with Bonferroni correction. All significance levels were set at a risk rate of less than 5% with a 2-tailed test.

### *Ethical Standards*

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and Ethical Guidelines for Epidemiological Research (Notification of Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare) as well as the study protocol. In order to protect the human rights and the safety of the participants as well as the reliability of the study data, the documents were submitted in advance to Ethical Committee of Nihonbashi Cardiology Clinic (Chairman of the Committee: Takayasu Omura, the Assistant Director of Nihonbashi Cardiology Clinic), and were reviewed and approved on April 7, 2011. Written informed consent was obtained from the participants prior to the screening tests, and the participants sufficiently understood the contents of the study plan and voluntarily expressed their willingness to participate in the study by submitting the consent form.

## *Results*

### *Analysis Set*

The tracking flow diagram for the participants is shown in ***Fig. 2***. Participant recruitment began on April 7, 2011. After undergoing screening tests, 14 participants were randomly assigned to either a 10 mg/day group or a 20 mg/day group (7 participants each) for participation in the main study. All participants completed the study, and none dropped out. All participants were included in the analysis, and none were ineligible. Baseline demographic and clinical characteristics for each group are shown in ***Table 2***.

### *Primary Endpoints*

Changes in the primary endpoints including high molecular weight adiponectin, total adiponectin, and high molecular weight/total adiponectin ratio are shown in ***Table 3***. There were no significant inter-group differences in the post-ingestion variations for these 3 parameters. No significant variation between pre-ingestion and post-ingestion was observed.

### *Secondary Endpoints*

In the secondary endpoints, significant variations or a trend between pre-ingestion and post-ingestion were observed in the following 4 parameters (***Table 3***).

1) Total cholesterol (TC)

TC in the 10 mg/day group showed a significant decrease as follows: before ingestion: 233.7 ± 34.7 mg/dL → 12 weeks after ingestion: 218.3 ± 28.4 mg/dL ($p = 0.038$, t-test). On the other hand, TC in the 20 mg/day group showed no significant variations before and after ingestion. There were no significant inter-group differences in the variations before and after ingestion. (***Fig. 3***)

2) Non-HDL cholesterol (non-HDL-C)

Non-HDL-C in the 10 mg/day group showed a decreasing trend as follows: before ingestion: 176.7 ± 29.0 mg/dL → 12 weeks after ingestion: 164.9 ± 24.0 mg/dL ($p = 0.067$, t-test). On the other hand, non-HDL-C in the 20 mg/day group showed no significant variations before and after ingestion. There were no significant inter-group differences in the variations before and after ingestion. (***Fig. 3***)

3) Insulin

Insulin in the 10 mg/day group showed a significant decrease as follows: before ingestion: 13.2 ± 4.4 μU/mL → 12 weeks after ingestion: 9.8 ± 3.7 μU/mL ($p = 0.022$, t-test). On the other hand, insulin in the 20 mg/day group showed no significant variations before and after ingestion. There were no significant inter-group differences in the variations before and after ingestion. (***Fig. 4***)

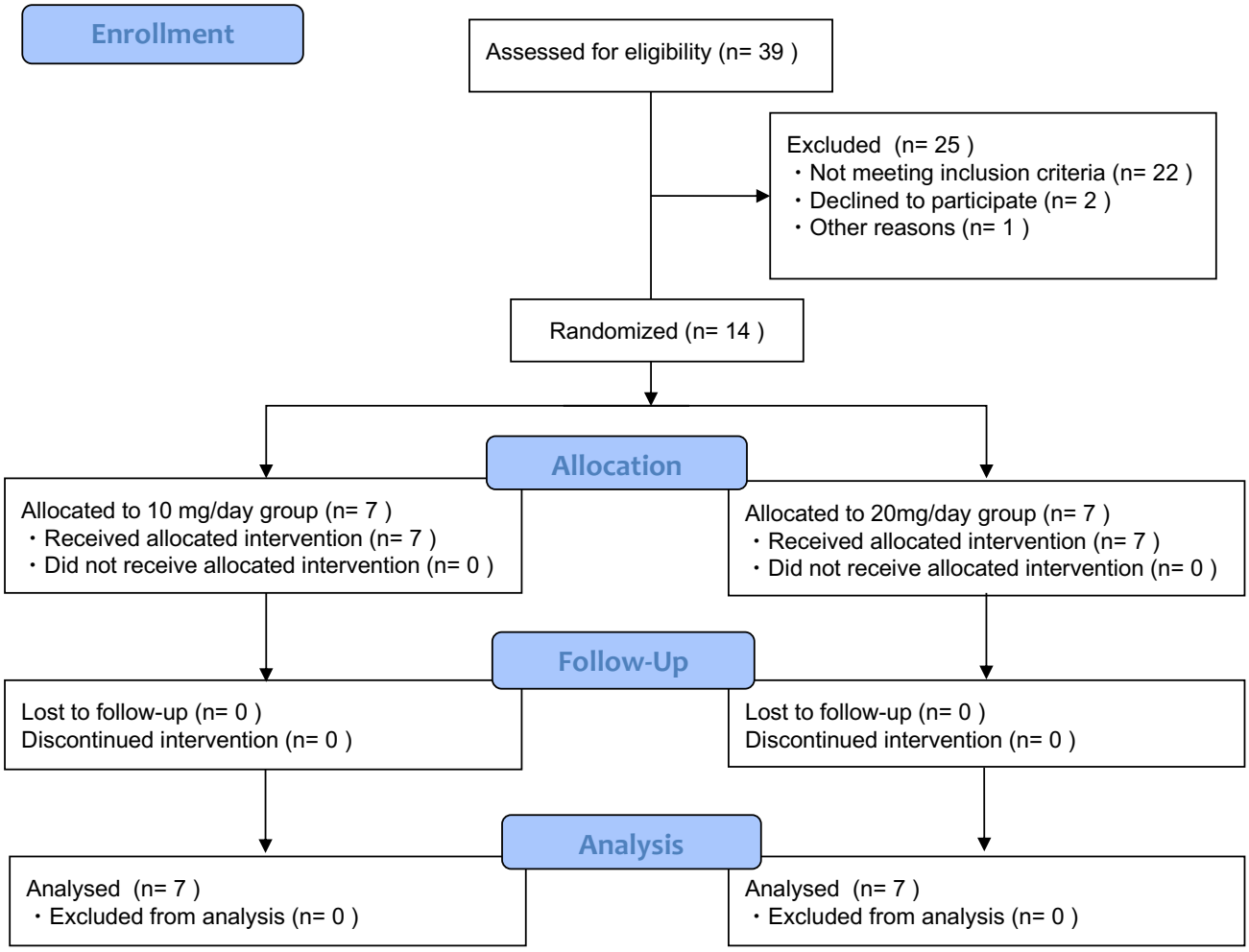


***Fig. 2.*** **Tracking flow diagram for trial participants.**

***Table 2. Baseline demographic and clinical characteristics.***

| | Unit | Auraptene 10 mg/day | Auraptene 20 mg/day | p-value (t-test) |
|---|---|---|---|---|
| No. of participants (male : female) | | 7 (7 : 0) | 7 (7 : 0) | — |
| Age | years of age | 53.4 ± 7.3 | 53.1 ± 8.1 | p = 0.94 |
| Height | cm | 169.3 ± 6.0 | 168.0 ± 6.3 | p = 0.70 |
| Weight | kg | 73.3 ± 7.0 | 72.6 ± 5.0 | p = 0.83 |
| BMI | $kg/m^2$ | 25.5 ± 1.6 | 25.7 ± 1.5 | p = 0.82 |
| LDL cholesterol | mg/dL | 147.3 ± 27.5 | 148.0 ± 27.5 | p = 0.97 |
| Total cholesterol | mg/dL | 233.7 ± 34.7 | 233.7 ± 38.9 | p = 0.83 |
| Triglycerides | mg/dL | 147.0 ± 20.7 | 143.7 ± 51.5 | p = 0.88 |
| Insulin | μU/mL | 13.2 ± 4.4 | 8.3 ± 2.7 | p = 0.027* |
| HOMA-IR | | 3.26 ± 1.09 | 2.01 ± 0.76 | p = 0.028* |

* There were coincidental inter-group differences. Measured value: mean ± standard deviation. BMI, body mass index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

***Table 3. Primary and secondary endpoints.***

| Parameter | | Unit | Reference range | Group (/day) | n | 0 W | 6 W | 12 W |
|---|---|---|---|---|---|---|---|---|
| Primary Endpoints | | | | | | | | |
| | Total adiponectin | μg/mL | – | 10 mg | 7 | 8.86 ± 3.55 | – | 8.53 ± 3.46 |
| | | | | 20 mg | 7 | 7.01 ± 2.61 | – | 6.99 ± 2.69 |
| | High molecular adiponectin | μg/mL | – | 10 mg | 7 | 2.76 ± 1.57 | – | 2.71 ± 1.57 |
| | | | | 20 mg | 7 | 1.99 ± 1.03 | – | 2.03 ± 1.31 |
| | High molecular weight / total adiponectin ratio | | – | 10 mg | 7 | 0.277 ± 0.102 | – | 0.289 ± 0.091 |
| | | | | 20 mg | 7 | 0.272 ± 0.051 | – | 0.272 ± 0.057 |
| Secondary endpoints | | | | | | | | |
| Lipid metabolism | LDL cholesterol | mg/dL | 70-139 | 10 mg | 7 | 147.3 ± 27.5 | 144.1 ± 27.6 | 135.1 ± 23.4 |
| | | | | 20 mg | 7 | 148.0 ± 27.5 | 149.2 ± 18.2 | 133.9 ± 26.1 |
| | HDL cholesterol | mg/dL | 40-77 | 10 mg | 7 | 57.0 ± 10.9 | 55.6 ± 11.3 | 53.4 ± 9.4 # |
| | | | | 20 mg | 7 | 57.0 ± 12.3 | 53.3 ± 8.9 | 54.4 ± 9.6 |
| | Total cholesterol | mg/dL | 130-219 | 10 mg | 7 | 233.7 ± 34.7 | 226.1 ± 36.0 | 218.3 ± 28.4 # |
| | | | | 20 mg | 7 | 233.7 ± 38.9 | 230.7 ± 25.4 | 221.1 ± 24.9 |
| | Non-HDL cholesterol | mg/dL | < 170 | 10 mg | 7 | 176.7 ± 29.0 | 170.6 ± 29.0 | 164.9 ± 24.0 † |
| | | | | 20 mg | 7 | 176.7 ± 29.4 | 177.4 ± 24.4 | 166.7 ± 21.8 |
| | L/H ratio | | < 2.5 | 10 mg | 7 | 2.63 ± 0.50 | 2.62 ± 0.38 | 2.57 ± 0.52 |
| | | | | 20 mg | 7 | 2.64 ± 0.45 | 2.86 ± 0.54 | 2.52 ± 0.59 |
| | Arteriosclerotic index | | < 2.5 | 10 mg | 7 | 3.16 ± 0.59 | 3.13 ± 0.58 | 3.14 ± 0.56 |
| | | | | 20 mg | 7 | 3.16 ± 0.52 | 3.42 ± 0.73 | 3.14 ± 0.62 |
| | Triglycerides | mg/dL | 40-149 | 10 mg | 7 | 147.0 ± 20.7 | 132.3 ± 63.1 | 148.7 ± 72.9 |
| | | | | 20 mg | 7 | 143.7 ± 51.5 | 141.3 ± 64.4 | 163.9 ± 84.6 |
| | Free fatty acids | mEq/L | 0.10-0.90 | 10 mg | 7 | 0.801 ± 0.222 | 0.803 ± 0.181 | 0.874 ± 0.299 |
| | | | | 20 mg | 7 | 1.050 ± 0.362 | 0.869 ± 0.265 | 1.003 ± 0.322 |
| Glucose metabolism | Fasting glucose | mg/dL | 70-109 | 10 mg | 7 | 100.9 ± 9.7 | 99.0 ± 10.9 | 100.4 ± 11.0 |
| | | | | 20 mg | 7 | 97.4 ± 7.3 | 99.1 ± 5.9 | 97.4 ± 8.5 |
| | Hemoglobin A1c (JDS) | % | 4.3-5.8 | 10 mg | 7 | 5.07 ± 0.20 | 5.13 ± 0.32 | 5.10 ± 0.31 |
| | | | | 20 mg | 7 | 4.86 ± 0.22 | 4.86 ± 0.31 | 4.83 ± 0.22 |
| | Insulin (IRI) | μU/mL | 1.7-11.8 | 10 mg | 7 | 13.2 ± 4.4 | 11.7 ± 3.3 | 9.8 ± 3.7 # |
| | | | | 20 mg | 7 | 8.3 ± 2.7 | 7.6 ± 1.8 | 6.9 ± 2.5 |
| | HOMA-IR | | < 2.5 | 10 mg | 7 | 3.26 ± 1.09 | 2.87 ± 0.96 | 2.44 ± 0.98 # |
| | | | | 20 mg | 7 | 2.01 ± 0.76 | 1.87 ± 0.47 | 1.66 ± 0.61 |

All male. Measured value: mean ± standard deviation. No significant differences were observed in inter-group comparison. # $p < 0.05$, † $p < 0.1$: Comparison with pre-ingestion (1-sample t-test with Bonferroni correction). L/H ratio, LDL cholesterol / HDL cholesterol ratio; JDS, The Japan Diabetes Society; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

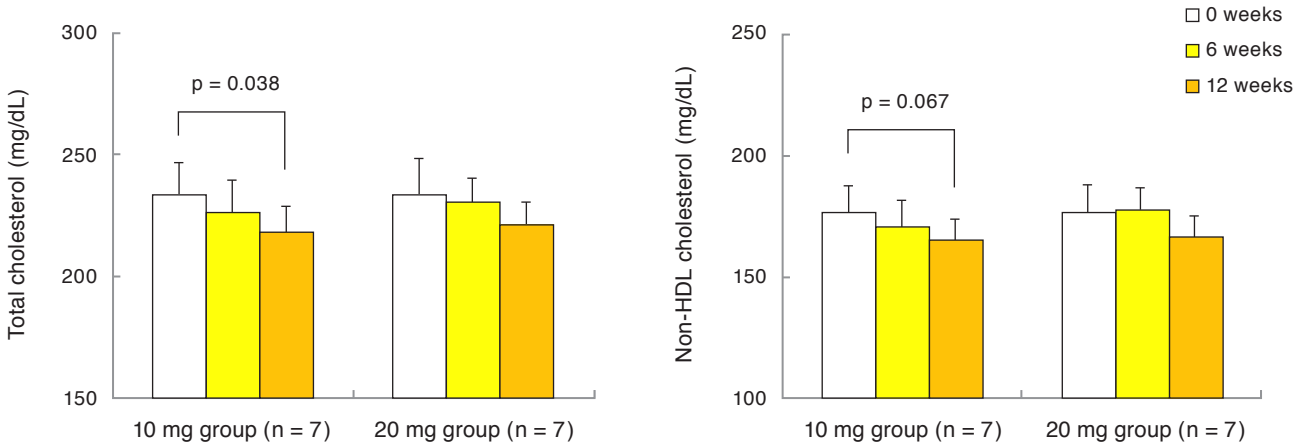


***Fig. 3.*** **Changes in total cholesterol and non-HDL cholesterol.**

Measured value: mean ± standard error of the mean. Analysis: 1-sample t-test with Bonferroni correction (intra-group) and 2-sample t-test (inter-group).

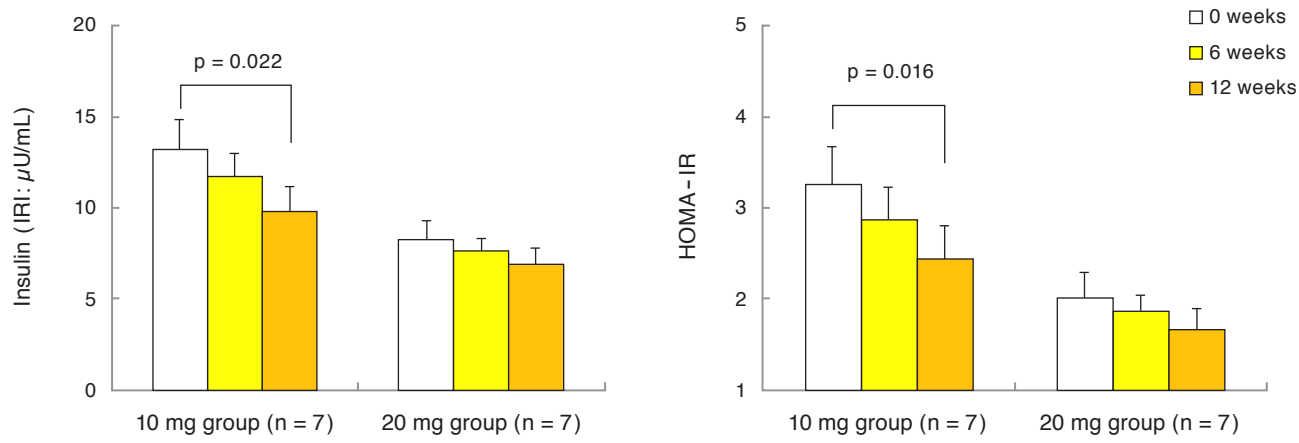


***Fig. 4.*** **Changes in insulin (IRI) and HOMA-IR.**

Measured value: mean ± standard error of the mean. Analysis: 1-sample t-test with Bonferroni correction (intra-group) and 2-sample t-test (inter-group). HOMA-IR, homeostatic model assessment for insulin resistance.

4) HOMA-IR

HOMA-IR in the 10 mg/day group showed a significant decrease as follows: before ingestion: 3.26 ± 1.09 → 12 weeks after ingestion: 2.44 ± 0.98 ($p = 0.016$, t-test). On the other hand, HOMA-IR in the 20 mg/day group showed no significant variations before and after ingestion. There were no significant inter-group differences in the variations before and after ingestion. (***Fig. 4***)

### *Safety Endpoints*

Adverse event occurrence based on participants' diaries and variations in clinical laboratory values were investigated. The adverse events of "lumbosacral strain", "common cold symptom", "abdominal pain" and "periodontal disease" occurred in 1 participant each, but these events disappeared shortly or were temporary changes. In blood biochemistry, hematology, urinalysis, physical examination, or medical interview, there were no abnormalities or abnormal variations that might be caused by the ingestion of the test food. The physician determined that there were no safety issues.

## *Discussion*

### *Primary endpoints*

In this study, no significant changes in adiponectin-related indicators, the primary endpoints were observed. However, in diabetic obese KK-$A^y$ mice, significant increases in mRNA expression levels of adiponectin in adipose tissues and adiponectin protein levels in plasma were observed[10]. The possible causes of these results were that the doses per body weight in the mice were approximately 500 to 1000-

fold greater that used in this study method and there was a species difference.

### *Secondary endpoints*

In the secondary endpoints, TC, insulin, and HOMA-IR significantly improved in the 10 mg/day group, and there was a trend towards improvement of non-HDL-C. On the other hand, in the 20 mg/day group, there were no significant or trending variations. Thus, dose dependency was not observed. As the reason for this, it was considered that there were incidental inter-group differences for insulin and HOMA-IR (***Table 2***). In accordance with the protocol, group matching was performed on LDL-C, body weight, and BMI, but insulin was not taken into account. More participants with higher insulin resistance were assigned to the 10 mg/day group, which led to greater improvement of insulin resistance due to the effects of auraptene (***Fig. 4***). As a result, it was thought that further improvement in cholesterol metabolism had been observed. The reason was that in diabetic and obese model mice (KK-$A^y$) fed with high-fat food, auraptene contributed to the increase in the expression of PPAR-α target genes that are related to fatty acid oxidation in the liver and the skeletal muscle[10], activation of PPAR-α improved insulin resistance[15], and the improvement of insulin resistance led to cholesterol improvement[16]. These were considered to be one of the mechanisms. In addition, in the experiments on obese adipose tissue models using the co-culture system of 3T3-L1 adipocyte and RAW264 macrophage, auraptene suppressed the production of inflammatory factors[17], which suggested a contribution to the alleviation of chronic inflammation and improvement of insulin resistance. Furthermore, it was inferred that glycative stress is suppressed through this insulin resistance improvement.

### *HDL cholesterol*

In the 10 mg/day group, HDL-C significantly decreased (57.0 → 53.4 mg/dL, p = 0.023). However, there were no participants whose HDL-C decreased below the lower limit of the reference range, but the variation was relatively small within the range. LDL transports cholesterol produced in the liver throughout the body, and HDL has the role of collecting unnecessary cholesterol from tissues and transporting it back to the liver. When LDL-C increases, HDL-C may increase in compensatory fashion to maintain homeostasis. Since non-HDL-C, which was high before the test food intake, tended to decrease by the intervention, it is believed that the compensatory increase in HDL-C was reduced. This is supported by the fact that there was no change in the L/H ratio. Therefore, it was thought that there were no clinical abnormalities in the balance of lipid metabolism.

### *Combining the both groups*

In this study, since there were only 7 participants per group, the 2 groups were combined and tested as a trial calculation. As a result, a significant decrease was observed in LDL-C, TC, insulin, and HOMA-IR, with a decreasing trend in non-HDL-C (data not shown). From this, it was thought that the 20 mg/day group also has the potential for improvement in the aforementioned parameters, and it may indicate that intake of auraptene 10 to 20 mg/day improves lipid and glucose metabolism.

### *Alleviation of glycative stress by test foods*

It has been suggested that not only carbohydrate-derived aldehydes generated following hyperglycemia[18] but also fatty acid-derived aldehydes[12, 13] play pathogenic roles in the effects of glycative stress on the body. These aldehydes modify proteins in blood vessels and cells in unphysiological manners. In pancreatic beta cells, insulin biosynthesis is affected by aldehyde modification, inhibiting the conversion of proinsulin to insulin. As a result, the proportion of inactive proinsulin increases and the proportion of active insulin decreases in immune-reactive insulin (IRI), which may increase insulin resistance[19]. It can be assumed that by lowering LDL-C, this test food reduced the production of fatty acid-derived aldehydes, reduced the effect of aldehydes on insulin biosynthesis in pancreatic beta cells, and increased the proportion of active insulin in the IRI. As a result, it is presumed that insulin activity was maintained even though the IRI value decreased, and the HOMA-Index (insulin resistance) improved. We plan to verify in the future whether this test food reduces fatty acid-derived aldehydes.

## *Conclusions*

A study was conducted on males with an abdominal circumference of 85 cm or more and LDL-C of 140 mg/dL or more who took 10 or 20 mg/day of auraptene for 12 weeks. The findings suggested that food containing Hassaku oil extract improves lipid and glucose metabolism via the effects of auraptene in the 10 mg/day group. These were consistent with the reported *in vitro* and *in vivo* studies, indicating that they may be effective in humans through a similar mechanism. From this fact, auraptene is expected to be effective in the prophylaxis and prevention of metabolic syndrome and arteriosclerosis, and suppression of glycative stress. Additionally, high safety was confirmed even for cases of continuous ingestion.

## *Conflict of Interest Declaration*

This study was fully funded by Arkray, Inc. and outsourced and conducted by TTC Co., Ltd. (currently, EP Mediate Co., Ltd.), a third-party organization. Hiroshige Kawai, Kenta Fujimoto, Eiji Yuasa, and Junichi Takimura are employees of Karada Lab, Inc., a subsidiary of Arkray Corporation. Yoshitaka Iwama is the principal investigator of this study at Nihonbashi Cardiology Clinic. There are no other conflicts of interest to disclose relevant to this article.

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