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Randomized, double-blind, crossover, placebo-controlled clinical trial to evaluate the effects of chicken hot water extract on insulin secretion

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Abstract. – OBJECTIVE: Essence of chicken (EOC), a hot water extract of chicken, is widely consumed in Southeast Asia as a beverage. EOC has an inhibitory effect on the elevation of blood glucose levels and a secretagogue effect on insulin. However, the mechanism by which EOC promotes insulin secretion is unknown. We aimed to verify the postprandial hyperglycemic inhibitory effect and the insulin secretory effect of EOC in healthy adults under appropriate placebo settings. In addition, we aimed to understand the mechanism underlying the insulin secretory effect of EOC.

PATIENTS AND METHODS: Thirty-four healthy Japanese adults were fed 68 mL of EOC or control food, followed by 200 g of cooked rice. Blood glucose and plasma insulin levels were measured at 30, 45, 60, 90, and 120 min after the participants ate cooked rice. The trial had a randomized, double-blind, crossover, placebo-controlled design.

RESULTS: The ingestion of EOC induced an increase in the maximum blood concentration (Cmax) of insulin and shortened the time required to reach the maximum blood concentration following rice consumption. Ingestion of the test beverage resulted in a significantly higher insulinogenic index than that obtained after ingestion of the control beverage. No side effects were observed in this study. Mechanistic experiments revealed that EOC stimulated significant ($p < 0.05$) secretion of GLP-1 from NCI-H716 human intestinal L cells at 0.1, 1, and 10 mg/mL.

CONCLUSIONS: Consuming EOC when eating rice supports pancreatic function. Daily consumption of EOC could elevate the early-phase insulin response; therefore, it could prevent diabetes in Asians with low insulin secretion.

Key Words:
Essence of chicken, Insulin, Placebo-controlled clinical trial.

Introduction

Over the last few decades, the prevalence of diabetes has considerably increased globally; currently, there are approximately 415 million patients with diabetes worldwide[1]. Type 2 diabetes is a complex disease. Chronic hyperglycemia is accompanied by endocrine and metabolic disorders such as retinopathy, nephropathy, and neuropathy[2]. Complications due to diabetes significantly reduce the quality of life. In addition, medical costs associated with diabetes care are a large burden on the patients. Therefore, efforts to prevent diabetes are important.

The risk factors for diabetes include advanced age, obesity, and decreased physical activity. Healthy eating habits and regular exercise are important to prevent or delay the onset of diabetes[3]. In particular, Asians, including Japanese, have a lower early-phase insulin response than that of Caucasians[4]. The early-phase insulin response is the secretion of insulin immediately after a meal. Insulin mediates glucose transport

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into the skeletal muscles, liver, and fat cells. The postprandial blood glucose level is higher in individuals with low early-phase insulin response compared to that in individuals with the normal response, even with the ingestion of the same amount of sugar[5]. Consequently, in these individuals, the pancreatic β cells do not function at the optimum level, resulting in diabetes[6]. According to the Diabetes Atlas and National Diabetes Statistics of the International Diabetes Federation, in 2021, 206 million patients from the Asia-Pacific region were diagnosed with diabetes. Therefore, to help prevent diabetes in Asians, it is essential to identify food ingredients that can be included in the daily diet to improve the early-phase insulin response.

Incretins play a pivotal role in the early-phase insulin response *in vivo*. Incretins are hormones secreted from the digestive tract in response to food intake. They promote insulin secretion in a blood glucose-dependent manner. Glucose-dependent insulinotropic polypeptide and glucagon-like polypeptide-1 (GLP-1) are known incretins. Incretins can strongly induce insulin secretion; however, they do so only at elevated levels of blood glucose[7]. Therefore, the risk of hypoglycemia is minimal. In clinical practice, GLP-1 analogs are used as hypoglycemic agents to promote blood glucose-dependent insulin secretion. These agents are successful in reducing the incidence of diabetes in prediabetic individuals (with impaired glucose tolerance (IGT) and/or impaired fasting blood glucose) by 84-96 % and achieving normal glucose tolerance (NGT)[8]. Therefore, identifying a daily use food ingredient that promotes GLP-1 secretion and enhances the early-phase insulin response will contribute considerably to the prevention of diabetes in Asians, including the Japanese population.

Essence of chicken (EOC), a hot water chicken extract, is a popular beverage in Southeast Asia. It is traditionally consumed to relieve anxiety, as well as to improve milk production in lactating mothers. EOC is prepared by processing chicken at a high temperature and pressure.

EOC inhibits the increase in blood glucose level[9,10] and exerts a secretagogue effect on insulin[10]. However, previous studies[11] had limitations, such as sex bias, small sample size, and the lack of control foods. EOC aids insulin function in KK-Ay mouse (an obesity model) and Goto-Kakizaki (GK) rat (an insulin secretory deficiency model); however, the mechanism that promotes insulin secretion is unknown. Therefore, the aim of the present study was to conduct a placebo-controlled trial to examine the acute antihyperglycemic effect of EOC and to ascertain whether it promotes GLP-1 secretion *in vitro*.

Patients and Methods

Study Participants

This study included healthy Japanese male and female volunteers aged 20-64 years. Candidate participants were screened based on specific eligibility criteria. Participants with a high blood glucose level, satisfying at least one of the following inclusion criteria were selected: in the rice load test, (i) the maximum blood glucose concentration C_{max} was equal to or higher than the average value of all the candidate participants, and (ii) the blood glucose level Δ120 min was equal to or higher than the average value of those who do not correspond to (i). The exclusion criteria were as follows: (i) pregnant (including possibly pregnant) or lactating women; (ii) participation in other clinical trials or within four weeks of completing other clinical trials; (iii) patients with diseases of the heart, liver, or kidneys; (iv) patients with a history of cardiovascular disease; (v) patients with diabetes; (vi) participants who regularly used medicines and quasi-drugs for the treatment of diseases, alleviation of symptoms, or maintenance of health; (vii) those who frequently consume alcohol or smoke; (viii) those with extremely irregular eating habits; (ix) those with a history of feeling sick during blood sampling, experiencing symptoms such as nausea and dizziness; (x) those allergic to the food ingredients used in this study; (xi) those with a fasting blood glucose level of 140 mg/dL or higher; and (xii) those judged by the investigator to be inappropriate for this study.

Study Design

This study was conducted in a double-blind, randomized, placebo-controlled crossover design. Participants selected based on the screening tests were randomly and evenly assigned to two groups, using sex, age, and blood glucose C_{max} in the rice load test as stratification factors. The test food allocation table was prepared by a person who was not involved in the study, and it was kept confidential. Blinding was maintained until the results were disclosed.

The participants were instructed to maintain usual eating and lifestyle habits during the study period and to refrain from consuming functional foods, which could affect blood glucose levels. In addition, unless unavoidable, the use of medicines for treating illnesses, alleviating symptoms, and maintaining health was prohibited. Simultaneously, guidelines for the prevention of coronavirus disease (COVID-19) infection were formulated. The participants were instructed to follow the guidelines to prevent infection and record their daily body temperature and physical condition. In case of a possible infection, such as an abnormal physical condition, visiting the examination center for the purpose of this study was prohibited.

This study was designed and funded by Suntory Global Innovation Center Ltd. The collection of data and management of the test site were conducted by Clinical Support Corporation Ltd., a contract research organization. The study period was from October to December 2020. The test site was Fukuoka Kinen PET Medical Examination Center. At the test site, COVID-19 infection control measures were followed based on endogenously created guidelines.

Study Food

The EOC used in this study was obtained from Suntory Beverage & Food Asia Pte. Ltd. The control beverage was prepared by flavoring a saline solution with amino acid-free chicken flavor and caramel coloring. The control beverage could not be distinguished from the test beverage by smell or appearance, thereby aiding the double-blind nature of the study. Table I shows the nutritional composition of the control beverage, test beverage, and the loaded food.

Study Outcomes

The primary endpoints were blood glucose C_{max} and blood glucose area under the curve $(AUC)_{0\text{-}120\ min}$. The secondary evaluation parameters were as follows: (i) plasma insulin level C_{max}; (ii) blood glucose level and plasma insulin level ΔC_{max}; (iii) blood glucose and plasma insulin level T_{max}; (iv) blood glucose and plasma insulin levels before and after the consumption of loaded foods at 30, 45, 60, 90, and 120 min; (v) changes in blood glucose and plasma insulin levels at 30, 45, 60, 90, and 120 min after the consumption of loaded foods compared to that before the consumption of loaded foods; (vi) plasma insulin level $AUC_{0-120\ min}$; and (vii) blood glucose and plasma insulin levels $\Delta AUC_{0\text{-}120\ min}$.

Table I. Nutrient content of essence of chicken (EOC), placebo, and test meals.

	Placebo	EOC	Test meal
Energy (kcal)	0	25	309
Protein (g)	0	6	4.62
Fat (g)	0	0	1.2
Carbohydrate (g)	0	< 1	68.3
Sodium (mg)	40	55	0.71

Nutritional data were obtained from the manufacturers. EOC, essence of chicken.

Data Collection

During the screening test and study duration, fasting blood glucose levels were measured prior to the testing, and those with a blood glucose level of 140 mg/dL or higher were excluded. Glutest Neo Alpha and Glutest Neo sensors (Sanwa Kagaku Kenkyusho, Aichi, Japan) were used for the measurements. Next, the participants were divided into groups A and B, and they underwent observation periods I and II (Figure 1). In the observation period I, rice load tests with EOC were conducted on participants in group A, and rice load tests with the control beverage were conducted on participants in group B. After a resting period of at least four days, the observation period II was initiated by interchanging EOC and the control beverage between the two groups. In each case, after consumption of the prescribed diet, the participant fasted overnight (minimum 12 h), and the blood sample was collected for biochemical examination and fasting glucose level measurement. The participants were then fed EOC or control foods and immediately fed 200 g of white rice as a loaded food. Blood was collected 30, 45, 60, 90, and 120 min after feeding the loaded food. Blood samples were collected in vacuum blood collection tubes NP-FN0205 (for blood glucose) and NP-SP1029 (for plasma insulin; both manufactured by Nipro Corporation, Osaka, Japan) and centrifuged at 2,300 × *g* for 5 min to obtain plasma. Blood glucose levels were measured using a JCA-BM9130 BioMajesty™ automatic analyzer (JEOL Ltd., Tokyo, Japan) and an L-type Wako Glu2 kit (FUJIFILM Wako Pure Chemical Corporation, Fukuoka, Japan). Plasma insulin levels were measured using an Architect analyzer i2000 SR (Abbott Japan Co., Ltd., Tokyo, Japan) and an Architect Insulin Kit (Abbott Japan Co., Ltd.).

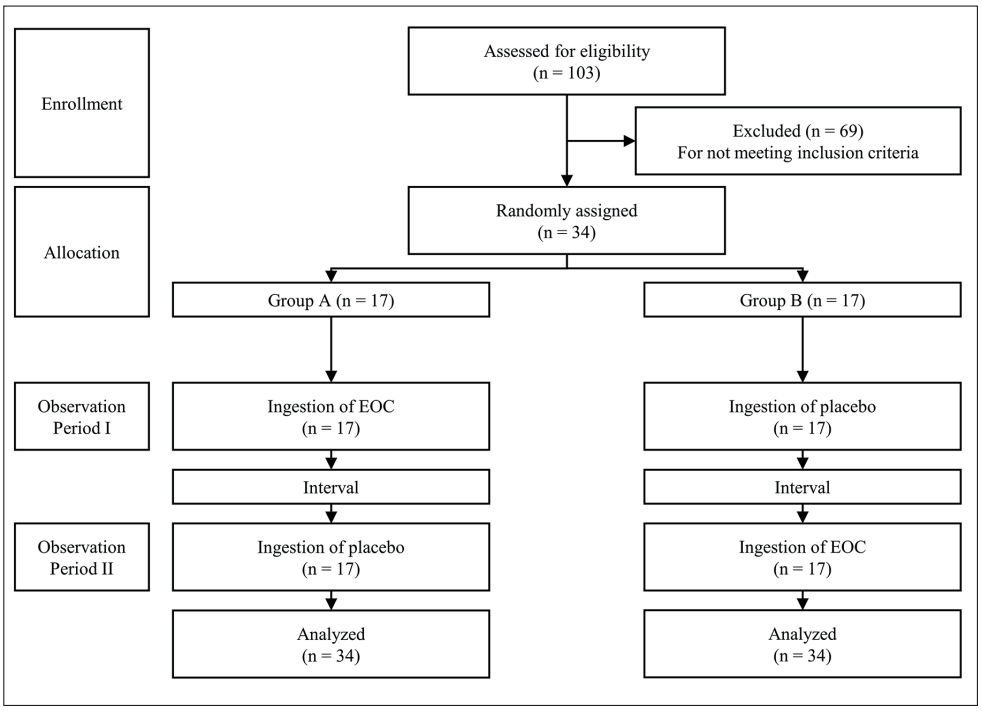

Figure 1. Schematic diagram of the study design.

Ethics

This study was conducted in compliance with the Declaration of Helsinki and the Ethical Guidelines for Medical Research for Humans. The study design was approved by the Institutional Review Board of Hokubukai Utsukushigaoka Hospital and registered in UMIN Clinical Trial Registration System (UMIN ID: UMIN000042100). Prior to the start of the study, written informed consent was obtained from all the participants.

Statistical Analysis

For the participant background, the average value, standard deviation, and number of participants were calculated as basic statistics. The AUC was calculated using the trapezoidal rule. When calculating ΔAUC, the area of the negative part was considered as 0. The crossover method was validated using the primary endpoint blood glucose level, $AUC_{0\text{-}120\ min}$ and logarithmically converted blood glucose level, $AUC_{0\text{-}120\ min}$. For normalization, the data for the observation periods I and II were summarized for each test food, and the blood glucose level $AUC_{0\text{-}120\ min}$ was examined using the Shapiro-Wilk test. If the normality of the data was not rejected, the paired *t*-test was used, otherwise, the Wilcoxon signed rank sum test was used. Each evaluation parameter was then compared. Microsoft Office Excel 2016 (Microsoft, Redmond, WA, USA) was used for aggregation and calculation of basic statistics, and SPSS Statistics 24 (IBM, Armonk, NY, USA) was used for other analyses. The statistical significance level was set at $p < 0.05$.

The insulinogenic index (IGI), which is an index of pancreatic β-cell function, was calculated using the following formula[12]:

$$IGI = \Delta IRI/\Delta BS$$

ΔIRI: Increased blood insulin levels before and 30 min after consumption of loaded food
ΔBS: Increased blood glucose levels before and 30 min after consumption of loaded food.

Cell Culture and GLP-1 Secretion Assay

NCI-H716 human intestinal L cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in a 75 cm^2 flask (AGC Techno Glass, Shizuoka, Japan) at 37°C in an atmosphere containing 5% CO_2 (1.0×10^5 cells/20 mL) using Roswell Park Memorial Institute (RPMI-16409) medium supplemented with L-glutamine (FUJIFILM Wako Pure Chemical Corporation, Fukuoka, Japan), 10% fetal bovine serum (FBS; Sigma, Kanagawa, Japan), and 1% penicillin-streptomycin mixed solution (10,000 unit/mL penicillin, 10 mg/mL streptomycin). Three days before the GLP-1 secretion assay, 5.0×10^5 cells were seeded in 96-well

culture plates, containing high glucose Dulbecco's Modified Eagle Medium (DMEM; supplemented with 4,500 mg/L glucose, L-glutamine, sodium pyruvate, sodium bicarbonate), 10% FBS, and 1% penicillin-streptomycin mixture. On the day of the assay, the cells were washed with phosphate-buffered saline (without Ca and Mg) and incubated at 37°C for 1 h with FBS-free DMEM. Simultaneously, EOC was freeze-dried and resuspended in FBS-free DMEM (0.01-10 mg/mL) and used for treating cells. Ionomycin was used as a positive control[13]. Active GLP-1 levels in the assay buffer were measured using a GLP-1 active (high sensitivity) assay kit (Immuno-Biological Laboratories, Shizuoka, Japan).

Results

Baseline Characteristics of the Study Participants

A screening test was conducted on 103 participants, who willingly provided written informed consent for the study. Among them, 34 participants were included in the study, based on the inclusion and exclusion criteria. All participants completed observation periods I and II. The investigators examined all the 34 participants before blind disclosure, to evaluate their suitability for the study (Figure 1). However, the 60-min insulin value of one participant at the time of ingestion of the control food was abnormal due to hemolysis; therefore, this particular value was excluded from the statistical analysis. Table II summarizes the baseline characteristics of the study population.

Primary Outcome

The results for the blood glucose level C_{max} are shown in Figure 2A. No significant differences in the blood glucose level C_{max} were observed between the groups that ingested the test beverage and the control beverage. In addition, no significant difference in the blood glucose level $AUC_{0\text{-}120\,min}$ was observed between the two groups (Figure 2B).

Secondary Outcome

The changes in the blood glucose levels at each time point after the ingestion of the loaded food is shown in Figure 2C. No significant difference was observed at any time point in the group that ingested the test beverage compared to that in the group that ingested the control beverage. The changes in the insulin levels at each time point after the ingestion of the loaded foods is shown in Figure 3A. Insulin levels at 30, 45, and 60 min after ingestion of the loaded food were significantly higher in the group that ingested the test beverage than that in the group that ingested the control beverage ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). In addition, the insulin level C_{max} (Figure 3B, $p < 0.01$) and IGI were significantly higher (Figure 3D, $p < 0.01$), and the insulin level T_{max} was significantly lower (Figure 3C, $p < 0.01$) in the test beverage group than that in the control beverage group.

Safety of the Study Participants

No side effects were observed in this study. The following ten adverse events occurred in ten participants during the study period. From the time of allocation to the intake of the test beverage, two adverse events (one each of myalgia

Table II. Baseline measurements of participants.

Parameters	Total	Group A	Group B
Study participants (Women/Men)	34 (14/20)	17 (7/10)	17 (7/10)
Age (years)	42.03 ± 12.90	42.82 ± 13.70	41.24 ± 12.42
Height (cm)	162.98 ± 9.47	163.63 ± 8.91	162.32 ± 10.22
Weight (kg)	58.53 ± 11.68	62.50 ± 12.59	54.55 ± 9.47
BMI (kg/m^2)	21.86 ± 2.66	23.14 ± 2.87	20.57 ± 1.69
Abdominal circumference (cm)	80.21 ± 8.21	84.57 ± 7.64	75.85 ± 6.34
Systolic blood pressure (mmHg)	118.85 ± 13.92	118.18 ± 13.31	119.53 ± 14.88
Diastolic blood pressure (mmHg)	75.50 ± 11.76	75.06 ± 11.97	75.94 ± 11.89
Pulse rate (bpm)	76.26 ± 7.47	73.76 ± 7.26	78.76 ± 7.00
Body temperature (°C)	36.30 ± 0.36	36.34 ± 0.34	36.26 ± 0.39
Fasting blood glucose (mg/dL)	87.12 ± 7.46	88.71 ± 9.10	85.53 ± 5.16

Values are presented as the mean ±standard error of mean (SEM); BMI indicates body mass index, calculated as the ratio between weight in kilograms and square of the height in meters.

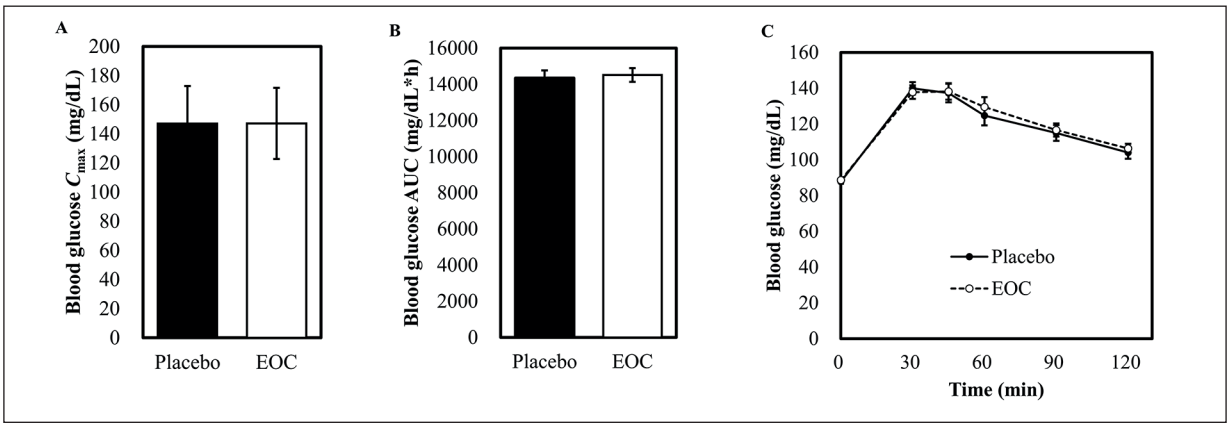

Figure 2. Mean changes in blood glucose levels after the consumption of the test meals. (**A**) Maximum blood concentration (C_{max}) of glucose, (**B**) area under the curve (AUC) of blood glucose, and (**C**) temporal curves of the average blood glucose response of 34 participants (14 women and 10 men) who consumed the same quantity of cooked rice. EOC, essence of chicken.

and migraine) occurred in two participants. After ingestion of the test beverage, four adverse events (one each of mood swings, myalgia, abdominal pain, and migraine) occurred in four participants. After ingestion of the control beverage, four adverse events (one each of fatigue, headache, runny nose, and myalgia) occurred in four participants. The incidence of adverse events was 11.8% (4/34) each, in both the test and control beverage ingestion groups, indicating no difference.

Mechanism Underlying EOC Effect

To verify whether EOC exerted a GLP-1 secretagogue effect, NCI-H716 human intestinal L cells (large granule cells) were treated with EOC, and the amount of secreted GLP-1 was measured. Initially, the amount of GLP-1 secreted from the untreated NCI-H716 cells was 19.5 ± 0.7 pM. Ionomycin stimulated the GLP-1 secretion to 142 ± 0.2 % that of the control ($p < 0.01$). EOC (0.1, 1, and 10 mg/mL) stimulated GLP-1 secretion to 114 ± 3.9 %, 130 ± 2.5 %, and 135 ± 0.9 % that of the control, respectively ($p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively, Figure 4). This suggests that EOC acts on L cells, which are enteroendocrine cells in the lower small intestine, and increases GLP-1 secretion.

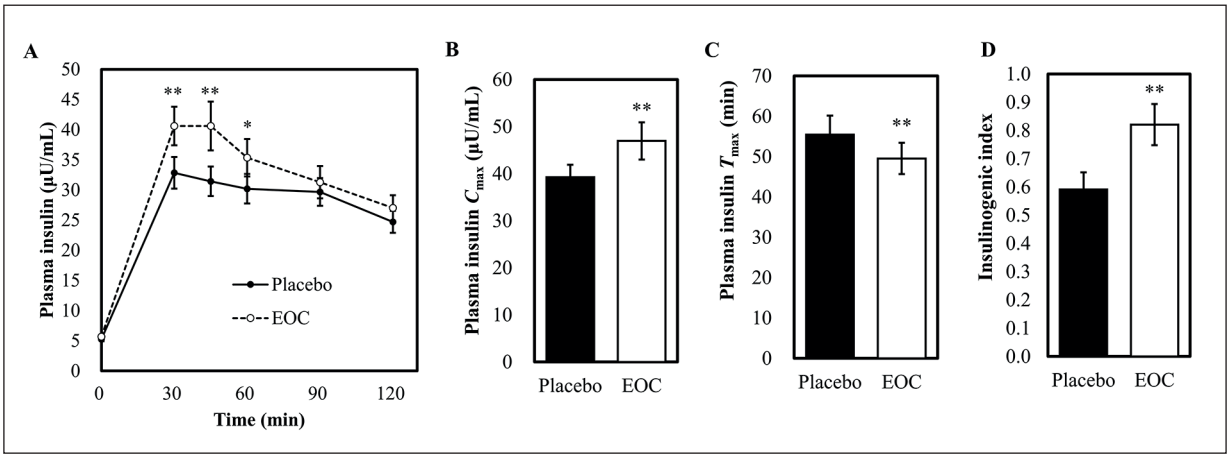

Figure 3. Mean changes in plasma insulin levels after the consumption of test meals. (**A**) Temporal curves of the average plasma insulin response, (**B**) maximum plasma concentration (C_{max}) of insulin, (**C**) T_{max}- time at which the C_{max} was observed, and (**D**) insulinogenic index of 34 participants (14 women and 10 men) who consumed the same quantity of cooked rice. *, $p < 0.05$ compared to the placebo group; **, $p < 0.01$ compared to the placebo group. EOC, essence of chicken.

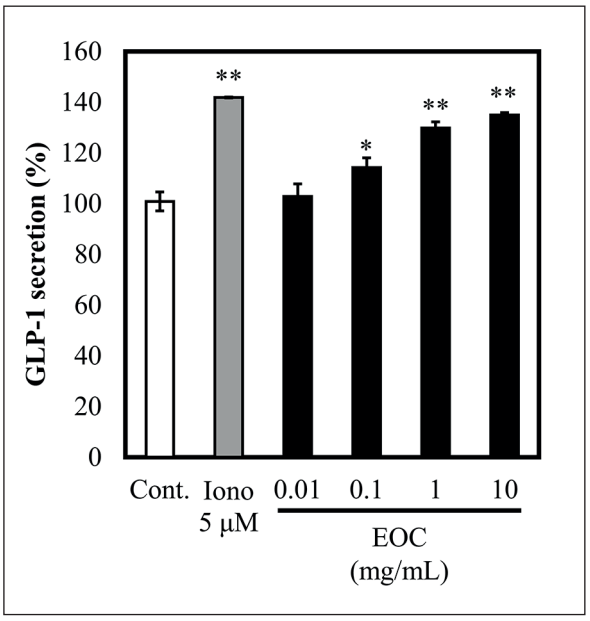

Figure 4. Effect of essence of chicken (EOC) on the secretion of glucagon-like peptide 1 (GLP-1). NCI-H716 cells were exposed to various concentrations of EOC and ionomycin for 60 min. The secretion of GLP-1 into the medium was expressed as a percentage of the control value. Data are expressed as the mean ± SEM (n = 5). Statistical analysis was performed using one-way analysis of variance, followed by Dunnett's multiple comparison test. *, $p < 0.05$ compared to the placebo group; **, $p < 0.01$ compared to control group. GLP-1, glucagon-like-peptide-1; Cont., control group; Iono, ionomycin; EOC, essence of chicken.

Discussion

To the best of our knowledge, this is the first study to report an improvement in the early-phase insulin response by EOC in a placebo-controlled trial with a sufficiently large sample size and no sex bias.

The role of EOC in human health has attracted attention because it exhibits promising results, such as suppressing hyperglycemia[9-13]; relieving mental fatigue[14] and anxiety symptoms[15]; and improving energy metabolism[16], the quality of breast milk[17], and cognitive function[18].

This study verified the inhibitory effect of EOC on the increase in postprandial blood glucose levels when ingested together with loaded foods. Ingestion of the test beverage led to no significant difference in the blood glucose level C_{max} and $AUC_{0\text{-}120\ min}$ compared to that after the ingestion of the control beverage. Therefore, no inhibitory effect on postprandial blood glucose levels was observed. However, ingestion of the loaded food led to significantly higher insulin levels in the test beverage group than that in the control beverage group at 30, 45, and 60 min after ingestion of the loaded food. In addition, the C_{max} and IGI were significantly higher and T_{max} was significantly lower in the test beverage group than that in the control beverage group. Therefore, EOC intake improved postprandial early-phase insulin response.

In this study, IGI was used in addition to T_{max} as an index of early-phase insulin response. IGI is the ratio of insulin increase to glucose increase within 30 min of consuming a meal[12,19,20]. Changes in IGI are positively correlated with changes in pancreatic β-cell function in NGT and prediabetes; therefore, it could be an alternative index for pancreatic β-cell function in healthy individuals[12]. In addition, IGI is lower in prediabetes and diabetes than in NGT[12,19,20]. In this study, a significant increase in IGI was observed when the test beverage was ingested than that when the control beverage was ingested. This indicates that consuming EOC with meals improves pancreatic β-cell function. In addition, EOC significantly reduced the T_{max}, which is the time taken for the insulin level to reach C_{max}. Therefore, the ingestion of EOC improved pancreatic β-cell function and enabled rapid insulin secretion after meals, thereby enhancing the early-phase insulin response. GLP-1, an incretin hormone, plays an important role in improving the early-phase insulin response. Here, we found that GLP-1 secretion in L cells was triggered by EOC. Therefore, EOC could act on L cells and stimulate GLP-1 secretion, promoting blood glucose-dependent insulin secretion and improving early-phase insulin response. In addition, EOC could inhibit dipeptidyl-peptidase-4, a GLP-1-degrading enzyme, and act directly on pancreatic β-cells to promote glycemic-dependent insulin secretion. Further research is needed to elucidate the mechanism of early insulin secretion by EOC.

EOC was proposed to have an inhibitory effect on the postprandial blood glucose elevation in humans; however, such an effect was not observed after the ingestion of EOC in this study. This could be because the postprandial blood glucose levels of the participants in the study were significantly different between the time of the screening and the main tests. When participants with such variations were excluded from the analysis, the increase in blood glucose level tended to be suppressed by EOC intake (data not shown). Therefore, in future studies, it may be

desirable to select participants with low intra-individual variation in postprandial blood glucose levels. Furthermore, in Asians, including Japanese populations, postprandial hyperglycemia is often attributed to low insulin secretion, which leads to the onset of diabetes[4]. EOC improves the early-phase insulin response; therefore, it could inhibit the increase in the postprandial blood glucose levels in individuals who are not insulin resistant but have low insulin secretory capacity.

This study has a few limitations. First, we could not examine the effects of EOC ingestion on GLP-1 secretion in humans. Second, the component of EOC contributing to the improvement of early-phase insulin response was not identified. Therefore, further research is needed to identify the active ingredient in EOC and elucidate the mechanism by which EOC enhances early-phase insulin response.

Conclusions

To the best of our knowledge, this is the first placebo-controlled trial to show that EOC enhances early-phase insulin response. Therefore, EOC intake may help prevent diabetes in Asians, including the Japanese population.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Author Contributions Statements

All authors have read and approved the final manuscript. The authors alone are responsible for the content and writing of the paper.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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