Circulating Concentrations of Insulin Resistance-Associated Hepatokines, Selenoprotein P and Leukocyte Cell-Derived Chemotaxin 2, during an Oral Glucose Tolerance Test in Humans

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INTRODUCTION

Hepatokine is a collective term for liver-derived secretory factors whose previously-unrecognized functions have been recently elucidated. We have rediscovered selenoprotein P (SeP) and leukocyte cell-derived chemotaxin 2 (LECT2) as hepatokines that are involved in the development of insulin resistance and hyperglycemia. The aim of this study was to determine whether and, if so, how oral glucose loading alters the two hepatokines in humans. We measured concentrations of serum SeP and plasma LECT2 during 75 g oral glucose tolerance test (OGTT) (n = 20) in people with various degrees of glucose tolerance. In OGTT, concentrations of both serum SeP and plasma LECT2 decreased at 120 min compared with the baseline values, irrespective of the severity of glucose intolerance. Decrement of serum SeP during OGTT showed no correlations to the clinical parameters associated with insulin resistance or insulin secretion. In multiple stepwise regression analyses, plasma cortisol was selected as the variable to explain the changes in plasma concentrations of LECT2. The current data reveal the acute inhibitory actions of oral intake of glucose on circulating SeP and LECT2 in humans, irrespective of the severity of glucose intolerance. This study suggests that circulating SeP is regulated by the unknown clinical factors other than insulin and glucose during OGTT.

Key words: selenoprotein P; leukocyte cell-derived chemotaxin 2; oral glucose tolerance test; hepatokine

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duction is increased in people with obesity. LECT2 emerged as a hepatokine whose hepatic expression levels positively correlate with body mass index in patients with type 2 diabetes. LECT2 was originally cloned as a neutrophil chemotactic factor. Gene expression for LECT2 (encoded by the Lect2 gene in mice) is selectively expressed by adult and fetal liver cells. Our experiments using recombinant LECT2 have revealed that LECT2 impairs insulin signal transduction in cultured myotubes by activating Jun NH2-terminal kinase (JNK). In contrast, genetic deletion of LECT2 attenuates skeletal muscle insulin resistance in dietary obese mice. This study reveals that LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance.

Gene expression for Lect2 in cultured hepatocytes is negatively regulated by adenosine monophosphate-activated protein kinase (AMPK), the energy depletion-sensing kinase. Consistent with this finding in hepatocytes, feeding of high fat diet decreases AMPK activity and increases gene expression for Lect2 in the liver of mice. Additionally, plasma LECT2 concentrations show the rapid response preceding body weight changes during diet-induced weight cycling in mice. However, similar to SeP, few papers were available on blood concentrations of LECT2 in humans during oral glucose loading test or daily diet intake.

Because both SeP and LECT2 are hepatokines that have great impacts on whole body glucose metabolism, we hypothesized that circulating concentrations of the two hepatokines are regulated by oral intake of glucose, as well as those of gut-derived hormones such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). In fact, many kinds of hormones, such as adiponectin, that play a major role in the regulation of glucose metabolism are known to be altered during oral glucose tolerance test. Additionally, people with abnormal glucose tolerance might show different profiles of circulating SeP and LECT2 during glucose loading. To test this hypothesis, we measured concentrations of serum SeP and plasma LECT2 during 75 g oral glucose tolerance test in people with various degrees of glucose tolerance.

**MATERIALS AND METHODS**

**Study Subjects and Protocol** For the study of oral glucose tolerance test (OGTT), the subjects were patients who visited outpatient department of Tsuruga Hospital. Twenty subjects were selected based on entry criteria and exclusion criteria. A total of 20 subjects (males/females: 10/10, mean age: 66 years) accepted the invitation. No subjects are treated with oral hypoglycemic agents, insulins nor GLP-1 therapy. After overnight fasting for at least 12 h, they underwent a 75 g OGTT. Peripheral venous blood samples were obtained for biochemical analyses and hepatokine measurement. In the OGTT, blood samples were collected before, at 30 min, 1 h and 2 h after ingestion of Trelan-G™ (75 g glucose in 225 mL water).

**Hepatokine Measurement** Concentrations of serum SeP were measured by a sol particle homogeneous immunooassay as we previously reported. We assessed serum levels of full-length SeP selectively by using two types of SeP monoclonal antibodies, one recognizing N-terminal domain of SeP and another recognizing the C-terminal domain. Plasma levels of LECT2 were measured by Ab-Match ASSEMBLY Mouse LECT2 kit (MBL).

![Fig. 1. Time–Course of Plasma Glucose](image-url)
Patient Eligibility  The eligibility criteria were as follows: >20 years of age; patients with diabetes; patients with impaired glucose tolerance; patients with normal glucose tolerance who suspected to have metabolic diseases (BMI >25, fasting plasma glucose >100mg/dL, postprandial plasma glucose >140mg/dL, HbA1c >6.0%, patients with dyslipidemia or fatty liver).

The exclusion criteria included: 1) poorly controlled unstable diabetes, 2) presence of a severe health problem and not suitable for the study, 3) pregnant, 4) patients taking corticosteroid treatments, 5) liver cirrhosis, 6) patients who were diagnosed as malignant diseases.

Human Rights Statement and Informed Consent  The current study was conducted in accordance with the principles in the Declaration of Helsinki of 1964 and later versions. The study protocol was approved by the Ethical Committee of Kanazawa University and the Ethical Committee of Municipal Tsuruga Hospital, respectively. All the people in current study provided written informed consent (date of the latest revision approval at UMIN: 14 October 2016, approval no. UMIN000024411).

Statistical Analysis  Numeric variables are expressed as means ± standard errors of mean (S.E.M.). For testing differences in time course of parameters, one-way repeated measures ANOVA were used and post hoc test were performed using the Bonferroni correction (Figs. 1C, D). To analyze difference in mean of three groups, we performed one-way ANOVA and Tukey–Kramer post-hoc test (Figs. 2, 3). In the study of 75g OGTT, we used stepwise multiple regression models to estimate the relationships between baseline levels of plasma LECT2 and the different components of the variables. A p value of less than 0.05 was considered statistically significant. All data were analyzed by using the Statistical Package for the Social Sciences version 22.0 (SPSS, Chicago, IL, U.S.A.).

RESULTS  Alteration of Blood Concentrations of SeP and LECT2 during OGTT Basal characteristics of the subjects are shown in Table 1. After a loading of 75g of glucose, con-
centrations of plasma glucose and serum insulin were altered as shown in Figs. 1A, 1B. No adverse effects associated with severe hyperglycemia were observed after 75 g OGTT. A repeated measures ANOVA with a Greenhouse–Geisser correction was performed and mean serum concentrations of SeP decreased at 60 min (p < 0.05). Post hoc tests using the Bonferroni correction revealed that concentrations of cortisol, baseline plasma concentrations of LECT2 and homeostasis model assessment of beta cell (HOMA-β) (Fig. 2A). Plasma cortisol concentrations, we selected 27 clinical variables and further clarify the mechanisms by which OGTT decreases concentrations, height, and LDL-C were selected as the significant variables to explain ΔLECT2

| n | 20 |
| --- | --- |
| Age | 65 ± 1.4 |
| Sex (male/female) | 10/10 |
| BMI (kg/m²) | 24.2 ± 0.5 |
| Waist circumference (cm) | 86.2 ± 1.8 |
| DM/IGT/NGT | 7/6/7 |

### Biochemical data

| Variable | Value |
| --- | --- |
| HbA1c (%) | 6.1 ± 0.1 |
| FPG (mg/dL) | 110.2 ± 5.1 |
| PPG 120 min (mg/dL) | 186.4 ± 22.1 |
| Total protein (g/dL) | 7.5 ± 0.1 |
| Albumin (g/dL) | 4.4 ± 0.1 |
| Aspartate transaminase (IU/L) | 24.2 ± 2.8 |
| Alanine transaminase (IU/L) | 27.1 ± 4.9 |
| Creatinine (mg/dL) | 0.78 ± 0.1 |
| Triglyceride (mg/dL) | 120.6 ± 16.2 |
| HDL-C (mg/dL) | 71.0 ± 4.4 |
| LDL-C (mg/dL) | 99.1 ± 8.0 |
| ACTH (pg/mL) | 28.3 ± 3.1 |
| Cortisol (µg/mL) | 13.2 ± 0.8 |
| HOMA-R | 2.36 ± 0.37 |
| HOMA-β | 78.0 ± 15.5 |

### Blood levels of hepatokines

| Variable | Value |
| --- | --- |
| Fasting serum levels of SeP (µg/mL) | 3.98 ± 0.15 |
| Fasting plasma levels of LECT2 (ng/mL) | 58.1 ± 4.2 |

Variables are expressed as n or means ± S.E.M. Abbreviation: BMI: body mass index; DM: diabetes mellitus; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; HbA1c: glycated hemoglobin A1c; HOMA-β: homeostasis model assessment of insulin resistance; HOMA-R: homeostasis model assessment of beta cell; SeP: selenoprotein P; LECT2: leukocyte cell-derived chemotaxin 2.

ΔSeP<sub>120-0</sub> and the other parameters (Table 2). ΔLECT2<sub>120-0</sub> correlated negatively with serum insulin (0, 60, 120 min), low density lipoprotein cholesterol (LDL-C), morning plasma concentrations of cortisol, baseline plasma concentrations of LECT2 and homeostasis model assessment of beta cell (HOMA-β) (Table 3).

### Models to Explain Decrement of LECT2 in OGTT

To further clarify the mechanisms by which OGTT decreases LECT2 concentrations, we selected 27 clinical variables and performed multivariable analyses using stepwise method to generate models to explain ΔLECT2<sub>120-0</sub>. Plasma cortisol concentrations, height, and LDL-C were selected as the significant variables to explain ΔLECT2<sub>120-0</sub> (Table 4).

### DISCUSSION

The current study shows the reduction of circulating hepatokines, SeP and LECT2, after the loading of glucose in humans. We have previously reported that both SeP and LECT2 attenuate insulin signal transduction in cultured myotubes, although the molecular mechanisms by which the two hepato...
gene expression in cultured hepatocytes.5,11,12) However, in the current study, ΔSeP120−0 during OGTT showed no significant correlations with the insulin- and glucose-associated parameters. Additionally, the participants with type 2 diabetes or impaired glucose tolerance, who had hyperglycemia or hyperinsulinemia during OGTT, respectively, showed a similar descending pattern of circulating SeP. These findings suggest that circulating SeP during OGTT in humans is regulated by the unknown clinical factors other than insulin and glucose. After oral glucose loading, blood levels of secretory factors other than insulin, such as gut-derived incretins, and activity of sympathetic nerve system fluctuate dramatically in humans.16,17,20) Further cellular or animal studies are needed to determine whether incretins and sympathetic nerve system regulate circulating levels of SeP during OGTT.

Multiple regression analysis reveals that fasting plasma cortisol is an independent explanatory variable of ΔLECT2120−0 in the OGTT study. Notably, fasting plasma cortisol did not significantly correlate with fasting plasma LECT2, but the participants with high concentrations of fasting cortisol showed a large decrement in plasma LECT2 during OGTT. However, as far as we know, there are no reports regarding the crosstalk between cortisol and LECT2. Additional cellular experiments are needed to determine whether cortisol affects LECT2 gene expression or LECT2 secretion in cultured hepatocytes.

A limitation of the current study is small numbers of the human subjects. In particular, the numbers may be insufficient to detect a statistically significant difference in the analyses classified by the severity of glucose intolerance. Further large-scale clinical studies are needed to confirm whether glucose intolerance alters the response of SeP or LECT2 during OGTT.

In conclusion, the present data demonstrate the acute inhibitory actions of oral intake of glucose on circulating SeP and LECT2 in humans. Response of SeP and LECT2 to oral glucose loading shows a similar pattern in people, irrespective of the severity of glucose intolerance.

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Conflict of Interest  The authors declare no conflict of interest.

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