Original Article

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Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver

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Summary **Leukocyte cell-derived chemotaxin 2 (LECT2) is a signaling molecule expressed in the liver and regulated by Wnt/β-catenin pathways implicated in hepatic metabolism. However, the clinical relevance of LECT2 in obesity and fatty liver is unknown. The objective of this study was to determine whether serum LECT2 levels are affected by of obesity and fatty liver. A cross sectional study comprising 231 Japanese adult subjects were tested for LECT2 using a highly sensitive assay. We evaluated the associations between LECT2 and the anthropometric or clinical markers of obesity and fatty liver. The mean serum LECT2 levels were 43.5 ± 13.6 ng/mL. LECT2 positively correlated with all the anthropometric measures of obesity: body mass index, waist circumference, waist-to-hip ratio, and waist-to-height ratio (W/Ht). Multiple regression analysis revealed that LECT2 is independently related to γ-glutamyl transpeptidase (γ-GTP), triglyceride, and age in males, whereas in females it was related to the homeostasis model assessment ratio, blood urea nitrogen, high-density lipoprotein cholesterol, and γ-GTP. Receiver operating characteristics curve analyses revealed that LECT2 correlated with obesity [area under the curve (AUC) 0.655, 95% confidence interval (CI) = 0.551-0.758, $p = 0.002$ in males; AUC 0.670, 95% CI = 0.570-0.770, $p < 0.001$ in females] and fatty liver (AUC 0.646, 95% CI = 0.544-0.749, $p = 0.004$ in males; AUC 0.733, 95% CI = 0.621-0.844, $p < 0.001$ in females). The present study indicates that serum LECT2 levels are increased by obesity and fatty liver, and suggests that LECT2 is a novel obesity-related protein.**

Keywords: Leukocyte cell-derived chemotaxin 2, obesity related protein, enzyme-linked immunosorbent assay

1. Introduction

The rapidly growing incidence of obesity worldwide is alarming because it is a major cause of morbidity and mortality. Obesity increases the risk of developing serious complications, including type 2 diabetes mellitus, hypertension, steatohepatitis, fatty liver, and dyslipidemia (*1*). Fatty liver is characterized by excessive triglyceride (TG) accumulation in the liver and is one of the most common forms of liver disease associated with obesity (*2*). Obesity alters hepatic metabolism as well as cytokine and growth factor production, which contribute to the development of obesity-associated complications. In a mouse model of high-fat diet-induced obesity, the Wnt/β-catenin signaling pathway was demonstrated to regulate hepatic metabolism (*3*). In contrast, in another study, liver-specific β-catenin knockout mice revealed increased susceptibility to methionine- and choline-deficient diet-induced steatohepatitis (*4*). These reports

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suggest that this pathway plays an important role in hepatic metabolism.

Leukocyte cell-derived chemotaxin 2 (LECT2) is one of the proteins regulated by hepatic β-catenin (*5*) and is primarily expressed by hepatocytes (*6,7*). It was initially identified *in vitro* as a neutrophil chemotactic factor (*8*). More recently, functional studies have reported that LECT2 may function in immunological events such as hepatitis (*9*) and arthritis (*10*), antihepatocarcinogenesis (*11,12*), and the negative regulation of the Wnt receptor signaling pathway in the small intestine (*13*). There is accumulating evidence indicating that LECT2 is a pleiotropic protein, as are many cytokines; however, the possible involvement of LECT2 in metabolic diseases has not been investigated.

The aim of the present study was to determine whether serum LECT2 levels may be affected by obesity and its related diseases such as fatty liver. We developed a highly sensitive enzyme-linked immunosorbent assay (ELISA) protocol to evaluate the association between serum LECT2 levels and anthropometric or clinical variables. Moreover, this study sought to provide evidence that LECT2 is specific for metabolic diseases and is not a predictor of other disorders such as chronic kidney disease (CKD).

2. Materials and Methods

2.1. *Study subjects*

The study included 231 Japanese subjects (113 males and 118 females; aged 40-69 years) who visited the Department of General Medicine at the National Center for Global Health and Medicine (Tokyo, Japan) between August 2010 and September 2012 for an annual health check-up. Subjects were categorized into obese [waist-to-height ratio (W/Ht) ≥ 0.5, n = 113] and lean (W/Ht < 0.5, n = 118) groups. The subjects receiving medication for diabetes mellitus, hypertension, or dyslipidemia or those with a provisional diagnosis of diabetes mellitus [fasting plasma glucose ≥ 126 mg/dL or hemoglobin A_{1c} (HbA1c) ≥ 6.5%] were excluded. This study was conducted with the approval from the ethical committee of the National Center for Global Health and Medicine. Written informed consent was obtained from each subject prior to study enrollment.

2.2. *Data collection*

Anthropometric measurements and blood sampling were performed after overnight fasting (for at least 12 h). Waist circumference (WC) at the level of the umbilicus and hip circumference at the level of the maximum protuberance of the buttocks were recorded with a measuring tape. Body mass index (BMI), waist-to-hip ratio (WHR), and W/Ht were calculated using the anthropometric measures. For the insulin resistance index, the homeostasis model assessment ratio (HOMA-R) was calculated from fasting plasma glucose (FPG) and fasting insulin values using the following equation: HOMA-R = [FPG (mg/dL) × fasting insulin (mU/L)]/405. A physician documented the medical history and alcohol consumption during a personal interview. A diagnosis of fatty liver was made on the basis of the results of an abdominal ultrasound performed by trained technicians. Fatty liver was defined as liver parenchyma with echogenicity higher than that of the kidney cortex, the presence of vascular blurring, and deep attenuation of ultrasound waves (*14*). Subjects were assessed for kidney disease by calculating the estimated glomerular filtration rate (eGFR) according to the Japanese formula using serum creatinine (SCr) (*15*). A diagnosis of CKD was made if eGFR was < 60 mL/min/1.73 m^2 (*16*).

2.3. *Measurement of serum LECT2 by ELISA*

The serum LECT2 levels were measured using a commercially available ELISA kit (Ab-Match ASSEMBLY Human LECT2 Kit with Ab-Match UNIVERSAL Kit; Medical & Biological Laboratories, Nagoya, Japan) with modifications to improve sensitivity. The LECT2 standard and serum samples (1:10 dilution) were diluted with sample diluent (provided with the kit) in the presence of 300 mM NaCl, which is an optimal concentration for sensitivity (Table S1, *http://www.biosciencetrends.com/docindex.php?year=2013&kanno=6*). These samples were incubated for 60 min at room temperature before adding to an ELISA plate. These conditions consistently generated LECT2 values that were 2-fold higher than those of the standard protocol (Table S2, *http://www.biosciencetrends.com/docindex.php?year=2013&kanno=6*).

2.4. *Statistical analyses*

The results were presented as mean ± standard deviation (S.D.). Data were analyzed using the IBM SPSS Statistics version 20 (IBM Corp., Armonk, NY, USA) and R version 2.8.1 (R Foundation for Statistical Computing, *http://www.r-project.org*). The continuous variables were analyzed using the Shapiro-Wilk test. A two-tailed unpaired Student's *t*-test was used to evaluate the differences in serum LECT2 levels between males and females and between obese and lean subjects. Comparisons of clinical parameters between males and females were performed using the two-tailed unpaired Student's *t*-test or the nonparametric Mann-Whitney *U* test, as appropriate. The relationship between serum LECT2 levels and anthropometric measures was evaluated using the two-tailed Pearson's correlation coefficient, whereas the interrelationships between the serum LECT2 levels and metabolic parameters, including age, were analyzed using the Spearman's

rank correlation coefficient. A partial correlation was used to evaluate these relationships, independent of the other variables. With regard to stepwise multiple linear regression analysis, because the levels of TG, high-density lipoprotein (HDL) cholesterol, C-reactive protein (CRP), aspartate transaminase (AST), alanine transaminase (ALT), and γ-glutamyl transpeptidase (γ-GTP) were not normally distributed, logarithmic transformations were used to approach normal distribution and to obtain equal variances. p values < 0.05 were considered statistically significant.

3. Results

3.1. *Clinical characteristics of the subjects*

A total of 231 subjects were included in this study, which included approximately equal numbers of males (n = 113) and females (n = 118), who were in their mid 50s (Table 1). Analysis of the anthropomorphic parameters revealed significantly lower BMI, WC, and WHR in females ($p < 0.001$). The mean systolic and diastolic blood pressures were within the normal range but significantly higher in males. With regard to systemic disease, there was no significant gender difference in the CRP levels. However, there were gender differences with regard to white blood cell counts. The other parameters were selected to characterize the subject population with regard to diabetes/obesity, dyslipidemia/fatty liver, and CKD. None of the subjects were diagnosed with diabetes mellitus. However, half of the subjects were classified as obese on the basis of W/Ht ≥ 0.5 (*17*), with a higher occurrence rate in males. With regard to liver diseases, the levels of AST, ALT, and γ-GTP were slightly higher in males. These findings are consistent with the fact that approximately 36% subjects were diagnosed with fatty liver, with a 2-fold higher incidence observed in males than females. Overall, these findings are consistent with

Table 1. Clinical characteristics of study subjects

Characteristics	Total (n = 231)	Males (n = 113)	Females (n = 118)	Gender tests p values
Serum LECT2 (ng/mL)	43.5 ± 13.6	43.6 ± 12.9	43.4 ± 14.3	NS
Age (years)	55.1 ± 8.7	55.0 ± 8.9	55.1 ± 8.4	NS
Anthropometric parameters				
Body mass index (BMI) (kg/m^2)	22.2 ± 3.1	23.4 ± 2.7	21.1 ± 3.0	< 0.001
Waist circumference (cm)	81.3 ± 9.0	84.7 ± 7.9	78.1 ± 8.9	< 0.001
Waist-to-hip ratio (WHR)	0.89 ± 0.06	0.91 ± 0.05	0.88 ± 0.07	< 0.001
Waist-to-height ratio (W/Ht)	0.50 ± 0.05	0.50 ± 0.04	0.50 ± 0.06	NS
Hypertension				
Systolic blood pressure (mm Hg)	122.1 ± 15.9	124.8 ± 16.1	119.5 ± 15.4	0.011
Diastolic blood pressure (mm Hg)	76.3 ± 11.3	80.1 ± 10.3	72.7 ± 11.0	< 0.001
Inflammation				
CRP (mg/dL)	0.114 ± 0.425	0.163 ± 0.585	0.067 ± 0.152	NS
White blood cells (10^3/μL)	5.32 ± 1.40	5.77 ± 1.31	4.90 ± 1.36	< 0.001
Diabetes mellitus				
Fasting plasma glucose (mg/dL)	92.2 ± 9.4	93.8 ± 9.1	90.7 ± 9.5	0.012
HbA1c (%)	5.6 ± 0.3	5.6 ± 0.3	5.6 ± 0.3	NS
Insulin (mU/L)	4.2 ± 2.6	4.4 ± 2.5	3.9 ± 2.6	NS
HOMA-R	0.96 ± 0.65	1.03 ± 0.62	0.89 ± 0.68	NS
Dyslipidemia				
Total cholesterol (mg/dL)	218.0 ± 36.1	211.1 ± 32.5	224.5 ± 38.3	0.005
TG (mg/dL)	102.2 ± 55.1	118.3 ± 58.5	86.8 ± 46.8	< 0.001
HDL cholesterol (mg/dL)	67.1 ± 18.0	60.2 ± 15.5	73.7 ± 17.8	< 0.001
LDL cholesterol (mg/dL)	126.9 ± 31.7	127.0 ± 28.4	126.9 ± 34.7	NS
Liver disease				
AST (IU/L)	22.9 ± 6.1	23.9 ± 6.6	21.9 ± 5.3	0.013
ALT (IU/L)	21.5 ± 8.8	23.5 ± 9.0	19.6 ± 8.2	< 0.001
γ-GTP (IU/L)	35.2 ± 28.1	44.6 ± 32.6	26.1 ± 19.1	< 0.001
Kidney disease				
UA (mg/dL)	5.4 ± 1.3	6.2 ± 1.1	4.6 ± 1.1	< 0.001
BUN (mg/mL)	13.7 ± 3.4	14.1 ± 3.1	13.3 ± 3.6	NS
SCr (mg/dL)	0.74 ± 0.16	0.85 ± 0.13	0.64 ± 0.11	< 0.001
eGFR (mL/min/1.73 m^2)	76.2 ± 14.3	76.2 ± 14.4	76.2 ± 14.3	NS
Diagnosis				
Obesity (W/Ht ≥ 0.5)	113 (48.9)	63 (55.8)	50 (42.4)	0.042
Fatty liver (by ultrasonic)	83 (35.9)	56 (49.6)	27 (22.9)	< 0.001
Chronic kidney disease (eGFR < 60)	21 (9.1)	8 (7.1)	13 (11.0)	NS
Alcohol consumption (male ≥40 g/day, female ≥20 g/day, ≥10 yr)	105 (45.5)	67 (59.3)	38 (32.2)	< 0.001

Data are presented as mean ± S.D. or proportion (%). LECT2, leukocyte cell-derived chemotaxin 2; CRP, C-reactive protein; HbA1c, hemoglobin A_{1c}; HOMA-R, homeostasis model assessment ratio; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptidase; UA, uric acid; BUN, blood urea nitrogen; SCr, serum creatinine; eGFR, estimated glomerular filtration rate. NS, nonsignificant for p values > 0.05.

the results of the general Japanese population (Report of National Health and Nutrition Survey in 2011; Ministry of Health, Labour and Welfare of Japan). Therefore, we separately analyzed the relationship between serum LECT2 and anthropometric and metabolic parameters in males and females.

3.2. *Impact of obesity on serum LECT2 levels*

Serum LECT2 levels were comparable in males and females (43.6 ± 12.9 *vs*. 43.4 ± 14.3 ng/mL) with an overall average of 43.5 ± 13.6 ng/mL. The average LECT2 levels were 47.4 ± 13.1 ng/mL and 39.7 ± 13.1 ng/mL for the obese and lean subjects, respectively ($p < 0.001$). When the data were further segregated on the basis of gender, we observed similar effects in males (46.8 ± 11.9 *vs.* 39.5 ± 13.1 ng/mL, $p = 0.002$) and females (48.2 ± 14.6 *vs.* 39.8 ± 13.1 ng/mL, $p = 0.001$), respectively. These data suggest that serum LECT2 levels presented no gender difference and were higher in obese subjects. Therefore, we conducted regression analyses between serum LECT2 and the anthropometric parameters: BMI, WC, WHR, and W/Ht. All the four parameters positively correlated with serum LECT2 levels (Figure 1). These data indicate that serum LECT2 levels increase in an obesity-dependent manner. The strongest correlation was observed between LECT2 and W/Ht adjusted for age and gender ($r = 0.349$, $p < 0.001$). As a result, W/Ht was subsequently employed as the sole anthropometric measure to simplify further analyses on obesity.

3.3. *Impact of liver and kidney diseases on serum LECT2*

Because several subjects were diagnosed with secondary complications of obesity, the Spearman's rank correlation analyses were conducted to determine whether LECT2 associations were selective for clinical parameters indicative of hypertension, inflammation, diabetes mellitus, dyslipidemia, liver diseases, or kidney disease (Table 2). Among the markers of obesity, high LECT2 levels simply correlated with CRP and AST levels only in males, and with HbA1c, LDL cholesterol, BUN, and SCr levels only in females. In addition, a positive correlation was observed between LECT2 and the markers of diabetes mellitus, dyslipidemia, liver disease, and kidney disease. These included, insulin, HOMA-R, TG, HDL cholesterol, ALT, γ-GTP, and uric acid (UA). These correlations suggest that high serum LECT2 levels are associated with the development of liver and kidney diseases.

3.4. *Multiple linear regression analysis of serum LECT2 levels*

All the parameters having significant simple correlation with LECT2 for at least one gender (Table 2) were

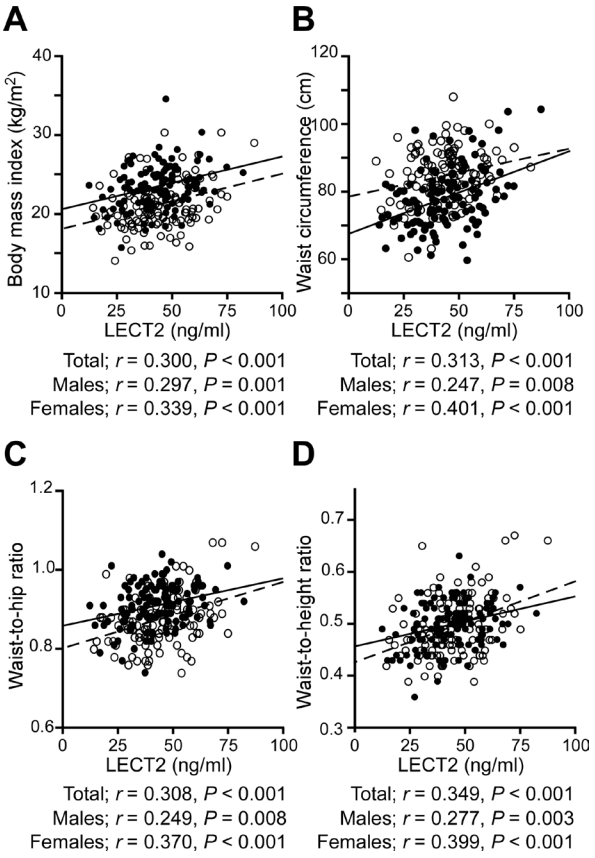

Figure 1. Relationship between serum LECT2 and anthropometric measurements. Distribution of serum LECT2 levels of males and females aged 40-69 years with regard to **(A)** body mass index (BMI), **(B)** waist circumference (WC), **(C)** waist-to-hip ratio (WHR), and **(D)** waist-to-height ratio (W/Ht). Males, filled circles and solid line; females, open circles and dashed line.

assessed for an independent relationship with LECT2 (CRP, HbA1c, insulin, HOMA-R, TG, HDL cholesterol, LDL cholesterol, AST, ALT, γ-GTP, UA, BUN, and SCr) as well as age and W/Ht. These multiple linear regression analyses were performed with serum LECT2 as a dependent variable for each gender (Table 3). In males, γ-GTP, TG, and age remained significant determinant of serum LECT2. On the other hand, in females, HOMA-R, BUN, HDL cholesterol, and γ-GTP were observed to significantly contribute to the determinant of serum LECT2 levels. These multiple regression analyses indicate that serum LECT2 is associated not only with obesity but also with liver diseases in both genders and with kidney dysfunction in females. In addition, these findings suggested that γ-GTP levels were strongly associated with serum LECT2 levels. We considered that this observation may arise if the serum LECT2 levels were also correlated with alcohol consumption. Therefore, we analyzed whether serum LECT2 and γ-GTP levels were correlated with alcohol consumption. Spearman's rank correlation analysis showed that serum LECT2 levels were not statistically correlated with alcohol consumption ($\rho = 0.065$, $p = 0.327$ in males, $\rho =$

Table 2. Spearman's rank correlation coefficient between serum LECT2 and the markers of obesity-related complications

	Total (n = 231)				Males (n = 113)				Females (n = 118)			
	Simple correlation		Age-, gender-, and W/Ht-adjusted		Simple correlation		Age- and W/Ht-adjusted		Simple correlation		Age- and W/Ht-adjusted	
	ρ	p value	Partial ρ	p value	ρ	p value	Partial ρ	p value	ρ	p value	Partial ρ	p value
Hypertension												
Systolic blood pressure	0.023	NS	− 0.032	NS	− 0.026	NS	− 0.072	NS	0.060	NS	− 0.005	NS
Diastolic blood pressure	0.057	NS	0.004	NS	0.041	NS	− 0.044	NS	0.070	NS	0.032	NS
Inflammation												
CRP	0.212	0.001	0.133	0.046	0.262	0.005	0.197	0.040	0.128	NS	0.054	NS
White blood cell	0.089	NS	0.050	NS	0.029	NS	0.002	NS	0.138	NS	0.102	NS
Diabetes mellitus												
Fasting plasma glucose	0.082	NS	0.038	NS	0.009	NS	− 0.014	NS	0.149	NS	0.090	NS
HbA1c	0.123	NS	0.079	NS	− 0.002	NS	− 0.039	NS	0.213	0.021	0.147	NS
Insulin	0.330	< 0.001	0.207	0.001	0.313	< 0.001	0.231	0.016	0.338	< 0.001	0.220	0.019
HOMA-R	0.337	< 0.001	0.217	0.001	0.308	< 0.001	0.229	0.017	0.352	< 0.001	0.239	0.011
Dyslipidemia												
Total cholesterol	0.063	NS	0.051	NS	0.066	NS	0.065	NS	0.071	NS	0.013	NS
TG	0.277	< 0.001	0.248	< 0.001	0.307	< 0.001	0.261	0.006	0.250	0.006	0.205	0.028
HDL cholesterol	− 0.274	< 0.001	− 0.204	0.002	− 0.205	0.030	− 0.141	NS	− 0.319	< 0.001	− 0.210	0.025
LDL cholesterol	0.190	0.004	0.136	0.042	0.128	NS	0.105	NS	0.236	0.010	0.137	NS
Liver disease												
AST	0.194	0.003	0.226	< 0.001	0.207	0.028	0.234	0.014	0.178	NS	0.231	0.015
ALT	0.261	< 0.001	0.215	0.001	0.220	0.019	0.174	NS	0.297	0.001	0.253	0.008
γ-GTP	0.270	< 0.001	0.270	< 0.001	0.340	< 0.001	0.300	0.002	0.261	0.004	0.274	0.004
Kidney disease												
UA	0.240	< 0.001	0.232	< 0.001	0.278	0.003	0.211	0.028	0.312	< 0.001	0.258	0.006
BUN	0.215	0.001	0.209	0.002	0.110	NS	0.137	NS	0.307	< 0.001	0.283	0.002
SCr	0.167	0.011	0.201	0.002	0.158	NS	0.180	NS	0.266	0.004	0.242	0.009

NS, nonsignificant for p values > 0.05.

Table 3. Multiple linear regression analyses with serum LECT2 as a dependent variable in Japanese subjects

Variables	B	(95% confidence interval)	Standardized β	t	p value	Partial correlation
Males (n = 113, R^2 = 0.214, adjusted R^2 = 0.192, p < 0.001)						
γ-GTP*	15.067	(6.188, 23.946)	0.295	3.363	0.001	0.307
TG*	16.346	(4.904, 27.788)	0.248	2.831	0.006	0.262
Age	− 0.258	(− 0.502, − 0.015)	− 0.178	− 2.100	0.038	− 0.197
Females (n = 118, R^2 = 0.355, adjusted R^2 = 0.332, p < 0.001)						
HOMA-R	5.217	(1.834, 8.601)	0.248	3.055	0.004	0.276
BUN	1.238	(0.629, 1.847)	0.308	4.030	< 0.001	0.354
HDL cholesterol*	− 41.955	(− 63.515, − 20.394)	− 0.306	− 3.855	< 0.001	− 0.341
γ-GTP*	14.299	(4.698, 23.900)	0.232	2.951	0.004	0.267

*, log scale.

Table 4. Receiver operating characteristic curve analysis for serum LECT2

Disease	Gender	cutoff values (ng/mL)	Area under the curve (95% confidence interval)	Significance	Sensitivity (%)	Specificity (%)
Obesity	Males	41.8	0.655 (0.551–0.758)	0.002	71.4	62.0
	Females	45.0	0.670 (0.570–0.770)	< 0.001	64.0	72.1
Fatty liver	Males	43.3	0.646 (0.544–0.749)	0.004	67.9	59.7
	Females	46.4	0.733 (0.621–0.844)	< 0.001	70.4	69.2

−0.064, $p = 0.488$ in females), whereas γ-GTP level was positively correlated with alcohol consumption in both genders ($\rho = 0.363$, $p < 0.001$ in males, $\rho = 0.193$, $p = 0.037$ in females). These results suggest that an increase in serum LECT2 level does not directly reflect liver damage due to alcohol abuse.

When the 231 subjects were divided on the basis of the diagnosis of fatty liver, the average LECT2 levels were 48.7 ± 13.6 ng/mL and 40.5 ± 12.8 ng/mL in subjects with and without fatty liver ($p < 0.001$), respectively. When categorized on the basis of gender, the average LECT2 levels were 46.8 ± 12.8 ng/mL and 40.4 ± 12.3 ng/mL ($p = 0.007$) in males with and without fatty liver, respectively, and 52.6 ± 14.7 ng/mL and 40.6 ± 13.1 ng/mL ($p < 0.001$) in females with and without fatty liver, respectively. However, no statistically significant difference was observed when the subjects were divided into a CKD group (48.1 ± 14.4 ng/mL) and a non-CKD group (43.0 ± 13.5 ng/mL) according to eGFR, even separated by gender (45.1 ± 12.9 ng/mL and 43.5 ± 13.0 ng/mL in males, 50.0 ± 15.5 ng/mL and 42.6 ± 14.1 ng/mL in females). Taken together, these results suggest that high serum LECT2 levels may indicate higher risks of developing obesity-related liver diseases.

3.5. *Receiving operating characteristic curve analyses*

Considering the relationships established between LECT2 levels and the markers of obesity and fatty liver, receiver operating characteristic (ROC) curve analysis was performed to check whether serum LECT2 levels could discriminate between lean and obese subjects, and subjects without and with fatty liver (Table 4). With regard to obesity, the area under curve (AUC) values for LECT2 levels were significant both in males (0.655, $p = 0.002$) and females (0.670, $p < 0.001$). The optimal cutoff values were 41.8 ng/mL (sensitivity, 71.4%; specificity, 62.0%) and 45.0 ng/mL (sensitivity, 64.0%; specificity, 72.1%) for males and females, respectively. Moreover, as presented in Table 4, ROC curve analysis revealed that AUCs of LECT2 levels for fatty liver were 0.646 ($p = 0.004$) and 0.733 ($p < 0.001$) for males and females, respectively. From these data, the optimal cutoff values of 43.3 ng/mL (sensitivity, 67.9%; specificity, 59.7%) and 46.4 ng/mL (sensitivity, 70.4%; specificity, 69.2%) were determined for males and females, respectively. Overall, these results indicated that serum LECT2 levels have predictive values with regard to the occurrence of obesity and fatty liver.

4. Discussion

The worldwide obesity epidemic is raising concerns because of the serious complications developing in these patients, including cardiovascular disease, diabetes mellitus, and liver diseases. In fact, the development of better preventive measures based on education and early detection and diagnosis have become a priority in the medical community. Unfortunately, the best predictors of obesity are anthropometric measures, which do not take the obesity-related complications into account. The present study demonstrates, for the first time, that serum LECT2 levels positively correlate with the markers of obesity and fatty liver.

The sensitivity of the ELISA assay currently available for serum LECT2 is too low to distinguish between different patient populations. Studies using this ELISA protocol reported the presence of LECT2 levels in human serum or plasma in the range of 1-15 ng/mL (*18-20*). Recently, Ando *et al*. reported LECT2 levels of 19.7 ± 3.4 ng/mL in heparinized plasma from healthy volunteers with the conventional ELISA protocol (*21*). In addition, we preliminarily determined that the serum LECT2 levels measured by conventional ELISA were lower than the estimated levels obtained by immunoblotting using recombinant LECT2 protein as a standard (data not revealed). These studies suggested that electrostatic interactions with other serum proteins may interfere with the LECT2 assay. One such candidate protein is transferrin, whose interaction with LECT2 has been reported in fish and mice (*22*). Another candidate protein is LECT2 itself, which can oligomerize *in vitro* under certain conditions as previously reported (*23*). Therefore, we resolved the electrostatic interference by adding 300 mM NaCl to the sample diluent, which increased the sensitivity of the assay by more than 2-fold. Using this modified protocol, we report overall LECT2 levels of 43.5 ± 13.6 ng/mL in human serum in a subject group representative of the general Japanese population. When the 231 subjects included in the present study were segregated on the basis of a diagnosis of obesity or fatty liver, the average LECT2 levels were 47.4 ± 13.1 ng/mL *vs.* 39.7 ± 13.1 ng/mL in the obese and lean subjects, respectively, and 48.7 ± 13.6 ng/mL *vs.* 40.5

± 12.8 ng/mL in subjects with and without fatty liver, respectively. The fact that differences between these values were statistically significant testifies to the high sensitivity of this modified ELISA assay. These results indicate a positive correlation between serum LECT2 levels and obesity and fatty liver.

Simple regression analysis established a positive correlation between serum LECT2 and all the four major anthropometric measures of obesity: BMI, WC, WHR, and W/Ht. In addition, the ROC analysis revealed the predictive accuracy of obesity detection in both genders. The hepatic Wnt/β-catenin signaling pathway plays an important role in the metabolism of hepatic glucose, glycogen, and lipids (*3*). Together with the Wnt/β-catenin signaling pathway that increases LECT2 expression (*5*), these studies and the present data are comparable with regard to the notion that LECT2 participates in the events leading to obesity.

The LECT2 levels of the subjects with fatty liver were significantly higher compared with those in the subjects without fatty liver. Moreover, the ROC curve analysis showed that serum LECT2 levels significantly discriminated between subjects with and without fatty liver in both genders. In addition, these data are consistent with the studies reporting an increase in hepatic CYP2E1 in obesity (*24*) and fatty liver (*25*), which is a direct hepatic β-catenin target gene as well as *LECT2* gene (*5*). In addition, insulin resistance is promoted and plays a key role during the progression of a nonalcoholic fatty liver disease. HOMA-R, an indicator of insulin resistance, was correlated with serum LECT2 levels. Therefore, further studies are proposed to investigate the association between serum LECT2 levels and steatohepatitis, cirrhosis, and hepatocarcinoma.

Furthermore, LECT2 levels were associated with the kidney disease-related factors UA, BUN, and SCr. However, in this study, LECT2 could not be determined as a predictor of renal function. These findings are supportive evidence that LECT2 is specific for obesity and obesity-related liver disease.

In conclusion, this study demonstrates that the more sensitive ELISA protocol, which we developed for human serum LECT2, will now allow discriminative studies among different patient populations. Using this assay, we demonstrated that serum LECT2 levels vary between subjects diagnosed with obesity and fatty liver. This clinical study may lead to the development of a new population screening strategy for the worldwide obesity epidemic and its major secondary complications.

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