

Obesity as an effect modifier of the association between leptin and diabetic kidney disease

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ABSTRACT

Aims/Introduction: Obesity has been shown to be a modifier of the association between leptin levels and cardiovascular events. We examined whether obesity modifies the association between serum leptin levels and the progression of diabetic kidney disease.

Materials and Methods: This was an observational longitudinal study on patients with type 2 diabetes. We enrolled 410 and 348 patients in the eGFR and ACR cohorts, respectively. Patients were classified into three groups by sex-specific tertile of leptin levels. Obesity was defined as body mass index ≥ 25 kg/m². Outcomes were the rate of change in estimated glomerular filtration rate (eGFR) and progression to a more advanced stage of albuminuria.

Results: In the eGFR cohort, the mean eGFR change during the median follow-up period of 4.7 years was -1.4 mL/min/1.73 m²/year. An interaction between leptin levels (low, medium or high) and obesity (present or absent) on the change in eGFR was detected (P interaction = 0.003). In the lean group, adjusted eGFR decline in patients with low leptin was steeper than that in patients with medium leptin (2.1 and 0.8 mL/min/1.73 m²/year, P = 0.023). In the obese group, patients with high leptin had a steeper adjusted eGFR decline than those with medium leptin (1.7 and 0.6 mL/min/1.73 m²/year, P = 0.044). In the ACR cohort, 29 patients showed progression of albuminuria during the median follow-up period of 3.9 years. There was no interaction between leptin levels and obesity on the outcome (P interaction = 0.094).

Conclusions: Obesity might modify the effects of leptin on kidney function decline in patients with type 2 diabetes.

INTRODUCTION

Diabetic kidney disease (DKD) is a serious complication with high cardiovascular morbidity and mortality, as well as being the major cause of end-stage kidney disease, worldwide^{1,2}. Therefore, the mechanisms underlying DKD should be clarified, and risk factors for DKD should be identified urgently. Leptin, a low-molecular-weight hormone (16 kDa) secreted by adipocytes and cloned in 1994, is a unique hormone with pleiotropic effects in multiple organ systems³. Leptin has been shown to induce nitric oxide production⁴, which has protective effects against DKD^{5,6}. In contrast, activation of the sympathetic nervous system and increase of oxidative stress have been documented to be involved with leptin^{7,8}, which promotes the progression of DKD^{9–11}. In

humans, the association of leptin with DKD has received little attention and remains controversial^{12–14}. Furthermore, there are few longitudinal studies regarding the association between leptin and the progression of DKD¹⁵.

Obesity is associated with resistance to many of the metabolic effects of leptin¹⁶. Exogenous leptin administration improved glycemic control in patients with congenital leptin deficiency and leptin deficiency as a result of lipodystrophy or HIV-induced lipodystrophy^{17–19}; however, obese patients do not respond to exogenous leptin administration²⁰. Recently, obesity has been shown to be a modifier of the association between leptin levels and cardiovascular (CV) events^{21,22}. Lower leptin levels predicted CV events in lean patients^{21,22}. In contrast, in obese patients, higher leptin level was a predictor of CV events²¹, or there were no relationship between leptin levels

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and CV events²². We, therefore, examined the hypothesis that obesity modifies the association between serum leptin levels and DKD progression in patients with type 2 diabetes.

MATERIALS AND METHODS

Participants

This was a hospital-based, single-center, observational, longitudinal, cohort study on adult Japanese patients with type 2 diabetes. The participants were recruited from the ambulatory and hospitalized patients presenting at the Diabetes Center, Tokyo Women's Medical University Hospital, in Tokyo, Japan, during the period between August 2003 and December 2009. Type 2 diabetes was diagnosed according to the Japan Diabetes Society (JDS) criteria²³.

At a regular ambulatory visit or at the time of hospitalization, participants underwent baseline anthropometric and physical examinations including height, weight and blood pressure. Laboratory examinations included serum lipids, creatinine and plasma hemoglobin A1C (HbA1c) levels in random spot blood samples, and urinary albumin excretion levels were measured using the first morning urine specimen. Patients with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m² were assessed for eligibility, because serum leptin levels increase as a result of decreased renal clearance. Patients who had malignant diseases or those with lower limb amputation were excluded (Figure 1). Patients were classified into three groups by sex-specific tertile of serum leptin levels in each cohort.

The study protocol was designed in adherence to the Declaration of Helsinki, and was approved by the Ethics Committee of Tokyo Women's Medical University Hospital.

Measurements

Serum leptin levels were determined by radioimmunoassay (Human Leptin RIA; Millipore, Billerica, MA, USA). The inter- and intra-assay coefficient of variation (CV) was 3.6–6.2% and

3.4–8.3%, respectively. Serum creatinine and total cholesterol levels were determined by enzymatic methods. HbA1c levels were measured by high-performance liquid chromatography (HPLC), using a set of calibrators assigned by the JDS (normal range 4.3–5.8%). In the present study, National Glycohemoglobin Standardization Program (NGSP)-equivalent values were obtained using the following equation: HbA1c (%) = 1.02 \times HbA1c (JDS) (%) + 0.25%²⁴.

Urinary albumin levels were measured using the latex agglutination method, and normalized using urinary creatinine levels. Urinary creatinine concentrations were determined using an enzymatic method. The stage of albuminuria was defined as normoalbuminuria [urinary albumin-to-creatinine ratio (ACR) <30 mg/g], microalbuminuria (30 \leq ACR < 300 mg/g) or macroalbuminuria (300 mg/g \leq ACR). Glomerular filtration rate (GFR) was estimated using the following modified three-variable equation, as proposed by the Japanese Society for Nephrology: eGFR (mL/min/1.73 m²) = 194 \times age (years)^{-0.287} \times serum creatinine level (in mg/dL)^{-1.094} \times (0.739 if female)²⁵.

Definition of Obesity

In the present study, obesity was defined as body mass index (BMI) ≥ 25 kg/m² according to the World Health Organization classification for Asian populations²⁶.

Outcome Measurements

The first outcome measurement of the present study was defined as the annual rate of change in eGFR. For each individual, the rate of change in eGFR per year was determined using a simple regression analysis, with eGFR as a function of time in years, applied to all eGFR values obtained during the follow-up period. Patients were excluded if their follow-up period was less than 2 years since entry into the study (Figure 1). This minimum observation period was selected based on the basis of a previous recommendation for an observation period of at

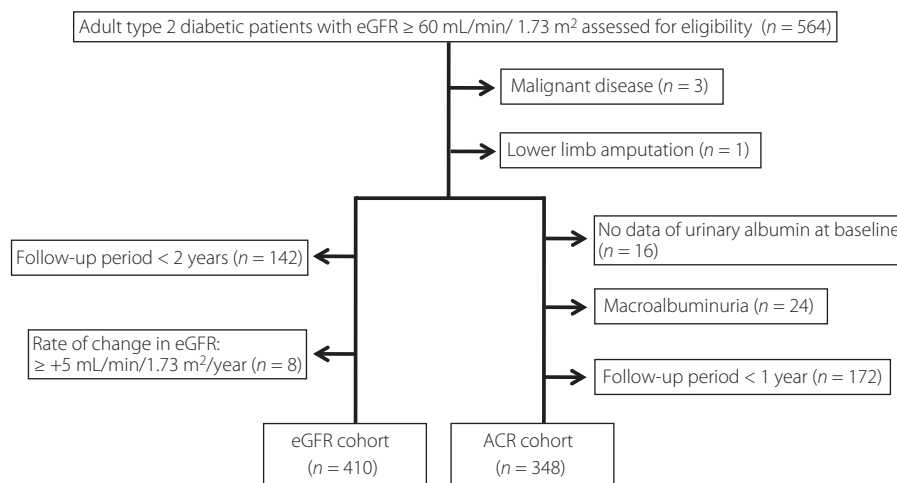


Figure 1 | Flow diagram of the study population.

least 2 years to assure valid determination of the rate of decline in GFR²⁷. In addition, patients were excluded if their rate of change in eGFR was equal to or more than 5 mL/min/1.73 m²/year, because such an increase is not a physiological change (Figure 1).

The second outcome measurement was defined as the transition from any given stage to a more advanced stage of albuminuria; that is, from normoalbuminuria to micro- or macroalbuminuria, or from microalbuminuria to macroalbuminuria, established using at least two consecutive urinary ACR measurements to reduce misclassification. Patients were excluded if their follow-up period was less than 1 year from enrolment into the study (Figure 1).

Statistical Analysis

Continuous variables were expressed as arithmetic mean \pm standard deviation (SD) or geometric mean with 95% confidence interval (CI), as appropriate according to the data distribution. Categorical data were expressed by percentages. For statistical analyses, Pearson's correlational analysis, Fisher's exact probability test, analysis of covariance (ANCOVA), multivariate regression analysis and Cox proportional hazard model analysis were used, as appropriate. Cumulative incidence of transition of albuminuria stage was estimated using the Kaplan–Meier method, and the statistical differences between the groups were compared by the log–rank test. In the multivariate regression analysis and multivariate Cox proportional hazard model analysis, a stepwise variable-selecting procedure was carried out, specifying the significant levels for entering another explanatory variable into the model as 0.25, and that for removing an explanatory variable from the model as 0.15, respectively. In the multivariate model, the following parameters were used as covariates: age, sex, systolic blood pressure, BMI, HbA1c, total cholesterol, triglycerides levels, eGFR, urinary ACR levels and use of renin–angiotensin system blockers at baseline. *P*-values <0.05 were considered significant. All statistical analyses were carried out using the SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Baseline Demographic and Clinical Characteristics

Of the 564 patients, 410 [160 women and 250 men: mean age 58 \pm 13 years (range 23–86 years)] who met the eligibility criteria, and 348 patients [138 women and 210 men: mean age 58 \pm 13 years (range 23–86 years)] were enrolled as the eGFR and ACR cohort, respectively (Figure 1). Baseline demographic and laboratory data in the eGFR and ACR cohort are presented in Table 1. Leptin levels in women were significantly higher than those in men in both cohorts (*P* <0.001). In the eGFR cohort, the sex-specific first and second tertile levels of serum leptin were 6.6 and 11.7 ng/mL, and 2.9 and 4.9 ng/mL in women and men, respectively. In the ACR cohort, the gender-specific first and second tertile levels of serum leptin were 6.3 and 11.7 ng/mL, and 2.9 and 4.8 ng/mL in women and men, respectively.

Table 1 | Baseline demographic and laboratory data in estimated glomerular filtration rate and albumin-to-creatinine ratio cohorts

	eGFR cohort (<i>n</i> = 410)	ACR cohort (<i>n</i> = 348)
Age (years)	58 \pm 13	58 \pm 13
Men (%)	61.0	60.3
Duration of diabetes (years)	11 \pm 9	11 \pm 8
BMI (kg/m ²)	25.9 \pm 5.5	25.7 \pm 5.3
Retinopathy, none/ simple/proliferative (%)	59.3/33.0/7.7	59.7/33.0/7.3
Diabetes therapy, none/OHA/insulin (%)	28.1/52.4/19.5	26.4/55.2/18.4
Systolic blood pressure (mmHg)	132 \pm 18	133 \pm 18
Diastolic blood pressure (mmHg)	76 \pm 12	77 \pm 12
Use of RAS blockers (%)	36.3	34.5
Use of other antihypertensive drug (%)	26.6	22.7
Use of lipid lowering agents (%)	32.0	32.5
History of cardiovascular disease (%)	18.9	17.6
Laboratory data		
Hemoglobin A1c (%)	9.0 \pm 2.0	9.0 \pm 1.9
Total cholesterol (mg/dL)	197 \pm 39	196 \pm 40
Triglycerides (mg/dL)	127 (120–134)	125 (118–133)
Creatinine (mg/dL)	0.69 \pm 0.15	0.69 \pm 0.14
Estimated GFR (mL/min/1.73 m ²)	84.9 \pm 18.2	84.8 \pm 17.4
Urinary albumin to creatinine ratio (mg/g)	18.0 (15.9–20.4)	15.1 (13.6–16.8)
Stage of albuminuria, normo/ micro-/macroalbuminuria (%)	72.5/23.0/4.5	74.1/25.9/–
Leptin (ng/mL)		
Women	8.8 (7.9–9.8)	8.5 (7.6–9.6)
Men	4.0 (3.7–4.3)	3.9 (3.6–4.3)

Data are expressed as percentage, mean \pm standard deviation, or geometric mean (95% confidence interval). ACR, albumin-to-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; OHA, oral hypoglycemic agents; RAS, renin–angiotensin system.

Interaction between Leptin Levels and Obesity on the Rate of Change in eGFR and Progression of Albuminuria

In the eGFR cohort, the median follow-up period was 4.7 years (range 2.0–8.2 years). The mean number of creatinine measurements used for determining the rate of change in eGFR was 16 \pm 10. The mean rate of change in eGFR was -1.4 ± 3.7 mL/min/1.73 m²/year. A significant interaction between serum leptin levels (low, medium or high) and obesity (present or absent) on the rate of change in eGFR was detected (*P* interaction = 0.005). Analysis adjusted by other covariates showed results similar to those aforementioned (*P* interaction = 0.003; Table 2).

During the median follow-up period of 3.9 years (range 1.0–8.2 years) in the ACR cohort, 22 of 258 patients with normoalbuminuria and five of 90 patients with microalbuminuria

Table 2 | Interaction between serum leptin levels and obesity on the annual rate of change in estimated glomerular filtration rate and the progression of albuminuria

Rate of change in eGFR	Standardized estimate	P-value
Leptin (low, medium or high) × obesity (present or absent)	-0.367	0.003 (P interaction)
Leptin (low, medium or high)	0.244	0.004
Obesity (present or absent)	0.286	0.002
Hemoglobin A1c (%)	-0.188	<0.001
eGFR (mL/min/1.73 m ²)	-0.130	0.010
Logarithmically transformed urinary ACR (mg/g)	-0.241	<0.001
Progression of albuminuria	Hazard ratio (95% CI)	P-value
Leptin (low, medium or high) × obesity (present or absent)	3.279 (0.816–13.181)	0.094 (P interaction)
Leptin (low, medium or high)	0.254 (0.087–0.736)	0.012
Obesity (present or absent)	0.364 (0.074–1.779)	0.212
Logarithmically transformed triglycerides (mg/dL)	6.084 (1.328–27.866)	0.020
Logarithmically transformed urinary ACR (mg/g)	2.251 (0.970–5.226)	0.059

In the analysis of the interaction between serum leptin levels and obesity on the annual rate of change in estimated glomerular filtration rate (eGFR), age, sex, systolic blood pressure, total cholesterol, logarithmically transformed triglycerides and use of renin-angiotensin system blockers were excluded from the model. In the analysis of the interaction between serum leptin levels and obesity on the progression of albuminuria, age, sex, systolic blood pressure, hemoglobin A1c, total cholesterol, eGFR and use of renin-angiotensin system blockers were excluded from the model. ACR, albumin-to-creatinine ratio; CI, confidence interval.

progressed to micro- and macroalbuminuria, respectively. Two patients with normoalbuminuria progressed to macroalbuminuria. There was no interaction between leptin levels and obesity on the progression of albuminuria (*P* interaction = 0.192). In the adjusted analysis, no interaction was observed (*P* interaction = 0.094, Table 2).

Association between Leptin Levels and the Annual Rate of Decline in eGFR in the Lean and Obese Group

In the lean group, the rate of decline in eGFR (mean ± standard error) in patients with low leptin levels was significantly steeper than that in patients with medium leptin levels in both the univariate and multivariate analyses (2.2 ± 0.3 and 0.7 ± 0.4 mL/min/1.73 m²/year, and 2.1 ± 0.3 and 0.8 ± 0.4 mL/min/1.73 m²/year, *P* = 0.006 and 0.023; Figure 2a). In contrast, patients with higher leptin levels had a steeper eGFR decline compared with those with medium leptin levels in both the univariate and multivariate analyses (1.7 ± 0.3 and 0.7 ± 0.4 mL/min/1.73 m²/year, and 1.7 ± 0.3 and 0.6 ± 0.4 mL/min/1.73 m²/year, *P* = 0.065 and 0.044; Figure 2b) in the obese group.

In the analysis treating leptin levels as a continuous variable, lower logarithmically transformed serum leptin levels were significantly associated with steeper eGFR decline in the lean group (Table 3). Meanwhile, in the obese group, higher logarithmically transformed leptin levels were risk factors of eGFR decline (Table 3).

Association between Leptin Levels and the Progression of Albuminuria in the Lean and Obese Group

The Kaplan–Meier method in the lean group showed a trend of negative graded relationships between the progression of albuminuria and three groups classified into sex-specific tertile of leptin levels; however, the difference was not significant (*P* = 0.077; Figure 2). In the obese group, no difference was observed in cumulative incidence of the outcome among the three groups (*P* = 0.764; Figure 3).

DISCUSSION

In this hospital-based, single-center, observational, longitudinal, cohort study, we have found, for the first time, a significant interaction between serum leptin levels and obesity on the rate of change in eGFR in patients with type 2 diabetes. This finding could suggest that the effects of leptin on kidney function decline are modified by the presence of obesity in patients with type 2 diabetes. In the lean group, lower serum leptin levels were associated with steeper eGFR decline; in contrast, higher leptin levels were risk factors of eGFR decline in the obese group. These associations were independent of other variables that are well-known risk factors for the development of DKD. These findings might be peculiar and important to the Asian population, as type 2 diabetic patients in Asia, including Japan, are not necessary obese, unlike those in Western countries^{28,29}. Meanwhile, there was no interaction between serum leptin levels and obesity on the progression of albuminuria.

Although the reasons for the association of leptin levels with eGFR decline were not able to be clarified in the present observational study, several explanations are speculated. Beneficial effects of leptin, such as inducing nitric oxide production, might affect kidney function rather than unfavorable actions, such as activation of the sympathetic nervous system, in lean diabetic patients, although we did not measure any marker of oxidative stress or endothelial dysfunction in this study. In the present study, patients with low leptin levels in the lean group showed a steep decline in eGFR, despite having lower blood pressure than the other groups (Table S1). A recent report clearly showed transgenic overexpression of leptin in Akita mice, a model of non-obese diabetes, to protectively affect against DKD, as well as to improve metabolic profiles³⁰. Furthermore, the reports on the association between serum leptin levels and CV events showed that lower leptin levels were risk factors of CV events in lean patients^{31,32}. These findings, including the present results, could suggest that a relative leptin deficiency is associated with the pathogenesis of DKD and CV events in lean subjects. Whereas, low leptin levels might

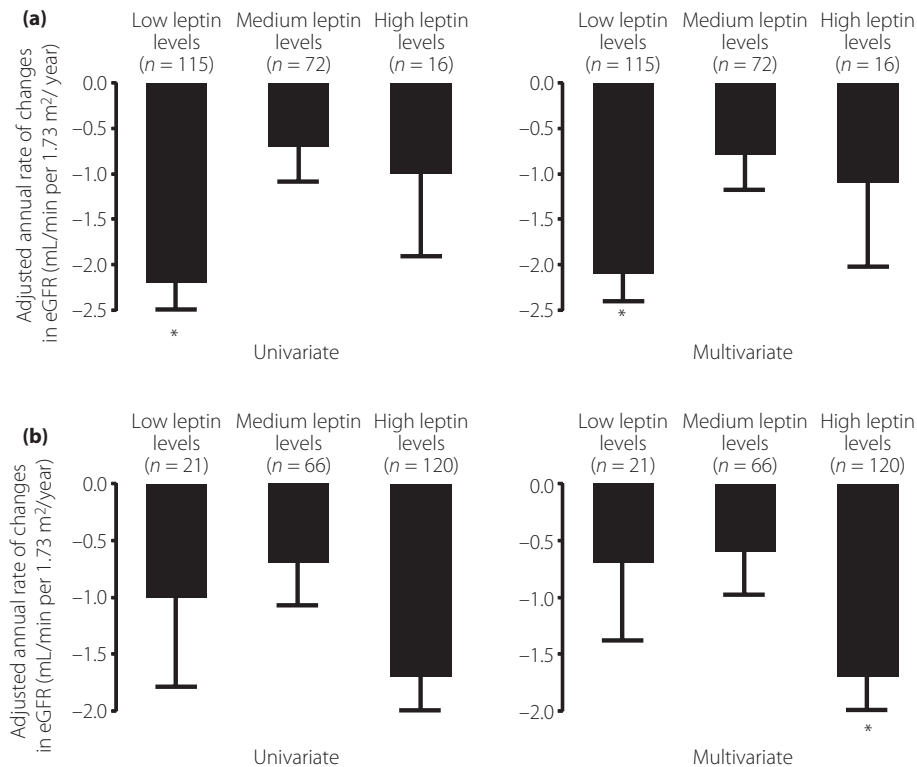


Figure 2 | (a) Comparison of the annual rate of change in estimated glomerular filtration rate (eGFR) among three groups classified into sex-specific tertiles of serum leptin levels in lean patients. (b) Comparison of the annual rate of change in eGFR among three groups classified into sex-specific tertiles of serum leptin levels in obese patients. In the multivariate models, the rate of change in eGFR was adjusted by age, sex, systolic blood pressure, body mass index, hemoglobin A1c, total cholesterol, logarithmically transformed triglycerides levels, eGFR, logarithmically transformed urinary albumin levels and use of renin–angiotensin system blockers at baseline (ANCOVA). **P* < 0.05 vs patients with medium leptin levels.

simply be markers of an undetected wasting condition, as leptin levels have been shown to be correlated with the amount of body fat³³; indeed, there were a significant difference of BMI among the three groups in the present study (Table S1). However, in this study, the rate of change in eGFR in each group was adjusted by covariates including BMI (Figure 2); and in the multivariate analysis using the rate of change in eGFR as a dependent variable, BMI was excluded by a step-wise variable-selecting procedure (Table 3). Furthermore, patients with obvious malignant disease were excluded, and there was no difference in the history of cardiovascular disease among the three groups (low, medium or high leptin levels; Table S1). Finally, a lower proportion of users of renin–angiotensin system blockers among patients with low leptin levels might contribute to our finding that lower leptin levels were associated with steeper eGFR decline, although ‘use of renin–angiotensin system blockers’ was used as a covariate in the multivariate models.

Meanwhile, higher leptin levels were associated with greater eGFR decline in obese patients despite a higher proportion of use of renin–angiotensin system blockers. This could suggest that high leptin levels affected eGFR decline beyond the renal protective effects of renin–angiotensin system blockers. In these

Table 3 | Association of serum leptin levels with the annual rate of change in estimated glomerular filtration rate in the lean and obese groups

Variables at baseline	Lean patients		Obese patients	
	Standardized estimate	<i>P</i> -value	Standardized estimate	<i>P</i> -value
Logarithmically transformed leptin (ng/mL)	0.229	0.004	−0.253	0.021
Men (vs women)	0.150	0.054	−0.207	0.025
BMI (kg/m ²)	Excluded	–	0.188	0.038
Hemoglobin A1c (%)	−0.290	<0.001	Excluded	–
Total cholesterol (mg/dL)	Excluded	–	−0.147	0.041
eGFR (mL/min/1.73 m ² /year)	Excluded	–	−0.164	0.032
Logarithmically transformed urinary ACR (mg/g)	−0.332	<0.001	−0.153	0.034
Use of RAS blockers (vs non-use)	Excluded	–	0.115	0.117

Other excluded variables were age, systolic blood pressure and logarithmically transformed triglycerides at baseline. ACR, albumin-to-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; RAS, renin–angiotensin system.

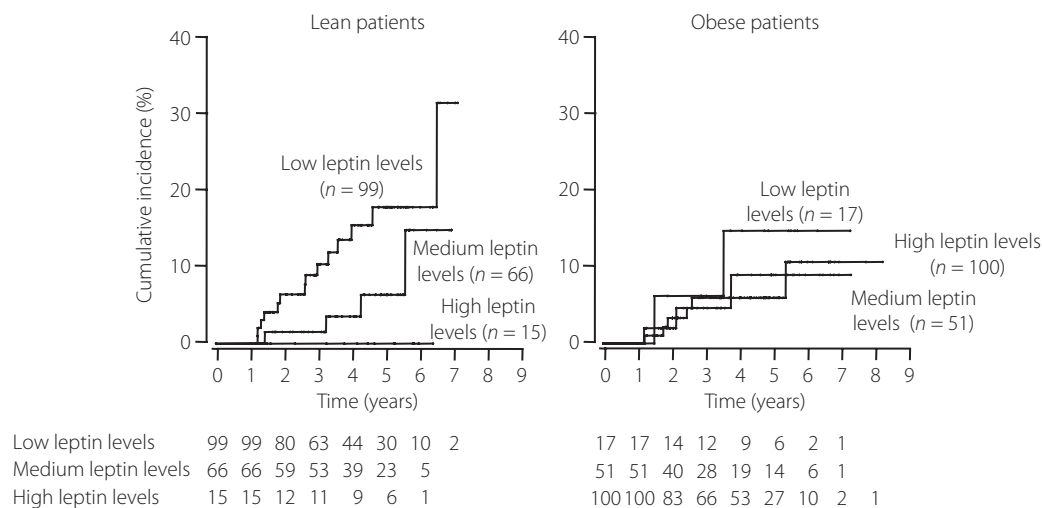


Figure 3 | Cumulative incidence of the progression of albuminuria in three groups classified into sex-specific tertiles of serum leptin levels in the lean and obese group. There was no difference among the three groups in both the lean and obese group (log-rank test; $P = 0.077$ and 0.764 , respectively).

patients, the unfavorable effects of leptin could exceed the beneficial effects on kidney function decline. One of the mechanisms underlying the aforementioned effect is speculated to be leptin resistance. Leptin resistance increases with obesity, resulting in high circulating leptin levels, but decreased leptin signaling³¹. Indeed, leptin administration in obese patients did not improve metabolic profiles²⁰. Reduction in the beneficial effects of leptin on kidneys as a result of leptin resistance might have affected the steep decline in eGFR in patients with high leptin levels in the present study. In addition, according to the concept of selective leptin resistance, basic research using a murine model of obesity showed that renal sympathetic activation of leptin is preserved despite impairment in the metabolic action and nitric oxide production of leptin^{32,34}. A previous report showed that higher serum leptin levels in obese patients receiving hemodialysis were associated with increased CV events²¹, which might support our findings.

In the present study, an interaction between leptin levels and obesity on the progression of albuminuria was not observed, unlike that in the case of kidney function decline. Although the reasons for the aforementioned results remain unclear, several explanations are speculated. First, this could show the differences of the mechanisms and the risk factors for two renal outcomes; that is, the progression of albuminuria and the GFR decline. The UK Prospective Diabetes Study reported the differences in the risk factors for the two renal outcomes³⁵. We have shown that leptin levels had different effects on kidney function decline and the progression of albuminuria in patients with type 2 diabetes¹⁵. Second, the number of patients reaching the end-point was small, yielding less statistical power to observe the interaction between leptin levels and obesity. This issue warrants further investigation.

The present study had several limitations. First, we did not evaluate time-dependent changes in serum leptin levels, HbA1c, lipid profiles, blood pressure or BMI during the follow-up period; therefore, the relationship between leptin levels and DKD progression will need to be confirmed by evaluating leptin levels during the follow-up period in future studies. Second, obesity was defined by BMI, and we did not evaluate waist circumference, hip circumference or direct measurement of fat amount. Third, GFR was estimated using only serum creatinine levels. The present findings need to be confirmed using other methods of estimating obesity and GFR, including cystatin C, in future studies. Fourth, serum leptin levels might need to be determined using blood samples at a certain time, because a recent study showed a circadian rhythm of leptin levels in healthy men³⁶. Fifth, the present study was carried out using a relatively small cohort, and the occurrences of events in the second outcome measurement were low. Finally, this was carried out in a single urban university hospital, which might not be representative of the entire Japanese patient population with type 2 diabetes.

In conclusion, the present single-center observational cohort study suggests that the effects of leptin levels on kidney function decline might depend on the presence of obesity in patients with type 2 diabetes. Lower serum leptin levels in lean patients and higher serum leptin levels in obese patients were risk factors of kidney function decline. These findings should be confirmed in studies with a larger sample size and a multicenter design.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Baseline demographic and laboratory data in six groups classified into lean and obesity and sex-specific tertile of serum leptin levels at baseline (estimated glomerular filtration cohort).