Association of Albuminuria With Intraglomerular Hydrostatic Pressure and Insulin Resistance in Subjects With Impaired Fasting Glucose or Impaired Glucose Tolerance

Akihiro Tsuda, Eiji Ishimura, Hideki Uedono, Akinobu Ochi, Shinya Nakatani, Tomoaki Morioka, Katsuhito Mori, Junji Uchida, Masanori Emoto, Tatsuya Nakatani and Masaaki Inaba

Description	【概要】 研究グループは、糖尿病前段階かつ肥満である人は腎臓に負担がかかっていることを明らかにしました。 糖尿病の合併症の代表として網膜症、腎症、神経障害があげられます。糖尿病は慢性腎臓病を発症し、透析を導入している患者の原疾患の 40%以上を占めています。このような合併症を引き起こさないためには、早期発見・早期治療が肝要です。これまで糖尿病性腎症の前段階であっても腎臓に負担がかかっている可能性は推測されていましたが、実際にヒトで検討することは困難とされていました。 研究グループは、合併症や既往歴、内服歴のない 54 名の腎移植ドナーを対象に Gomez の式を用いて肥満度、インスリン抵抗性、糸球体内圧、アルブミン尿の関連性について検討しました。その結果、糖尿病前段階であっても肥満である人は糸球体内圧が高く、腎症の判断基準となるアルブミン尿が多いことが明らかになりました。本研究成果により、糖尿病前段階であっても腎臓への負担を把握することで慢性腎臓病を引き起こす前に防ぐことが期待されます。 ‘肥満であると糖尿病の前段階でも腎臓に負担がかかっていると判明’. 大阪市立大学. https://www.osaka-cu.ac.jp/ja/news/2018/180914. (参照 2018-09-14)

TSUDA A, et al. (2018). Association of Albuminuria With Intraglomerular Hydrostatic Pressure and Insulin Resistance in Subjects With Impaired Fasting Glucose or Impaired Glucose Tolerance. *Diabetes Care*. 41, 2414-2420. https://doi.org/10.2337/dc18-0718

American Diabetes Association. Diabetes Care

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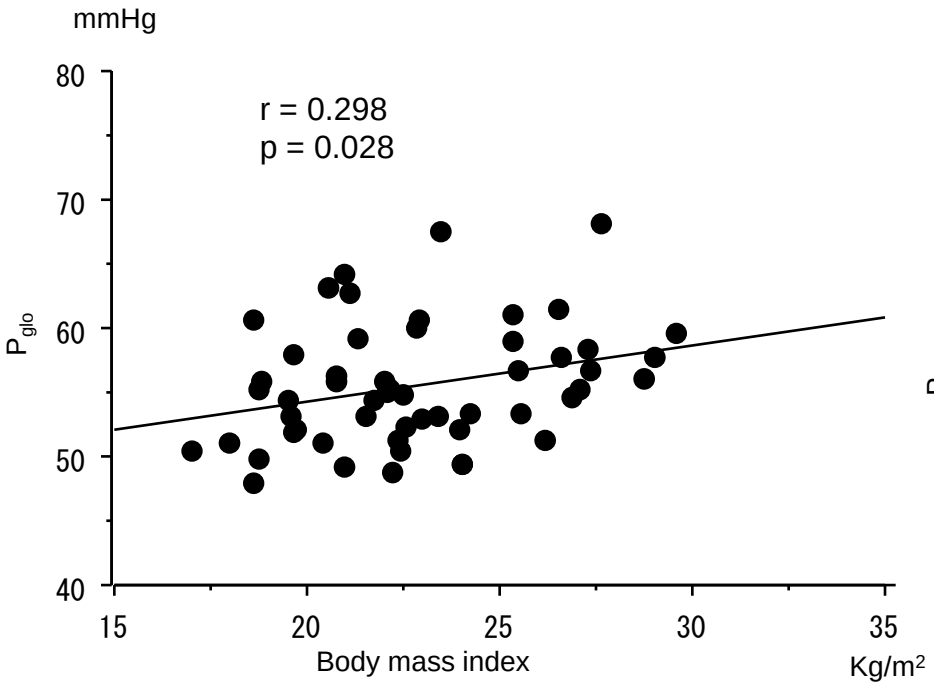
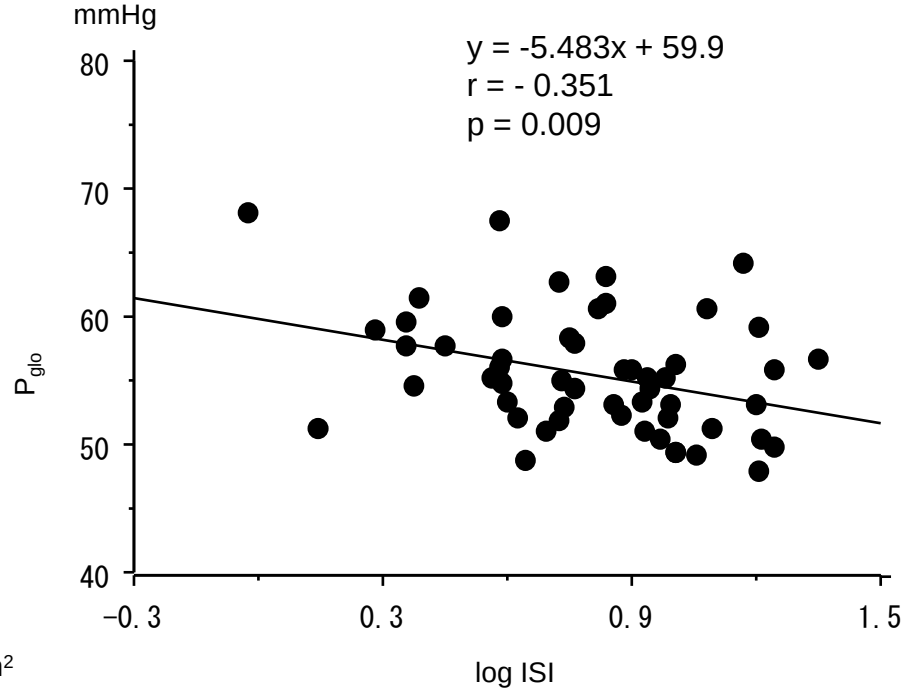
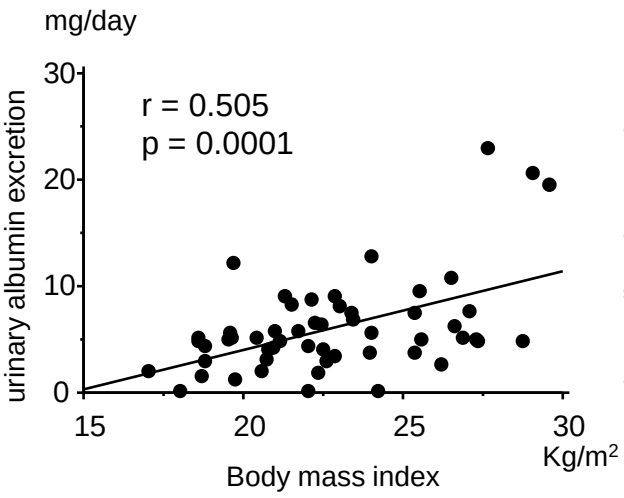
Journal:	*Diabetes Care*
Manuscript ID	DC18-0718.R1
Manuscript Type:	Original Article: Cardiovascular and Metabolic Risk
Date Submitted by the Author:	n/a
Complete List of Authors:	Tsuda, Akihiro; Osaka City University Graduate School of Medicine, Metabolism, Endocrinology and Molecular Medicine Ishimura, Eiji; Osaka City University Graduate School of Medicine, Department of Nephrology Uedono, Hideki; Osaka City University Graduate School of Medicine, Department of Metabolism, Endocrinology and Molecular Medicine Ochi, Akinobu; Osaka City University Graduate School of Medicine, Department of Metabolism, Endocrinology and Molecular Medicine Nakatani, Shinya ; Osaka City University Graduate School of Medicine, Department of Metabolism, Endocrinology and Molecular Medicine Mori, Katsuhito; Osaka City University Graduate School of Med., Dept Metabolism Endocrinology & Molecular Med. Morioka, Tomoaki; Osaka City University Graduate School of Medicine, Metabolism, Endocrinology and Molecular Medicine Uchida, Junji ; Osaka City University Graduate School of Medicine, Department of Urology Emoto, Masanori; Osaka City University Graduate School of Med., Dept Metabolism Endocrinology & Molecular Med. Nakatani, Tatsuya ; Osaka City University Graduate School of Medicine, Department of Urology Inaba, Masaaki; Osaka City University Graduate School of Med., Dept Metabolism Endocrinology & Molecular Med.


mmHg
80
70
60
50
40
P_{glo}
r = 0.298
p = 0.028
15
20
25
30
35
Body mass index
Kg/m²


mmHg
y = -5.483x + 59.9
r = - 0.351
p = 0.009
80
70
60
50
40
P_{glo}
−0. 3
0. 3
0. 9
1. 5
log ISI


mg/day
urinary albumin excretion
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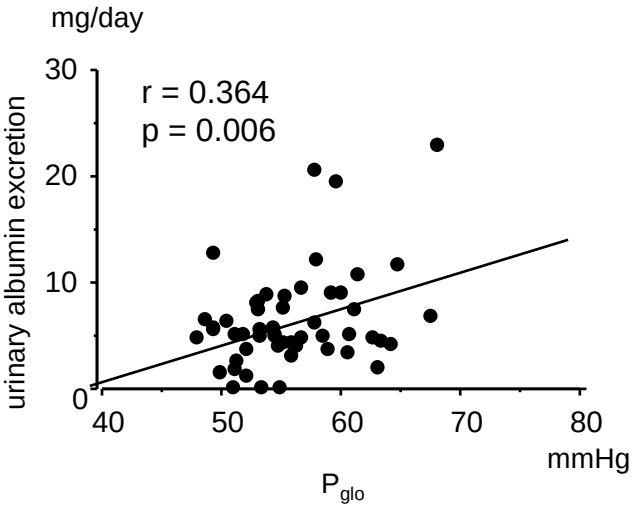
20
10
0
r = 0.505
p = 0.0001
15
20
25
30
Kg/m2
Body mass index


mg/day
urinary albumin excretion
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20
10
0
r = 0.364
p = 0.006
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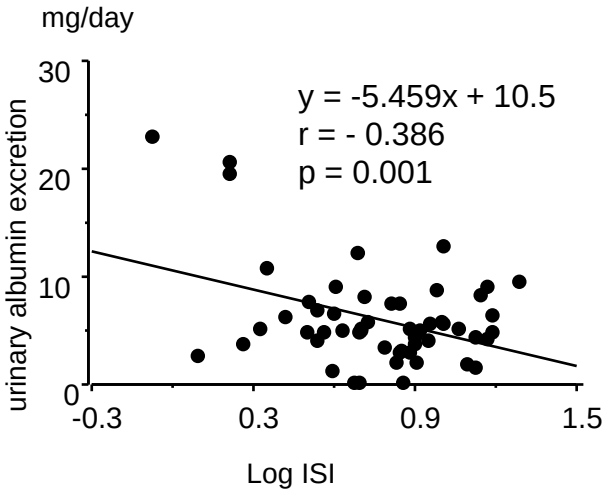
50
60
70
80
mmHg
Pglo


mg/day
urinary albumin excretion
30
20
10
0
y = -5.459x + 10.5
r = - 0.386
p = 0.001
-0.3
0.3
0.9
1.5
Log ISI

Association of albuminuria with intraglomerular hydrostatic pressure and insulin resistance in subjects with impaired fasting glucose and/or impaired glucose tolerance

Short running title: Albuminuria, glomerular hemodynamics, and insulin resistance

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Keywords: insulin resistance, albuminuria, glomerular hemodynamics

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Word count: 3212 words

2 tables and 2 figures

Abstract

Objecive – Little is known about the relationships between insulin resistance, intrarenal hemodynamics, and urinary albumin excretion (UAE) in humans with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). The aim of the present study was to examine intrarenal hemodynamic abnormalities, insulin resistance, and UAE, in subjects with IFG and/or IGT. We hypothesized that intrarenal hemodynamic abnormalities would be associated with insulin resistance.

RESEARCH DESIGN AND METHODS – Fifty-four kidney donors underwent 75 g oral glucose tolerance, and inulin and para-aminohippuric acid clearance testing. Insulin sensitivity index (ISI) was evaluated by the Matsuda Index. Intrarenal hemodynamic parameters were calculated by Gomez's formulae.

RESULTS - Of the 54 subjects, 33 exhibited IFG and/or IGT, and 31 exhibited normal glucose tolerance (NGT). Glomerular hydrostatic pressure (P_{glo}) and UAE were significantly higher in the IFG and/or IGT subjects with obesity (p=0.015 and 0.0001, respectively). Log ISI correlated significantly and negatively with P_{glo} (r= -0.351, p=0.009) in all subjects. In multiple regression analyses among all subjects, log ISI was associated significantly and independently with P_{glo} (β=-0.316, p=0.015), after adjustment for age, gender, and systolic blood pressure. Further, BMI (β=0.517, p=0.0004), P_{glo} (β=0.420, p=0.004) and log ISI (β= -0.366, p=0.008) were each associated significantly and independently with UAE, after adjustment.

CONCLUSIONS – We demonstrated that increased insulin resistance is associated with increased P_{glo} and UAE in IFG and/or IGT subjects. These hemodynamic burdens and insulin resistance may cause injury to the glomeruli even in subjects with IFG and/or IGT.

It has been reported that the development and progression of diabetic nephropathy are associated with glomerular hypertension and hyperfiltration in both type 1 and type 2 diabetes patients (1; 2). Glomerular hyperfiltration in diabetic patients contributes to the onset of nephropathy, its progression, and loss of renal function (3; 4). Increased albuminuria is associated with obesity and diabetes, and is a risk factor for cardiovascular and renal diseases (5; 6). Further, albuminuria in the high normal range (10–30 μg/mg) has been identified as a risk factor for cardiovascular disease (7). Recently, several studies have shown that sodium glucose cotransporter (SGLT) 2 inhibitors, which have been demonstrated to reduce glomerular hypertension, slow the progression of decline of the estimated glomerular filtration rate, and that they decrease albuminuria in patients with type 2 diabetes (8; 9). Obese and type 2 diabetes patients are well known to exhibit insulin resistance (10). These data suggest that there may be relationships between glomerular hypertension, insulin resistance, and urinary albumin excretion (UAE).

However, as little is known about the precise intrarenal hemodynamic abnormalities in subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), the relationships between glomerular hypertension, insulin resistance, and urinary albumin excretion remain to be elucidated. Glomerular hemodynamics can be examined using Gomez's formula (11; 12), in which both inulin and para-aminohippuric acid clearance (C_{in} and C_{PAH}, respectively) are measured simultaneously. We recently reported a significant association between poor glycemic control and increased efferent arteriolar resistance in diabetic patients using Gomez's formula (13).

Thus, we hypothesized that intrarenal hemodynamic abnormalities would be

associated with increased urinary albumin excretion (UAE) and insulin resistance in pre-diabetic subjects. The aim of the present study was to evaluate intrarenal hemodynamic abnormalities by measuring C_{in} and C_{PAH} in subjects with normal glucose tolerance (NGT) and in those with IFG and/or IGT. We further investigated the relationships between intrarenal hemodynamic abnormalities, insulin resistance, and urinary albumin excretion in these subjects.

RESEARCH DESIGN AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (#3955). Kidney donor candidates were admitted to Osaka City University Hospital between January 2006 and March 2017 for the evaluation of suitability for transplantation. A total of 54 subjects were enrolled consecutively after providing written informed consent. Two-hour 75-g oral glucose tolerance test (75g-OGTT), C_{in}, and C_{PAH} were determined in all subjects. None of the subjects were under treatment with medication, including anti-hypertensives, i.e., angiotensin-converting-enzyme inhibitors and/or angiotensin Π receptor blockers.

Body mass index (BMI) was calculated as the body weight (BW) in kilograms divided by the square of the height (Ht) in meters; BMI = BW (kg)/Ht $(m)^2$. Obesity was defined as BMI > 25, according to the Japan Society for the Study of Obesity, which has defined obesity among Japanese subjects based upon a number of epidemiological studies (14). Subjects were divided into 4 groups; NGT subjects without obesity (group 1), NGT with obesity (group 2), IFG and/or IGT without obesity

(group 3, and IFG and/or IGT with obesity (group 4).

Measurements of C_{in} and C_{PAH}, and calculation of intrarenal hemodynamic parameters

Glomerular filtration rate (GFR) and renal plasma flow (RPF), as measured by C_{in} and C_{PAH}, respectively, were determined simultaneously by the input clearance technique (13; 15-17), using constant infusion of 1% inulin (Inulead®, Fuji Yakuhin Co. Ltd., Saitama, Japan) and 0.5% para-aminohypuric acid (PAH) (Sodium p-Aminohippurate®, Daiichi-Sankyo Co. Ltd., Tokyo, Japan), respectively. Inulin concentrations were measured enzymatically (16). PAH concentrations were measured photometrically, by means of the N-1 naphthylethylenediamine and the anthrone method using a Corning 258 spectrophotometer (18). Since the direct measurement of glomerular hemodynamics parameters is not feasible in humans, the formulae introduced by Gomez (11) allows indirect assessment of glomerular hemodynamics, as reported previously by others (12; 19; 20) and ourselves (13; 15; 21; 22). The Gomez formulae were calculated from the original report (11), as described in detail in our previous studies (13; 15; 21; 22). In the formulae, GFR (C_{in}), afferent arteriolar resistance (R_a), efferent arteriolar resistance (R_e), and glomerular hydrostatic pressure (P_{glo}) are calculated. Filtration fraction was calculated by dividing the GFR (C_{in}) by the RPF (C_{PAH}). According to Gomez's formulae, in which inulin clearance greater than 60 ml/min could be applied (11), we excluded those with inulin clearance values that were less than 60 ml/min (n = 5) from further analyses.

The Gomez formulae were according to the original paper, as follows:

$\Delta P_F = GFR/K_{FG}$

$P_{glo} = \Delta P_F + P_{Bow} + \pi G$

$\pi G = 5 \cdot (C_M - 2)$

$C_M = TP/FF \cdot \ln (1/(1-FF))$

In the above formulae, ΔP_F was the filtration pressure across the glomerular capillary. K_{FG} (the gross filtration coefficient) was estimated as 0.0406 mL/sec · mm Hg per kidney. P_{Bow} (the hydrostatic pressure in Bowman's space) was estimated as 10 mm Hg; πG (the oncotic pressure within the glomerular capillaries) was obtained from the C_M (plasma protein concentration within the glomerular capillaries), and calculated from the TP (total protein concentration) and filtration fraction (FF).

From Ohm's law:

$R_a = ((MBP-P_{glo})/RBF) \cdot 1328$

$R_e = (GFR/K_{FG} \cdot (RBF-GFR)) \cdot 1328$

RBF can be calculated from the RPF and hematocrit (Ht) using the standard formula:

$RBF = RPF/(1-Ht)$

In the above, the conversion factor to dyne · sec · cm^{-5} is 1328; GFR (glomerular filtration rate), RPF (renal plasma flow), and RBF (renal blood flow) are expressed in mL/sec; and the mean blood pressure (MBP) is calculated as (2 x diastolic BP + systolic BP)/3.

Oral glucose tolerance test and insulin sensitivity index

Two-hour 75-g OGTT was performed in the morning after an overnight fast. Blood was collected *via* an intravenous catheter before and 30, 60 and 120 min after glucose ingestion, for measurement of plasma glucose and insulin levels.

According to the American Diabetes Association, the diagnosis of prediabetes (IGT and/or IFG) is made on the basis of one of the following clinical biochemistry criteria: 1) 2-h plasma glucose of 140–199 mg/dL during OGTT for IGT; *2*) fasting plasma glucose of 100–125 mg/dL for IFG (23; 24).

For the assessment of insulin resistance, the insulin sensitivity index (ISI) was evaluated by the Matsuda Index, and calculated from the pre- and 120-min data from the 75-g OGTT (25), according to the following formula:

$$ISI = 10000/[\ (FPG * FPI) * (\bar{G} * \bar{I}\ )\ ]^{\wedge 0.5}$$

FPG; fasting plasma glucose, FPI; fasting plasma insulin, $\bar{G}$; mean plasma glucose concentration during 75g-OGTT; $\bar{I}$; mean plasma insulin concentration during the 75g-OGTT.

Biochemical and Physiological Parameters

Blood and urine samples were obtained after overnight fasting. Plasma glucose levels were measured with the glucose oxidase method. Plasma insulin and urinary albumin were determined by electrochemical luminescence immunoassay (Roche Co., Tokyo, Japan) and turbidimetric immunoassay (Wako Co., Tokyo, Japan), respectively.

Statistical methods

The results are expressed as the mean ± standard deviation (SD). Multiple comparisons of the differences of the characteristics between each of the groups (group 1: NGT subjects without obesity, group 2: NGT subjects with obesity, group 3: IFC and/or IGT subjects without obesity, and group 4: IFC and/or IGT subjects with obesity)

were evaluated by two-way ANOVA and Scheffé's multiple means comparisons. Correlations between variables were examined using Pearson's correlation coefficient. Multiple regression analyses were performed to examine the relationships between P_{glo} and the clinical parameters, and between urinary albumin excretion and the clinical parameters. All analyses were performed using Stat View 5 (SAS Institute Inc., Cary, NC, USA) for Windows. The level of statistical significance was set at $p < 0.05$.

Results

The characteristics of the 54 subjects examined in the present study are presented in Table 1. The patients were 56.2 ± 12.0 years, and 29 (51.9 %) were male. As shown in Table 1, of the 54 subjects who underwent 75g-OGTT, 26 exhibited normal glucose tolerance (NGT) without obesity (group 1), 5 exhibited NGT with obesity (group 2), 12 exhibited IFG and/or IGT without obesity (group 3), and 11 exhibited IFG and/or IGT with obesity (group 4).

P_{glo} ($p = 0.0015$) , urinary albumin excretion ($p = 0.0001$) and Filtration fraction (FF) ($p = 0.016$) were significantly higher in the IFG and/or IGT subjects with obesity (group 4) than in the other groups. R_e in the IFC and/or IGT subjects with obesity (group4) tended to be higher than in the other groups (FF; $p = 0.088$, R_e; $p = 0.066$). ISI in subjects with obesity (group 2 and group 4) was significantly lower than in those without obesity ($p < 0.0001$, *vs.* group 1 and group 3). It is well known that insulin resistance is related to obesity (26; 27). When we examined the association between BMI and ISI, log ISI was associated significantly and negatively with BMI ($r = - 0.593$, $p< 0.0001$). As shown in Figure 1, P_{glo} correlated significantly and positively with BMI ($r = 0.298$, $p = 0.028$), and correlated significantly and negatively with the log ISI (r = -

0.351, p = 0.009). There were no significant relationships between the logISI and the following parameters; GFR (r = 0.067, p = 0.628), RPF (r = 0.149, p = 0.283), R_a (r = 0.023, p = 0.867), R_e (r = 0.103, p = 0.460), and FF (r = 0.094, p = 0.498). Since there was a significant association between BMI and log ISI , we performed multiple regression analyses, in which BMI and log ISI were entered as independent variables. In these analyses, BMI tended to be associated with P_{glo} (β = 0.269, p = 0.064; Table 2, Model 1), and the log ISI was associated significantly and independently with P_{glo} (β = - 0.316, p = 0.015; Table 2, Model 2), after adjustment for age, gender, and systolic blood pressure.

Next, we examined the associations between urinary albumin excretion and several factors. As shown in Figure 2, BMI (r = 0.505, p = 0.0001) and P_{glo} (r = 0.364, p = 0.006) correlated significantly and positively, and log ISI (r = - 0.386, p = 0.001) correlated significantly and negatively with urinary albumin excretion. In order to analyze the factors associated with urinary albumin excretion, multiple regression analyses were performed after adjustment for age, gender, and systolic blood pressure. Since P_{glo} was associated significantly with BMI and log ISI (Figure 1), multiple regression analyses were performed in which BMI, P_{glo}, and log ISI were entered as independent variables. As shown in Model 3, Model 4, and Model 5 in Table 2, BMI (β = 0.517, p = 0.0004), P_{glo} (β = 0.420, p = 0.004), and log ISI (β = - 0.366, p = 0.008) were each significantly and independently associated with urinary albumin excretion, after these adjustments.

Conclusion

In the present study, we examined C_{in} and C_{PAH}, and calculated the intrarenal

hemodynamic parameters in kidney donor candidates using Gomez formulae, as described in detail in our previous studies (13; 21). In all subjects, GFR, as measured by C_{in}, was greater than 60 mL/min, and urinary albumin excretion was less than 30 mg/day, *i.e.*, within the normal range. Glucose metabolism pattern was evaluated by 2-hour 75-g OGTT in all subjects, and insulin resistance was evaluated by calculation of the insulin sensitivity index (Matsuda Index) (25). In this study, we demonstrated that P_{glo} and the degree of urinary albumin excretion, even within a normal range (13.7 ± 8.4 mg/day), were significantly higher in obese IFG and/or IGT patients (group 4) compared with the other groups. We also demonstrated that the R_e and FF in the obese IFG and/or IGT subjects (group 4) tended to be higher than in the other groups. Further, we demonstrated that log ISI and BMI were associated significantly with P_{glo}, and that ISI, BMI and P_{glo} were associated significantly with urinary albumin excretion. These results indicate that lower ISI, *i.e.*, higher insulin resistance, even in IFG and/or IGT subjects, is associated with increased P_{glo}, and suggest that increased P_{glo} may, in turn, be associated with increased urinary albumin excretion, even within the normal range. BMI was associated with insulin resistance in the present study, which has been well established (28). Thus, the associated increased insulin resistance in IFG and/or IGT subjects likely increases the burden on the glomeruli, and thus, affecting deterioration of the glomeruli in prediabetic patients.

Increased GFR has been reported previously in diabetic nephropathy (1; 29). Glomerular hyperfiltration is observed in the early stages of most patients with diabetes mellitus, and is considered to precede the development of microalbuminuria by several years (1; 30). In animals, the increase in GFR in diabetes is caused by imbalances of afferent and efferent arteriolar tone with a disproportionate decrease in afferent

arteriolar resistance and relatively higher efferent arteriolar tone, leading to increases in glomerular capillary pressure (30). However, imbalances between afferent and efferent arteriolar resistance have not been demonstrated clinically in the early stages in humans with diabetes mellitus. We recently demonstrated that poor glycemic control is associated significantly with glomerular hemodynamic abnormalities in humans (13). In our previous study, we reported that poor glycemic control increased the filtration fraction, P_{glo}, and R_e, but not R_a (13). While all of these experimental and clinical studies have examined diabetic patients or diabetic experimental animals, to date, there has been no report in which renal hemodynamic abnormalities were evaluated by C_{in} and C_{PAH} with respect to the relationship with insulin resistance in non-diabetic human subjects. Further, there have been no reports in which urinary albumin excretion was examined in relation to glomerular hemodynamics and insulin resistance in these subjects. Thus, this is the first study to demonstrate that insulin resistance is associated significantly with glomerular hemodynamic changes and urinary albumin excretion in IFG and/or IGT subjects.

Based upon the current findings, the mechanism underlying increased P_{glo}, under the status of insulin resistance remains unknown. Evidence suggests that insulin resistance and obesity could activate the intrarenal renin angiotensin system (RAS) (2). RAS activation increases efferent arteriolar resistance, leading to glomerular hypertension and hyperfiltration (1; 31). From the results of the present study, we consider that intrarenal RAS may be activated, even in subjects with IFG and/or IGT in obesity. Concerning P_{glo}, in a model in which both BMI and log ISI were entered simultaneously as independent variables, neither BMI nor log ISI were significantly, independently associated with P_{glo} (data not shown). We consider that the results of

these additional analyses could indicate that there is a high level of confounding between BMI and log ISI, leading to the non-significant associations between the two variables and P_{glo}.

On the other hand, it is well known that urinary albumin excretion is caused by glomerular hypertension induced by poor glycemic control and/or obesity in animal studies (32). It has been reported that urinary albumin excretion was preceded by glomerular hypertension under high glucose, through increased shear stress (33). Nakagawa *et al.* reported that the reduction of nitric oxide synthesis from endothelial cells in the glomeruli, as a consequence of insulin resistance, induces continuous increases in renal vascular endothelial growth factor (VEGF) expression and marked macrophage infiltration in an animal model (34). Furthermore, in humans, Pistrosch *et al.* reported that an insulin-sensitizing drug, rosiglitazone, ameliorated glomerular hyperfiltration and reduced urinary albumin excretion in patients with early type 2 diabetes with microalbuminuria (35). Denic *et al.* demonstrated that obesity was associated with a higher single-nephron GFR in chronic kidney disease among otherwise healthy adult kidney donor candidates (36). Bjornstad *et al.* demonstrated relationships between whole-body, central adiposity, and intrarenal hemodynamic function in adults with long-standing type 1 diabetes (37). We consider that mechanisms similar to those described above may be underlying the increase in UAE in the present study, in which IFG and/or IGT subjects were examined.

Concerning urinary albumin excretion (UAE), additional analyses, in which two or three measures, out of BMI, P_{glo}, and log ISI, were included simultaneously as independent variables, BMI and P_{glo} were independently, significantly associated with UAE (data not shown). However, log ISI was not associated significantly with UAE

(data not shown). These results may indicate that BMI, which is very strongly associated with log ISI, may be a stronger factor that is associated with UAE. We consider that obesity itself may affect the increased UAE. However, the analyses presented in Table 1 demonstrate that, among four groups, only group 4 (IFG and/or IGT with obesity) showed significantly higher P_{glo} and UAE. We consider that the result may indicates that the presence of IFG and/or IGT and obesity cause significantly increased P_{glo} and UAE. Thus, we consider that a significant increase in P_{glo} is caused by the presence of both increased log ISI (as represented by IFG and/or IGT) and increased BMI. In the present study, we demonstrated, for the first time, that increased P_{glo} is associated with increased UAE in human; and that obesity is also significantly associated with UAE, as has been reported previously by others (38; 39). The reason obesity is associated with increased UAE remains unknown from the results of present study. We consider that the presence of both insulin resistance and obesity may cause increased P_{glo}, which may be followed by increased UAE.

In the current report, we showed that P_{glo}, insulin resistance, and BMI were associated significantly with urinary albumin excretion in IFG and/or IGT subjects, even within the normal range. Some reports have shown that higher levels of urinary albumin excretion, even within the normal range, predict the decline in glomerular filtration rate in diabetic patients (40), as well as the development of cardiovascular disease (41), and coronary heart disease (42). These studies and the results of the present study may support the need to redefine the range of microalbuminuria, even at the levels currently considered to be normal. The current findings suggest that albuminuria, even at levels currently considered to be within the normal range, may be increased as a consequence of insulin resistance in subjects with IFG and/or IGT, likely

through increasing the intraglomerular hydrostatic pressure.

There are some limitations to the present study. First, the study was performed in a small number of Japanese subjects, and a large-scale study is needed to confirm the relationship between urinary albumin excretion, insulin resistance, and glomerular hemodynamic abnormalities in IFG and/or IGT subjects. Secondly, this is a cross sectional study. Further studies may be needed to explore the consequence of insulin resistance on glomerular hypertension and urinary albumin excretion. However, this is the first study in which insulin resistance was associated significantly with P_{glo} and urinary albumin excretion in human subjects with IFG and/or IGT. Thirdly, we were not able to directly measure R_a, R_e, and P_{glo} in the present study, as it is not possible to directly measure these parameters in humans; compared with animal studies. Thus, we used Gomez's formulae to assess intrarenal hemodynamics, as in the previous studies reported by others (12; 19; 20; 43) and by ourselves (13; 15; 21; 22).

In conclusion, in the present study, by measuring C_{in} and C_{PAH}, we showed that increased insulin resistance is associated significantly with increased P_{glo} and urinary albumin excretion, even at levels currently considered to be within the normal range in human subjects with IFG and/or IGT. These hemodynamic burdens may lead to glomerular injury in IFG and/or IGT subjects. We also suggest that the clinically significant levels of microalbuminuria, as currently defined, could be redefined based upon several human studies, including the present study.

Acknowledgement

A.T. and E.I. performed the research, acquired the data and wrote the manuscript. A.T., E.I., H.U., A.O.,S.N., T.M.,K.M., J.U., M.E., T.N., and M.I. contributed to the

discussion, and reviewed the manuscript. A.T. and E.I. had full access to all of the study data and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the reported research. This study was not funded by any grant from a funding agency in the public, commercial, or not-for-profit sector.

This study was supported by Grant from the Osaka Kidney Foundation (OKF18-0019).

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Table 1 Clinical characteristics of the 54 subjects.

	NGT		IFG and/or IGT		
	Without obesity (group 1)	With obesity (group 2)	Without obesity (group 3)	With obesity (group 4)	p value
N (54)	26	5	12	11	---
Age (years)	55.5 ± 13.1	61.0 ± 12.7	56.3 ± 11.9	55.5 ± 3.1	n.s.
Gender (male/female)	11/15	4/1	3/9	6/3	0.037 #1a, 0.021 #1b
Body mass index (kg/m^2)	21.0 ± 1.8	26.2 ± 1.8	21.5 ± 2.1	27.1 ± 1.3	<0.0001 #2
Mean arterial pressure (mmHg)	87.9 ± 13.3	87.3 ± 10.3	83.0 ± 12.3	95.3 ± 14.0	0.033 #3
Systolic blood pressure (mmHg)	122.3 ± 19.3	117.8 ± 12.9	117.9 ± 16.5	128.6 ± 14.2	n.s.
Diastolic blood pressure (mmHg)	70.9 ± 12.3	71.4 ± 8.7	67.3 ± 11.6	78.6 ± 14.3	0.033 #3
Blood urea nitrogen (mg/dL)	13.2 ± 3.9	12.4 ± 1.1	13.3 ± 2.9	12.7 ± 1.7	n.s.
Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.01 #4
Estimated GFR (mL/min/1.73m^2)	78.7 ± 11.9	70.9 ± 5.3	81.1 ± 16.4	74.0 ± 11.7	n.s.
GFR (C_{in})(mL/min)	80.4 ± 18.9	78.3 ± 9.3	85.8 ± 25.5	88.2 ± 18.9	n.s
GFR (C_{in})(mL/min/1.73m^2)	89.2 ± 18.7	81.3 ± 10.8	95.5 ± 25.4	88.9 ± 18.6	n.s.
RPF (C_{PAH}) (mL/min)	380.9 ± 118.0	371.2 ± 71.8	402.1 ± 124.4	362.5 ± 74.2	n.s.
Urinary albumin excretion (mg/day)	5.2 ± 2.8	6.0 ± 2.0	4.4 ± 3.6	10.1 ± 7.4	0.0001 #5
filtration fraction (FF) (%)	0.22 ± 0.03	0.21 ± 0.03	0.22 ± 0.04	0.25 ± 0.04	0.016 #6
glomerular hydrostatic pressure (P_{glo}) (mmHg)	54.3±4.5	54.8 ± 3.5	55.9 ± 5.0	58.4 ± 4.4	0.015 #7
afferent arteriole resistance (R_a) (dyne□sec□cm^{-5})	4629.3 ± 2233.2	4267.0 ± 1536.8	3648.0 ± 2532.4	5001.0 ± 2441.8	n.s.
efferent arteriole resistance (R_e) (dyne□sec□cm^{-5})	2440.5 ± 366.5	2376.2 ± 491.9	2476.9 ± 547.9	2783.1 ± 723.0	0.066 #8
fasting plasma glucose (mg/dL)	89 ± 6	90 ± 3	100 ± 8	104 ± 12	0.007 #9
hemoglobin A1c (%)	5.6 ± 0.3	5.5 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	n.s.
2 hour plasma glucose in 75-g OGTT	102 ± 20	104 ± 22	150 ± 41	139 ± 35	<0.0001 #10
Log insulin sensitivity index (the MatsudaIndex)	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.4 ± 0.2	<0.0001 #11

NGT: normal glucose tolerance, IFG: impared fasting glucose, IGT: impared glucose tolerance, GFR : glomerular filtration rate, C_{in} : inulin clearance, RPF : renal plasma flow, C_{PAH} : para-aminohippuric acid clearance, P values were evaluated by two-way ANOVA and Scheffé multiple means comparisons. #1a, p = 0.037 (group1 *vs*. group2); #1b p = 0.021 (group 2 *vs*. group3); #2, p <0.0001 (group 1 and 3 *vs*. group2 and 4); #3, p = 0.033 (group3 *vs*. group4), #4. P = 0.01 (group1 and group3 *vs.* group 4), #5, p = 0.0001 (group 1, 2, and 3 *vs*. group 4), #6, p = 0.016 (group 1 *vs*. group 4), #7, p = 0.015 (group 1 *vs*. group 4), #8, p = 0.066 (group 1 *vs*. group 4), #9 p = 0.007 (group 1 and 2 *vs*. group 3 and 4), #10, p < 0.0001 (group 1 and 2 *vs*. group 3 and 4), #11, p < 0.0001 (group 1, 2, and 3 *vs*. group 4)

Table 2. Factors associated with the glomerular hydrostatic pressure (P_{glo}) and urinary albumin excretion.

	P_{glo}					urinary albumin excerection					
	Model 1		Model 2			Model 3		Model 4		Model 5	
	β	p	β	p		β	p	β	p	β	p
age (years)	-0.357	0.010	-0.327	0.015	age (years)	0.061	0.633	0.222	0.124	0.103	0.452
gender (male = 0, female =1)	-0.083	0.565	-0.188	0.146	gender (male = 0, female =1)	- 0.004	0.974	- 0.137	0.314	- 0.211	0.119
systolic blood pressure (mmHg)	0.143	0.299	0.108	0.427	systolic blood pressure (mmHg)	- 0.103	0.436	- 0.124	0.375	- 0.124	0.380
body mass index (kg/m^2)	0.269	0.064	---	---	body mass index (kg/m^2)	0.517	0.0004	---	---	---	---
log ISI	---	---	- 0.316	0.015	P_{glo}	---	---	0.420	0.004	---	---
					log ISI	---	---	---	---	-0.366	0.008
R^2/p	0.357/0.010		0.327/0.015		R^2/p	0.265/0.004		0.201/0.024		0.181/0.041	

P_{glo} ; glomerular hydrostatic pressure, ISI ; insulin sensitivity index (the Matsuda index)

Figure legends

Figure 1. Relationship between glomerular hydrostatic pressure (P_{glo}) and body mass index, and between P_{glo} and insulin sensitivity index (ISI). There was a significant and positive correlation between body mass index and P_{glo}, and a significant and negative correlation between log ISI and P_{glo}.

Figure 2. Relationship between urinary albumin excretion and body mass index, insulin sensitivity index (ISI), and glomerular hydrostatic pressure (P_{glo}). There were significant and positive correlations between body mass index and urinary albumin excretion and between P_{glo} and urinary albumin excretion. There was a significant and negative correlation between log ISI and urinary albumin excretion.

Association of albuminuria with intraglomerular hydrostatic pressure and insulin resistance in subjects with impaired fasting glucose and/or impaired glucose tolerance

Short running title: Albuminuria, glomerular hemodynamics, and insulin resistance

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Keywords: insulin resistance, albuminuria, glomerular hemodynamics

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Word count: 3212 words

2 tables and 2 figures

Abstract

Objecive – Little is known about the relationships between insulin resistance, intrarenal hemodynamics, and urinary albumin excretion (UAE) in humans with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). The aim of the present study was to examine intrarenal hemodynamic abnormalities, insulin resistance, and UAE, in subjects with IFG and/or IGT. We hypothesized that intrarenal hemodynamic abnormalities would be associated with insulin resistance.

RESEARCH DESIGN AND METHODS – Fifty-four kidney donors underwent 75 g oral glucose tolerance, and inulin and para-aminohippuric acid clearance testing. Insulin sensitivity index (ISI) was evaluated by the Matsuda Index. Intrarenal hemodynamic parameters were calculated by Gomez's formulae.

RESULTS - Of the 54 subjects, 33 exhibited IFG and/or IGT, and 31 exhibited normal glucose tolerance (NGT). Glomerular hydrostatic pressure (P_{glo}) and UAE were significantly higher in the IFG and/or IGT subjects with obesity (p=0.015 and 0.0001, respectively). Log ISI correlated significantly and negatively with P_{glo} (r= -0.351, p=0.009) in all subjects. In multiple regression analyses among all subjects, log ISI was associated significantly and independently with P_{glo} (β=-0.316, p=0.015), after adjustment for age, gender, and systolic blood pressure. Further, BMI (β=0.517, p=0.0004), P_{glo} (β=0.420, p=0.004) and log ISI (β= -0.366, p=0.008) were each associated significantly and independently with UAE, after adjustment.

CONCLUSIONS – We demonstrated that increased insulin resistance is associated with increased P_{glo} and UAE in IFG and/or IGT subjects. These hemodynamic burdens and insulin resistance may cause injury to the glomeruli even in subjects with IFG and/or IGT.

It has been reported that the development and progression of diabetic nephropathy are associated with glomerular hypertension and hyperfiltration in both type 1 and type 2 diabetes patients (1; 2). Glomerular hyperfiltration in diabetic patients contributes to the onset of nephropathy, its progression, and loss of renal function (3; 4). Increased albuminuria is associated with obesity and diabetes, and is a risk factor for cardiovascular and renal diseases (5; 6). Further, albuminuria in the high normal range (10–30 μg/mg) has been identified as a risk factor for cardiovascular disease (7). Recently, several studies have shown that sodium glucose cotransporter (SGLT) 2 inhibitors, which have been demonstrated to reduce glomerular hypertension, slow the progression of decline of the estimated glomerular filtration rate, and that they decrease albuminuria in patients with type 2 diabetes (8; 9). Obese and type 2 diabetes patients are well known to exhibit insulin resistance (10). These data suggest that there may be relationships between glomerular hypertension, insulin resistance, and urinary albumin excretion (UAE).

However, as little is known about the precise intrarenal hemodynamic abnormalities in subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), the relationships between glomerular hypertension, insulin resistance, and urinary albumin excretion remain to be elucidated. Glomerular hemodynamics can be examined using Gomez's formula (11; 12), in which both inulin and para-aminohippuric acid clearance (C_{in} and C_{PAH}, respectively) are measured simultaneously. We recently reported a significant association between poor glycemic control and increased efferent arteriolar resistance in diabetic patients using Gomez's formula (13).

Thus, we hypothesized that intrarenal hemodynamic abnormalities would be

associated with increased urinary albumin excretion (UAE) and insulin resistance in pre-diabetic subjects. The aim of the present study was to evaluate intrarenal hemodynamic abnormalities by measuring C_{in} and C_{PAH} in subjects with normal glucose tolerance (NGT) and in those with IFG and/or IGT. We further investigated the relationships between intrarenal hemodynamic abnormalities, insulin resistance, and urinary albumin excretion in these subjects.

RESEARCH DESIGN AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (#3955). Kidney donor candidates were admitted to Osaka City University Hospital between January 2006 and March 2017 for the evaluation of suitability for transplantation. A total of 54 subjects were enrolled consecutively after providing written informed consent. Two-hour 75-g oral glucose tolerance test (75g-OGTT), C_{in}, and C_{PAH} were determined in all subjects. None of the subjects were under treatment with medication, including anti-hypertensives, i.e., angiotensin-converting-enzyme inhibitors and/or angiotensin Π receptor blockers.

Body mass index (BMI) was calculated as the body weight (BW) in kilograms divided by the square of the height (Ht) in meters; BMI = BW (kg)/Ht $(m)^2$. Obesity was defined as BMI > 25, according to the Japan Society for the Study of Obesity, which has defined obesity among Japanese subjects based upon a number of epidemiological studies (14). Subjects were divided into 4 groups; NGT subjects without obesity (group 1), NGT with obesity (group 2), IFG and/or IGT without obesity

(group 3, and IFG and/or IGT with obesity (group 4).

Measurements of C_{in} and C_{PAH}, and calculation of intrarenal hemodynamic parameters

Glomerular filtration rate (GFR) and renal plasma flow (RPF), as measured by C_{in} and C_{PAH}, respectively, were determined simultaneously by the input clearance technique (13; 15-17), using constant infusion of 1% inulin (Inulead®, Fuji Yakuhin Co. Ltd., Saitama, Japan) and 0.5% para-aminohypuric acid (PAH) (Sodium p-Aminohippurate®, Daiichi-Sankyo Co. Ltd., Tokyo, Japan), respectively. Inulin concentrations were measured enzymatically (16). PAH concentrations were measured photometrically, by means of the N-1 naphthylethylenediamine and the anthrone method using a Corning 258 spectrophotometer (18). Since the direct measurement of glomerular hemodynamics parameters is not feasible in humans, the formulae introduced by Gomez (11) allows indirect assessment of glomerular hemodynamics, as reported previously by others (12; 19; 20) and ourselves (13; 15; 21; 22). The Gomez formulae were calculated from the original report (11), as described in detail in our previous studies (13; 15; 21; 22). In the formulae, GFR (C_{in}), afferent arteriolar resistance (R_a), efferent arteriolar resistance (R_e), and glomerular hydrostatic pressure (P_{glo}) are calculated. Filtration fraction was calculated by dividing the GFR (C_{in}) by the RPF (C_{PAH}). According to Gomez's formulae, in which inulin clearance greater than 60 ml/min could be applied (11), we excluded those with inulin clearance values that were less than 60 ml/min (n = 5) from further analyses.

The Gomez formulae were according to the original paper, as follows:

$\Delta P_F = GFR/K_{FG}$

$P_{glo} = \Delta P_F + P_{Bow} + \pi G$

$\pi G = 5 \cdot (C_M - 2)$

$C_M = TP/FF \cdot \ln (1/(1-FF))$

In the above formulae, ΔP_F was the filtration pressure across the glomerular capillary. K_{FG} (the gross filtration coefficient) was estimated as 0.0406 mL/sec · mm Hg per kidney. P_{Bow} (the hydrostatic pressure in Bowman's space) was estimated as 10 mm Hg; πG (the oncotic pressure within the glomerular capillaries) was obtained from the C_M (plasma protein concentration within the glomerular capillaries), and calculated from the TP (total protein concentration) and filtration fraction (FF).

From Ohm's law:

$R_a = ((MBP-P_{glo})/RBF) \cdot 1328$

$R_e = (GFR/K_{FG} \cdot (RBF-GFR)) \cdot 1328$

RBF can be calculated from the RPF and hematocrit (Ht) using the standard formula:

$RBF = RPF/(1-Ht)$

In the above, the conversion factor to dyne · sec · cm^{-5} is 1328; GFR (glomerular filtration rate), RPF (renal plasma flow), and RBF (renal blood flow) are expressed in mL/sec; and the mean blood pressure (MBP) is calculated as (2 x diastolic BP + systolic BP)/3.

Oral glucose tolerance test and insulin sensitivity index

Two-hour 75-g OGTT was performed in the morning after an overnight fast. Blood was collected *via* an intravenous catheter before and 30, 60 and 120 min after glucose ingestion, for measurement of plasma glucose and insulin levels.

According to the American Diabetes Association, the diagnosis of prediabetes (IGT and/or IFG) is made on the basis of one of the following clinical biochemistry criteria: 1) 2-h plasma glucose of 140–199 mg/dL during OGTT for IGT; *2*) fasting plasma glucose of 100–125 mg/dL for IFG (23; 24).

For the assessment of insulin resistance, the insulin sensitivity index (ISI) was evaluated by the Matsuda Index, and calculated from the pre- and 120-min data from the 75-g OGTT (25), according to the following formula:

$$ISI = 10000/[\ (FPG * FPI) * (\bar{G} * \bar{I}\ )\ ]^{\wedge 0.5}$$

FPG; fasting plasma glucose, FPI; fasting plasma insulin, $\bar{G}$; mean plasma glucose concentration during 75g-OGTT; $\bar{I}$; mean plasma insulin concentration during the 75g-OGTT.

Biochemical and Physiological Parameters

Blood and urine samples were obtained after overnight fasting. Plasma glucose levels were measured with the glucose oxidase method. Plasma insulin and urinary albumin were determined by electrochemical luminescence immunoassay (Roche Co., Tokyo, Japan) and turbidimetric immunoassay (Wako Co., Tokyo, Japan), respectively.

Statistical methods

The results are expressed as the mean ± standard deviation (SD). Multiple comparisons of the differences of the characteristics between each of the groups (group 1: NGT subjects without obesity, group 2: NGT subjects with obesity, group 3: IFC and/or IGT subjects without obesity, and group 4: IFC and/or IGT subjects with obesity)

were evaluated by two-way ANOVA and Scheffé's multiple means comparisons. Correlations between variables were examined using Pearson's correlation coefficient. Multiple regression analyses were performed to examine the relationships between P_{glo} and the clinical parameters, and between urinary albumin excretion and the clinical parameters. All analyses were performed using Stat View 5 (SAS Institute Inc., Cary, NC, USA) for Windows. The level of statistical significance was set at $p < 0.05$.

Results

The characteristics of the 54 subjects examined in the present study are presented in Table 1. The patients were 56.2 ± 12.0 years, and 29 (51.9 %) were male. As shown in Table 1, of the 54 subjects who underwent 75g-OGTT, 26 exhibited normal glucose tolerance (NGT) without obesity (group 1), 5 exhibited NGT with obesity (group 2), 12 exhibited IFG and/or IGT without obesity (group 3), and 11 exhibited IFG and/or IGT with obesity (group 4).

P_{glo} ($p = 0.0015$) , urinary albumin excretion ($p = 0.0001$) and Filtration fraction (FF) ($p = 0.016$) were significantly higher in the IFG and/or IGT subjects with obesity (group 4) than in the other groups. R_e in the IFC and/or IGT subjects with obesity (group4) tended to be higher than in the other groups (FF; $p = 0.088$, R_e; $p = 0.066$). ISI in subjects with obesity (group 2 and group 4) was significantly lower than in those without obesity ($p < 0.0001$, *vs.* group 1 and group 3). It is well known that insulin resistance is related to obesity (26; 27). When we examined the association between BMI and ISI, log ISI was associated significantly and negatively with BMI ($r = - 0.593$, $p< 0.0001$). As shown in Figure 1, P_{glo} correlated significantly and positively with BMI ($r = 0.298$, $p = 0.028$), and correlated significantly and negatively with the log ISI ($r = -$

0.351, p = 0.009). There were no significant relationships between the logISI and the following parameters; GFR (r = 0.067, p = 0.628), RPF (r = 0.149, p = 0.283), R_a (r = 0.023, p = 0.867), R_e (r = 0.103, p = 0.460), and FF (r = 0.094, p = 0.498). Since there was a significant association between BMI and log ISI , we performed multiple regression analyses, in which BMI and log ISI were entered as independent variables. In these analyses, BMI tended to be associated with P_{glo} (β = 0.269, p = 0.064; Table 2, Model 1), and the log ISI was associated significantly and independently with P_{glo} (β = - 0.316, p = 0.015; Table 2, Model 2), after adjustment for age, gender, and systolic blood pressure.

Next, we examined the associations between urinary albumin excretion and several factors. As shown in Figure 2, BMI (r = 0.505, p = 0.0001) and P_{glo} (r = 0.364, p = 0.006) correlated significantly and positively, and log ISI (r = - 0.386, p = 0.001) correlated significantly and negatively with urinary albumin excretion. In order to analyze the factors associated with urinary albumin excretion, multiple regression analyses were performed after adjustment for age, gender, and systolic blood pressure. Since P_{glo} was associated significantly with BMI and log ISI (Figure 1), multiple regression analyses were performed in which BMI, P_{glo}, and log ISI were entered as independent variables. As shown in Model 3, Model 4, and Model 5 in Table 2, BMI (β = 0.517, p = 0.0004), P_{glo} (β = 0.420, p = 0.004), and log ISI (β = - 0.366, p = 0.008) were each significantly and independently associated with urinary albumin excretion, after these adjustments.

Conclusion

In the present study, we examined C_{in} and C_{PAH}, and calculated the intrarenal

hemodynamic parameters in kidney donor candidates using Gomez formulae, as described in detail in our previous studies (13; 21). In all subjects, GFR, as measured by C_{in}, was greater than 60 mL/min, and urinary albumin excretion was less than 30 mg/day, *i.e.*, within the normal range. Glucose metabolism pattern was evaluated by 2-hour 75-g OGTT in all subjects, and insulin resistance was evaluated by calculation of the insulin sensitivity index (Matsuda Index) (25). In this study, we demonstrated that P_{glo} and the degree of urinary albumin excretion, even within a normal range (13.7 ± 8.4 mg/day), were significantly higher in obese IFG and/or IGT patients (group 4) compared with the other groups. We also demonstrated that the R_e and FF in the obese IFG and/or IGT subjects (group 4) tended to be higher than in the other groups. Further, we demonstrated that log ISI and BMI were associated significantly with P_{glo}, and that ISI, BMI and P_{glo} were associated significantly with urinary albumin excretion. These results indicate that lower ISI, *i.e.*, higher insulin resistance, even in IFG and/or IGT subjects, is associated with increased P_{glo}, and suggest that increased P_{glo} may, in turn, be associated with increased urinary albumin excretion, even within the normal range. BMI was associated with insulin resistance in the present study, which has been well established (28). Thus, the associated increased insulin resistance in IFG and/or IGT subjects likely increases the burden on the glomeruli, and thus, affecting deterioration of the glomeruli in prediabetic patients.

Increased GFR has been reported previously in diabetic nephropathy (1; 29). Glomerular hyperfiltration is observed in the early stages of most patients with diabetes mellitus, and is considered to precede the development of microalbuminuria by several years (1; 30). In animals, the increase in GFR in diabetes is caused by imbalances of afferent and efferent arteriolar tone with a disproportionate decrease in afferent

arteriolar resistance and relatively higher efferent arteriolar tone, leading to increases in glomerular capillary pressure (30). However, imbalances between afferent and efferent arteriolar resistance have not been demonstrated clinically in the early stages in humans with diabetes mellitus. We recently demonstrated that poor glycemic control is associated significantly with glomerular hemodynamic abnormalities in humans (13). In our previous study, we reported that poor glycemic control increased the filtration fraction, P_{glo}, and R_e, but not R_a (13). While all of these experimental and clinical studies have examined diabetic patients or diabetic experimental animals, to date, there has been no report in which renal hemodynamic abnormalities were evaluated by C_{in} and C_{PAH} with respect to the relationship with insulin resistance in non-diabetic human subjects. Further, there have been no reports in which urinary albumin excretion was examined in relation to glomerular hemodynamics and insulin resistance in these subjects. Thus, this is the first study to demonstrate that insulin resistance is associated significantly with glomerular hemodynamic changes and urinary albumin excretion in IFG and/or IGT subjects.

Based upon the current findings, the mechanism underlying increased P_{glo}, under the status of insulin resistance remains unknown. Evidence suggests that insulin resistance and obesity could activate the intrarenal renin angiotensin system (RAS) (2). RAS activation increases efferent arteriolar resistance, leading to glomerular hypertension and hyperfiltration (1; 31). From the results of the present study, we consider that intrarenal RAS may be activated, even in subjects with IFG and/or IGT in obesity. Concerning P_{glo}, in a model in which both BMI and log ISI were entered simultaneously as independent variables, neither BMI nor log ISI were significantly, independently associated with P_{glo} (data not shown). We consider that the results of

these additional analyses could indicate that there is a high level of confounding between BMI and log ISI, leading to the non-significant associations between the two variables and P_{glo}.

On the other hand, it is well known that urinary albumin excretion is caused by glomerular hypertension induced by poor glycemic control and/or obesity in animal studies (32). It has been reported that urinary albumin excretion was preceded by glomerular hypertension under high glucose, through increased shear stress (33). Nakagawa *et al.* reported that the reduction of nitric oxide synthesis from endothelial cells in the glomeruli, as a consequence of insulin resistance, induces continuous increases in renal vascular endothelial growth factor (VEGF) expression and marked macrophage infiltration in an animal model (34). Furthermore, in humans, Pistrosch *et al.* reported that an insulin-sensitizing drug, rosiglitazone, ameliorated glomerular hyperfiltration and reduced urinary albumin excretion in patients with early type 2 diabetes with microalbuminuria (35). Denic *et al.* demonstrated that obesity was associated with a higher single-nephron GFR in chronic kidney disease among otherwise healthy adult kidney donor candidates (36). Bjornstad *et al.* demonstrated relationships between whole-body, central adiposity, and intrarenal hemodynamic function in adults with long-standing type 1 diabetes (37). We consider that mechanisms similar to those described above may be underlying the increase in UAE in the present study, in which IFG and/or IGT subjects were examined.

Concerning urinary albumin excretion (UAE), additional analyses, in which two or three measures, out of BMI, P_{glo}, and log ISI, were included simultaneously as independent variables, BMI and P_{glo} were independently, significantly associated with UAE (data not shown). However, log ISI was not associated significantly with UAE

(data not shown). These results may indicate that BMI, which is very strongly associated with log ISI, may be a stronger factor that is associated with UAE. We consider that obesity itself may affect the increased UAE. However, the analyses presented in Table 1 demonstrate that, among four groups, only group 4 (IFG and/or IGT with obesity) showed significantly higher P_{glo} and UAE. We consider that the result may indicates that the presence of IFG and/or IGT and obesity cause significantly increased P_{glo} and UAE. Thus, we consider that a significant increase in P_{glo} is caused by the presence of both increased log ISI (as represented by IFG and/or IGT) and increased BMI. In the present study, we demonstrated, for the first time, that increased P_{glo} is associated with increased UAE in human; and that obesity is also significantly associated with UAE, as has been reported previously by others (38; 39). The reason obesity is associated with increased UAE remains unknown from the results of present study. We consider that the presence of both insulin resistance and obesity may cause increased P_{glo}, which may be followed by increased UAE.

In the current report, we showed that P_{glo}, insulin resistance, and BMI were associated significantly with urinary albumin excretion in IFG and/or IGT subjects, even within the normal range. Some reports have shown that higher levels of urinary albumin excretion, even within the normal range, predict the decline in glomerular filtration rate in diabetic patients (40), as well as the development of cardiovascular disease (41), and coronary heart disease (42). These studies and the results of the present study may support the need to redefine the range of microalbuminuria, even at the levels currently considered to be normal. The current findings suggest that albuminuria, even at levels currently considered to be within the normal range, may be increased as a consequence of insulin resistance in subjects with IFG and/or IGT, likely

through increasing the intraglomerular hydrostatic pressure.

There are some limitations to the present study. First, the study was performed in a small number of Japanese subjects, and a large-scale study is needed to confirm the relationship between urinary albumin excretion, insulin resistance, and glomerular hemodynamic abnormalities in IFG and/or IGT subjects. Secondly, this is a cross sectional study. Further studies may be needed to explore the consequence of insulin resistance on glomerular hypertension and urinary albumin excretion. However, this is the first study in which insulin resistance was associated significantly with P_{glo} and urinary albumin excretion in human subjects with IFG and/or IGT. Thirdly, we were not able to directly measure R_a, R_e, and P_{glo} in the present study, as it is not possible to directly measure these parameters in humans; compared with animal studies. Thus, we used Gomez's formulae to assess intrarenal hemodynamics, as in the previous studies reported by others (12; 19; 20; 43) and by ourselves (13; 15; 21; 22).

In conclusion, in the present study, by measuring C_{in} and C_{PAH}, we showed that increased insulin resistance is associated significantly with increased P_{glo} and urinary albumin excretion, even at levels currently considered to be within the normal range in human subjects with IFG and/or IGT. These hemodynamic burdens may lead to glomerular injury in IFG and/or IGT subjects. We also suggest that the clinically significant levels of microalbuminuria, as currently defined, could be redefined based upon several human studies, including the present study.

Acknowledgement

A.T. and E.I. performed the research, acquired the data and wrote the manuscript. A.T., E.I., H.U., A.O.,S.N., T.M.,K.M., J.U., M.E., T.N., and M.I. contributed to the

discussion, and reviewed the manuscript. A.T. and E.I. had full access to all of the study data and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the reported research. This study was not funded by any grant from a funding agency in the public, commercial, or not-for-profit sector.

This study was supported by Grant from the Osaka Kidney Foundation (OKF18-0019).

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Table 1 Clinical characteristics of the 54 subjects.

	NGT		IFG and/or IGT		
	Without obesity (group 1)	With obesity (group 2)	Without obesity (group 3)	With obesity (group 4)	p value
N (54)	26	5	12	11	---
Age (years)	55.5 ± 13.1	61.0 ± 12.7	56.3 ± 11.9	55.5 ± 3.1	n.s.
Gender (male/female)	11/15	4/1	3/9	6/3	0.037 #1a, 0.021 #1b
Body mass index (kg/m^2)	21.0 ± 1.8	26.2 ± 1.8	21.5 ± 2.1	27.1 ± 1.3	<0.0001 #2
Mean arterial pressure (mmHg)	87.9 ± 13.3	87.3 ± 10.3	83.0 ± 12.3	95.3 ± 14.0	0.033 #3
Systolic blood pressure (mmHg)	122.3 ± 19.3	117.8 ± 12.9	117.9 ± 16.5	128.6 ± 14.2	n.s.
Diastolic blood pressure (mmHg)	70.9 ± 12.3	71.4 ± 8.7	67.3 ± 11.6	78.6 ± 14.3	0.033 #3
Blood urea nitrogen (mg/dL)	13.2 ± 3.9	12.4 ± 1.1	13.3 ± 2.9	12.7 ± 1.7	n.s.
Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.01 #4
Estimated GFR (mL/min/1.73m^2)	78.7 ± 11.9	70.9 ± 5.3	81.1 ± 16.4	74.0 ± 11.7	n.s.
GFR (C_{in})(mL/min)	80.4 ± 18.9	78.3 ± 9.3	85.8 ± 25.5	88.2 ± 18.9	n.s
GFR (C_{in})(mL/min/1.73m^2)	89.2 ± 18.7	81.3 ± 10.8	95.5 ± 25.4	88.9 ± 18.6	n.s.
RPF (C_{PAH}) (mL/min)	380.9 ± 118.0	371.2 ± 71.8	402.1 ± 124.4	362.5 ± 74.2	n.s.
Urinary albumin excretion (mg/day)	5.2 ± 2.8	6.0 ± 2.0	4.4 ± 3.6	10.1 ± 7.4	0.0001 #5
filtration fraction (FF) (%)	0.22 ± 0.03	0.21 ± 0.03	0.22 ± 0.04	0.25 ± 0.04	0.016 #6
glomerular hydrostatic pressure (P_{glo}) (mmHg)	54.3±4.5	54.8 ± 3.5	55.9 ± 5.0	58.4 ± 4.4	0.015 #7
afferent arteriole resistance (R_a) (dyne□sec□cm^{-5})	4629.3 ± 2233.2	4267.0 ± 1536.8	3648.0 ± 2532.4	5001.0 ± 2441.8	n.s.
efferent arteriole resistance (R_e) (dyne□sec□cm^{-5})	2440.5 ± 366.5	2376.2 ± 491.9	2476.9 ± 547.9	2783.1 ± 723.0	0.066 #8
fasting plasma glucose (mg/dL)	89 ± 6	90 ± 3	100 ± 8	104 ± 12	0.007 #9
hemoglobin A1c (%)	5.6 ± 0.3	5.5 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	n.s.
2 hour plasma glucose in 75-g OGTT	102 ± 20	104 ± 22	150 ± 41	139 ± 35	<0.0001 #10
Log insulin sensitivity index (the MatsudaIndex)	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.4 ± 0.2	<0.0001 #11

NGT: normal glucose tolerance, IFG: impared fasting glucose, IGT: impared glucose tolerance, GFR : glomerular filtration rate, C_{in} : inulin clearance, RPF : renal plasma flow, C_{PAH} : para-aminohippuric acid clearance, P values were evaluated by two-way ANOVA and Scheffé multiple means comparisons. #1a, p = 0.037 (group1 *vs*. group2); #1b p = 0.021 (group 2 *vs*. group3); #2, p <0.0001 (group 1 and 3 *vs*. group2 and 4); #3, p = 0.033 (group3 *vs*. group4), #4. P = 0.01 (group1 and group3 *vs*. group 4), #5, p = 0.0001 (group 1, 2, and 3 *vs*. group 4), #6, p = 0.016 (group 1 *vs*. group 4), #7, p = 0.015 (group 1 *vs*. group 4), #8, p = 0.066 (group 1 *vs*. group 4), #9 p = 0.007 (group 1 and 2 *vs*. group 3 and 4), #10, p < 0.0001 (group 1 and 2 *vs*. group 3 and 4), #11, p < 0.0001 (group 1, 2, and 3 *vs*. group 4)

Table 2. Factors associated with the glomerular hydrostatic pressure (P_{glo}) and urinary albumin excretion.

	P_{glo}					urinary albumin excerection					
	Model 1		Model 2			Model 3		Model 4		Model 5	
	β	p	β	p		β	p	β	p	β	p
age (years)	-0.357	0.010	-0.327	0.015	age (years)	0.061	0.633	0.222	0.124	0.103	0.452
gender (male = 0, female =1)	-0.083	0.565	-0.188	0.146	gender (male = 0, female =1)	- 0.004	0.974	- 0.137	0.314	- 0.211	0.119
systolic blood pressure (mmHg)	0.143	0.299	0.108	0.427	systolic blood pressure (mmHg)	- 0.103	0.436	- 0.124	0.375	- 0.124	0.380
body mass index (kg/m^2)	0.269	0.064	---	---	body mass index (kg/m^2)	0.517	0.0004	---	---	---	---
log ISI	---	---	- 0.316	0.015	P_{glo}	---	---	0.420	0.004	---	---
					log ISI	---	---	---	---	-0.366	0.008
R^2/p	0.357/0.010		0.327/0.015		R^2/p	0.265/0.004		0.201/0.024		0.181/0.041	

P_{glo} ; glomerular hydrostatic pressure, ISI ; insulin sensitivity index (the Matsuda index)

Figure legends Figure 1. Relationship between glomerular hydrostatic pressure (P_{glo}) and body mass index, and between P_{glo} and insulin sensitivity index (ISI). There was a significant and positive correlation between body mass index and P_{glo}, and a significant and negative correlation between log ISI and P_{glo}.Figure 2. Relationship between urinary albumin excretion and body mass index, insulin sensitivity index (ISI), and glomerular hydrostatic pressure (P_{glo}). There were significant and positive correlations between body mass index and urinary albumin excretion and between P_{glo} and urinary albumin excretion. There was a significant and negative correlation between log ISI and urinary albumin excretion.