Research Article

Increased Glomerular Hydrostatic Pressure is Associated with Tubular Creatinine Reabsorption in Healthy Subjects

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Keywords

Inulin clearance · Tubular creatinine secretion and reabsorption · Intrarenal hemodynamics · Living kidney donor

Abstract

Background: Cr is secreted by the proximal tubules and thus Cr clearance (C_{Cr}) can overestimate inulin clearance (C_{in}). However, in some cases, C_{Cr} can even underestimate C_{in} . This suggests that Cr could be reabsorbed in the tubuli. We examined the clinical parameters that are associated with tubular Cr reabsorption. **Methods:** In 80 kidney donor candidates (53.9 ± 13.2 years, 29 males), C_{in} and para-aminohippuric acid clearance were measured simultaneously. Intrarenal hemodynamic parameters were calculated by Gomez's formulae. To quantify the secretory component of C_{Cr} (SF_{Cr}), it was calculated as follows: $SF_{Cr} = (C_{Cr} - C_{in})/C_{Cr}$. **Results:** Twenty-five subjects (31.3%) showed SF_{Cr} values <0 . SF_{Cr} that correlated significantly and negatively with efferent arteriolar resistance (R_e) and glomerular hydrostatic pressure (P_{glo}) (R_e : $r = -0.30$, $p = 0.008$; P_{glo} : $r = -0.28$, $p = 0.025$). In multiple regression analyses, R_e and P_{glo} were significantly and negatively associated with SF_{Cr} after adjustment for other confounders. **Conclusions:** These findings suggest that tubular reabsorption of Cr can occur in some cases. Intrarenal glomerular hemodynamic burden may be related to tubular creatinine reabsorption, which possibly leads to lower C_{Cr} values.

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Introduction

With the continuing increase in the number of patients with ESRD, accurate evaluation of renal function is necessary for early diagnosis and strict control of renal dysfunction. In addition to microalbuminuria or proteinuria, evaluation of the glomerular filtration rate (GFR) is generally believed to provide a clinically useful index for the assessment of the progression of renal dysfunction and cardiovascular risk in patients with CKD [1, 2]. The gold standard in the determination of GFR is the measurement of inulin clearance (C_{in}). However, in general, serum Cr, estimated GFR (eGFR) formulae, and Cr clearance (C_{cr}) are widely used to estimate GFR.

Therefore, the Japanese Society of Nephrology issued a new equation for eGFR based upon the measurement of C_{in} in 763 Japanese subjects [3]. However, serum Cr has been reported to be affected by muscle mass, gender, and age [4, 5]. It has been reported that the evaluation of eGFR by serum Cr can also be inaccurate, especially in diabetics [6–8], and even in healthy subjects [9, 10]. We previously reported that poor glycemic control is a major factor in the overestimation of GFR in diabetic patients [8]. Horio et al. [9] and Kakuta et al. [7] also reported that eGFR underestimated the measured GFR in kidney donor candidates, that is, healthy subjects, when eGFR was calculated using serum Cr. These reports suggest that this was possibly caused by higher muscle mass or higher protein intake. However, the mechanism underlying the underestimation of eGFR in healthy subjects still remains unknown.

On the other hand, in general, Cr clearance (C_{cr}) overestimates C_{in} , by 20–30% due to the secretion of Cr in the proximal tubuli. It has been reported that progressive increases in Cr secretion also occur with a decline in GFR [11, 12]. However, in some cases, C_{cr} underestimates GFR [7], although few reports have explored this matter in detail [7, 9]. The underestimation of measured C_{cr} compared with C_{in} may not be explained solely by muscle mass, protein intake, and/or aging. This phenomenon suggests the possibility that Cr is reabsorbed by the tubuli. In several reports, the possibility of tubular Cr reabsorption has been reported in animals [13–15] and in humans [15, 16].

However, to date, there are no available data regarding the tubular reabsorption of Cr, even though a small number of studies have compared the measurement of C_{cr} and C_{in} in humans [17–19]. Furthermore, there is currently no detailed data, in either animals or humans, regarding the association of tubular Cr reabsorption with clinical parameters, including intrarenal hemodynamics. Thus, in this study, we hypothesized that intrarenal hemodynamic parameters could be associated with Cr handling in the tubuli.

The aim of the present study was to examine which clinical parameters, including intrarenal hemodynamic parameters, were potentially associated with tubular Cr secretion and reabsorption. An additional aim was to assess how Cr handling in the tubuli changes according to GFR changes in examining the variability of the tubular secretory component of Cr in subjects in whom C_{in} and C_{cr} were measured both before and 1 year after the kidney donation.

Research Design and Methods

Subjects

The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (#3955) for opt out study. Kidney donor candidates were admitted to Osaka City University Hospital between January 2010 and March 2018 for evaluation of their suitability as kidney donors. The design was a single-center, retrospective, cross-sectional observation study that was conducted at Osaka City University Hospital.

Fig. 1. Schema of the protocol of measurements of plasma C_{in} and C_{PAH} . C_{in} and C_{PAH} were measured simultaneously using the double infusion technique of 1% inulin and 0.5% PAH according to the method of Horio et al. [9]. C_{in} , inulin clearance; PAH, para-aminohippurate; C_{PAH} , para-aminohippurate clearance.

In Japan, the guideline for kidney donor candidates recommends the accurate evaluation of GFR by C_{in} [20]. In Japan, inulin and para-aminohippuric acid (PAH) clearances (C_{in} and C_{PAH}) can be measured under the Japanese National Health Insurance system. Inulin (Inulead®, Fuji Yakuhin Co., Ltd., Saitama, Japan) and PAH (Sodium p-aminohippurate®, Daiichi-Sankyo Co., Ltd., Tokyo, Japan) are commercially available. Therefore, C_{cr} , C_{in} , and C_{PAH} were determined in all subjects.

According to Gomez's formulae, in which $C_{in} > 60$ mL/min could be applied [21], we excluded those with C_{in} values < 60 mL/min from further analyses. None of the subjects were undergoing treatment with medications, including antihypertensives, that is, angiotensin-converting-enzyme inhibitors and/or angiotensin II receptor blockers. Independent from antihypertensive drugs, none of the subjects were treated with any other drugs (e.g., cimetidine) that could modify the tubular handling of Cr.

Measurements of C_{cr} , C_{in} , and C_{PAH}

The present study examined subjects upon admission to the Departments of Urology and Nephrology of our university hospital, to precisely examine the renal function and determine their suitability for kidney donation. Each subject underwent medical checkup by expert urologists and nephrologists prior to the clearance study. None of the subjects exhibited any urination disorders. When examining the C_{cr} of the subjects, in routine clinical practice in our departments, the attending doctors and nurses instructed all subjects to completely void their bladder immediately after getting up in the morning and then instructed them to collect all the urine throughout the day until the first urination of the next day. All of the 24-h urine samples for evaluation of the C_{cr} were collected from the study subjects. Serum Cr levels were measured using an enzymatic method [3, 22]. C_{cr} was assessed based on the serum Cr levels and 24-h urinary Cr excretion. C_{cr} was calculated according to the following formula:

24-h $C_{cr} = \{(\text{urine Cr concentration [mg/dL]} \times \text{urine volume [mL/day]}) / (\text{serum Cr concentration [mg/dL]})\} \times 1,440$.

GFR, that is, C_{in} and renal plasma flow (RPF), that is, C_{PAH} were determined simultaneously by the constant input clearance technique using inulin and PAH, respectively. According to the method reported by Horio et al. [23–26], continuous intravenous infusion of inulin and PAH was administered simultaneously in the morning, under a fasting state. As shown in Figure 1, continuous intravenous infusion of 1% inulin and 0.5% PAH in saline was administered via the antecubital vein in the morning after an overnight fast, based on the method of Horio et al. [23–26]. C_{in} and C_{PAH} were measured simultaneously using a simple method based on a single urine collection. In brief, patients received 500 mL of water orally 15 min before infusion. For the priming dose of inulin and PAH, the rates of infusion were set at 300 mL/h for the first 30 min and thereafter at 100 mL/h for the remaining stabilization period [23–26]. To maintain hydration, 180 mL of water was given orally. Patients completely emptied their bladder 45 min after the start of the test, and urine was collected for measurement of urinary inulin and PAH. The urine collection period was set at 90 min to increase the accuracy of the clearance study [27]. Blood samples for the serum inulin and PAH measurements were taken both at the beginning and at the end of the clearance study. Inulin concentrations were measured enzymatically [23]. PAH concentrations were measured photometrically, by means of the *N*-1 naphthylethylenediamine and the anthrone method using a Corning 258 spectrophotometer [28].

Calculation of Intrarenal Hemodynamic Parameters

Since direct measurement of glomerular hemodynamics parameters is not feasible in humans, the formulae introduced by Gomez [21] allows indirect assessment of glomerular hemodynamics, as reported previously by others [29–33] and ourselves [24, 26, 34–36]. The Gomez formulae were calculated according to the original report [21], as described in detail in our previous studies [24, 26, 34, 35]. In the formulae, GFR (C_{in}), afferent arteriolar resistance (R_a), efferent arteriolar resistance (R_e), and glomerular hydrostatic pressure (P_{glo}) were calculated. Filtration fraction was calculated by dividing the GFR (C_{in}) by the RPF (C_{PAH}). According to Gomez's formulae, in which $C_{in} > 60$ mL/min could be applied [21], we excluded those with C_{in} values that were < 60 mL/min from further analyses.

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

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

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

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

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

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/s 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

$$R_a = ((MBP - P_{glo}) / RBF) \times 1,328$$

$$R_e = (GFR / K_{FG} \times (RBF - GFR)) \times 1,328$$

Renal blood flow (RBF) was calculated from the RPF and hematocrit (Ht) according to the standard formula:

$$RBF = RPF / (1 - Ht)$$

Table 1. Clinical characteristics of all 80 subjects, 55 subjects with $SF_{cr} > 0$, and 25 subjects with $SF_{cr} < 0$

	All subjects (N = 80)	$SF_{cr} > 0$ (N = 55)	$SF_{cr} < 0$ (N = 25)	p value evaluated by Mann-Whitney U test
Age, years	53.9±12.0	53.6±11.3	54.7±13.5	ns
Gender, males/females	29/51	20/35	9/16	ns
BMI, kg/m ²	22.8±3.1	23.4±3.2	21.6±3.2	0.012
Mean arterial pressure, mm Hg	89.8±13.2	89.8±13.1	89.8±13.4	ns
Systolic blood pressure, mm Hg	133.8±16.5	121.9±15.5	124.8±18.5	ns
Diastolic blood pressure, mm Hg	73.3±13.4	73.7±13.5	72.3±13.5	ns
Serum Cr, mg/dL	0.68±0.3	0.68±0.14	0.68±0.13	Ns
C_{cr} , mL/min	101.6±28.4	112.1±24.3	78.4±22.5	<0.0001
GFR (C_{in}), mL/min	88.9±18.0	86.0±21.3	95.3±21.3	0.085
Renal plasma flow, mL/min	416±125	403±101	444±164	Ns
Renal blood flow, mL/min	696±207	687±173	735±266	Ns
Glomerular hydrostatic pressure, mm Hg	56.6±4.5	56.0±4.2	58.1±4.8	0.053
R_a , dyne s cm ⁻⁵	4,150±2,078	4,227±1,974	3,980±2,325	Ns
R_e , dyne s cm ⁻⁵	2,464±431	2,406±344	2,588±563	ns
Filtration fraction, %	0.2±0.03	0.2±0.03	0.2±0.04	ns
SF_{cr}	0.073±0.26	–	–	–
Fasting plasma glucose, g/dL	90.9±10.8	89.7±8.9	93.0±13.9	ns
Hemoglobin A1c, %	5.5±0.3	5.5±0.3	5.5±0.3	ns

SF_{cr} , secretory component of Cr clearance; C_{cr} , Cr clearance; GFR, glomerular filtration rate; C_{in} , inulin clearance; R_a , afferent arteriolar resistance; R_e , efferent arteriolar resistance.

In the above-mentioned formulae, the conversion factor to dyne s cm⁻⁵ was 1,328; GFR, RPF, and RBF were expressed in mL/s; and the mean blood pressure (MBP) was calculated as $(2 \times \text{diastolic BP} + \text{systolic BP})/3$.

Measurement of Cr Handling in the Tubuli

As described in the textbook of Guyton and Hall [37], “If the clearance rate of the substance is greater than C_{in} , the substance must be secreted by nephron and tubuli, and if the clearance rate is less than the inulin clearance, the substance must have been reabsorbed.”

Serum Cr is excreted into urine via glomerular filtration and tubular secretion [38]. C_{cr} is considered to represent the net effect of glomerular Cr clearance and tubular creatinine secretion [38]. C_{in} is considered to be equivalent to GFR since inulin is not secreted from the renal tubuli [39, 40]. Accordingly, the formula for the secretory component of tubular Cr was calculated as follows:

$$(SF_{cr}: (C_{cr} - C_{in})/C_{cr}).$$

This formula is considered to be the ratio of tubular Cr secretion and/or reabsorption [38].

Statistical Methods

The data are expressed as the mean ± standard deviation. Correlations between 2 variables were examined using simple regression analysis by Pearson correlation coefficient test. The results are expressed as the mean ± standard deviation. Mann-Whitney U test was used to compare the differences of each of the parameters in the subjects with $SF_{cr} > 0$ and in the subjects with the $SF_{cr} < 0$. Wilcoxon signed-rank test was used to compare SF_{cr} between before and 1 year after kidney donation in 20 subjects. All analyses were performed using Stat View 5 (SAS Institute Inc., Cary, NC, USA) on a Windows computer. The level of statistical significance was set at $p < 0.05$.

Fig. 2. Relationship between C_{in} and C_{cr} . The dashed line shows $C_{cr}/C_{in} = 1$. There were 25 subjects in whom C_{cr}/C_{in} was <1 and 55 in whom C_{cr}/C_{in} was >1 . The former (i.e., those that are above the line) are those in whom Cr is theoretically considered to be secreted, and the latter (i.e., those that are below the line) are those in whom Cr is theoretically considered to be reabsorbed in the tubuli. C_{in} , inulin clearance; C_{cr} , Cr clearance.

Fig. 3. Relationships between R_a and SF_{cr} (a), between R_e and SF_{cr} (b), and between P_{glo} and SF_{cr} (c). While significant and negative correlations were observed between R_e and SF_{cr} (b), and between P_{glo} and SF_{cr} (c), there was no significant correlation between R_a and SF_{cr} (a). Dashed lines: $SF_{cr} = 0$, ns, not significant. R_a , afferent arteriolar resistance; SF_{cr} , secretory component of Cr clearance; R_e , efferent arteriolar resistance; P_{glo} , glomerular hydrostatic pressure.

Results

Clinical Characteristics, Renal Function, and Intrarenal Hemodynamic Parameters

The clinical characteristics of the 80 subjects enrolled in the present study are shown in Table 1. The mean age of all subjects was 53.7 ± 13.3 years; 29 (36.3%) were male. The mean serum Cr, C_{cr} , and C_{in} values were 0.68 ± 0.13 mg/dL, 101.6 ± 28.4 mL/min, and 88.9 ± 18.0 mL/min, respectively. The measured GFR based on the C_{in} was >60 mL/min in all subjects. Of them, 25 subjects (31.3%) showed SF_{cr} values <0 , which indicated the underestimation of C_{in} compared to C_{cr} (Fig. 2). Those subjects who exhibited $SF_{cr} < 0$ with normal kidney function are considered to exhibit Cr reabsorption, according to the textbook of Guyton and Hall [37].

We compared the clinical parameters, including intrarenal hemodynamics, between the subjects with $SF_{cr} > 0$ and those with $SF_{cr} < 0$ (Table 1). There were no significant differences in RPF and RBF between those with $SF_{cr} > 0$ and $SF_{cr} < 0$. C_{in} tended to be lower in the subjects with $SF_{cr} > 0$ than in those with $SF_{cr} < 0$ (86.0 ± 21.3 vs. 95.3 ± 21.3 mL/min; $p = 0.085$).

Table 2. Factors associated with SF_{cr} (multiple regression analyses)

	SF_{cr}					
	Model 1		Model 2		Model 3	
	β	<i>p</i> value	β	<i>p</i> value	β	<i>p</i> value
Age (years)	0.135	0.267	−0.004	0.972	−0.246	0.019
Gender, male = 0, female = 1	−0.059	0.600	−0.002	0.987	−0.187	0.045
BMI (kg/m ²)	0.233	0.049	0.325	0.005	−0.019	0.839
Systolic blood pressure (mm Hg)	−0.234	0.062	−0.175	0.158	0.144	0.164
R_e (dyne s cm ^{−5})	−0.301	0.012	–	–	−0.173	0.072
P_{glo} (mm Hg)	–	–	−0.269	0.019	−0.660	<0.0001
R^2/p value	0.164/0.022		0.163/0.019		0.454/<0.00001	

SF_{cr} , secretory component of Cr clearance; R_e , efferent arteriole resistance; P_{glo} , glomerular hydrostatic pressure.

Conversely, C_{cr} was significantly higher in subjects with $SF_{cr} > 0$ than in those with $SF_{cr} < 0$ (112.1 ± 24.3 vs. 78.4 ± 22.5 ; $p < 0.0001$). P_{glo} in subjects with $SF_{cr} > 0$ tended to be lower than in those with $SF_{cr} < 0$ (56.0 ± 4.2 vs. 58.1 ± 4.8 ; $p = 0.053$).

Factors Associated with SF_{cr}

We next evaluated the factors associated with SF_{cr} . We first examined the association of SF_{cr} with several clinical parameters, including renal hemodynamic parameters, using simple regression analyses. Although there were no significant correlations between SF_{cr} and age, gender, BMI, blood pressure, or R_a (Fig. 3a), there were significant and negative correlations between SF_{cr} and R_e and between SF_{cr} and P_{glo} (R_e : $r = -0.30$, $p = 0.008$; P_{glo} : $r = -0.39$, $p = 0.001$) (Fig. 3b, c).

Multiple regression analyses were performed to examine whether R_e and P_{glo} were independently associated with SF_{cr} after adjustment for age, gender, BMI, and blood pressure. R_e and P_{glo} were significantly and negatively associated with SF_{cr} after the adjustments (R_e : $\beta = -0.301$, $p = 0.012$; P_{glo} : $\beta = -0.269$, $p = 0.019$) (Table 2, Model 1 and Model 2). In another model in which R_e and P_{glo} were included simultaneously as independent variables, only P_{glo} was independently and significantly associated with SF_{cr} (R_e : $\beta = -0.173$, $p = 0.072$; P_{glo} : $\beta = -0.660$, $p < 0.0001$) (Table 2, Model 3).

Changes in SF_{cr} One Year after Kidney Donation

We next examined changes in SF_{cr} (ΔSF_{cr}) in subjects in whom C_{in} and C_{cr} were measured both before (pre-donation) and 1 year after kidney donation (post-donation), to evaluate how Cr handling in the tubuli varies with the changes in GFR. We were able to evaluate C_{in} and C_{cr} , before and 1 year after the donation in 20 out of the 80 subjects. There were no significant differences in the clinical parameters between the 20 subjects and the remaining 60 subjects (data not shown). Mean serum Cr, C_{cr} , and C_{in} post-donation were 1.10 ± 0.25 mg/dL, 61.8 ± 20.8 mL/min, and 50.6 ± 19.2 mL/min, respectively (Table 3). The pre-donation SF_{cr} was <0 in 7 (35%) out of the 20 subjects. On the other hand, the post-donation SF_{cr} was <0 in 5 (25%) out of the 20 subjects. All of the 5 subjects with $SF_{cr} < 0$ one year after kidney donation were included among the 7 subjects with $SF_{cr} < 0$ before donation. Among the 5 subjects with $SF_{cr} < 0$ one year after donation, none of the patients switched from $SF_{cr} < 0$ to $SF_{cr} > 0$. The post-donation SF_{cr} was significantly higher than the pre-donation SF_{cr} (0.289 ± 0.45 vs. 0.069 ± 0.27 , $p = 0.045$).

Table 3. Clinical characteristics of 20 subjects in whom C_{in} and C_{cr} were measured both before (pre-donation) and 1 year after kidney donation (post-donation)

	N = 20		p value evaluated by Wilcoxon signed-rank test
	pre-donation	post-donation	
Age, years	58.9±11.5	–	
Gender, male/female	7/13	–	
BMI, kg/m ²	23.4±2.6	23.8±2.8	0.019
Mean arterial pressure, mm Hg	91.2±14.3	87.0±12.6	0.073
Systolic blood pressure, mm Hg	125.6±16.2	118.4±15.9	0.015
Diastolic blood pressure, mm Hg	74.0±14.4	71.3±12.5	0.015
Serum Cr, mg/dL	0.7±0.2	1.1±0.2	<0.0001
C_{cr} , mL/min	98.8±27.7	61.8±20.8	<0.0001
GFR (C_{in}), mL/min	86.4±15.4	50.6±19.2	<0.0001
Renal plasma flow, mL/min	407±127	247±94	<0.0001
Renal blood flow, mL/min	691±212	379±197	<0.0001
SF _{cr}	0.069±0.27	0.289±0.45	0.045
The number of SF _{cr} < 0	7 (35%)	5 (25%)	ns
Fasting plasma glucose, g/dL	91±8	88.7±20.3	ns
Hemoglobin A1c, %	5.6±0.2	5.7±0.3	ns

C_{cr} , Cr clearance; GFR, glomerular filtration rate; C_{in} , inulin clearance; SF_{cr}, secretory component of Cr clearance.

Fig. 4. Relationship between the changes in C_{in} (ΔC_{in}) (%) and those of SF_{cr} (ΔSF_{cr}) (%), 1 year after kidney donation (post-donation). ΔC_{in} correlated significantly and negatively with ΔSF_{cr} , indicating that as C_{in} of post-donation decreases, SF_{cr} increases, and suggesting that the decrease in C_{cr} of post-donation may be caused by increased reabsorption of Cr in the tubuli. C_{in} , inulin clearance; SF_{cr}, secretory component of Cr clearance; C_{cr} , Cr clearance.

We next evaluated the factors associated with the changes of SF_{cr} (ΔSF_{cr}). We examined simple regression analyses of the changes of the various clinical parameters. The change of C_{in} (ΔC_{in}) correlated significantly and negatively with ΔSF_{cr} ($r = -0.473$, $p = 0.035$) (Fig. 4), indicating that SF_{cr} increases as the post-donation C_{in} decreases.

Discussion

In the present study, we examined C_{in} , C_{PAH} , and C_{cr} and calculated intrarenal hemodynamic parameters using Gomez's formulae, as described in detail in our previous reports [26, 35]. We also calculated tubular Cr handling, that is, SF_{cr}, in healthy living kidney donors.

According to the textbook by Guyton and Hall [37], it is considered that, in subjects with $SF_{cr} > 0$, Cr is secreted in the urine, and, in subjects with $SF_{cr} < 0$, Cr is reabsorbed from the tubuli [37]. In this study, we demonstrated that 25 (31.3%) out of 80 subjects exhibited SF_{cr} values < 0 . Furthermore, although no differences in RPF and RBF were observed, C_{in} tended to be higher in subjects with $SF_{cr} < 0$ than in those with $SF_{cr} > 0$ ($p = 0.085$). However, C_{cr} in subjects with $SF_{cr} < 0$ was significantly lower than in those with $SF_{cr} > 0$. Additionally, in 20 subjects, we evaluated the changes in SF_{cr} (ΔSF_{cr}) before and 1 year after kidney donation. We demonstrated that SF_{cr} increased, and that the proportion of subjects with $SF_{cr} < 0$ decreased, 1 year after the kidney donation. The decline in GFR (ΔC_{in}) 1 year after kidney donation was significantly and negatively associated with the increase in SF_{cr} (ΔSF_{cr}), indicating that, as C_{in} decreases post-donation, SF_{cr} increases, suggesting that the decrease in C_{cr} post-donation may be caused by increased reabsorption of Cr in the tubuli. These data suggest that tubular Cr reabsorption exists in some healthy subjects, and that this phenomenon decreases as GFR declines, in a cross-sectional and longitudinal study, respectively, in the present study.

From the findings of the present study, the physiological meaning and mechanism underlying decreased $SF_{cr} < 0$ in healthy subjects remains unknown. However, C_{in} and P_{glo} tended to be higher in subjects with $SF_{cr} < 0$ than in those with $SF_{cr} > 0$. Furthermore, R_e and P_{glo} were significantly and negatively associated with SF_{cr} . In the model in which R_e and P_{glo} were included simultaneously as independent variables, only P_{glo} was independently and significantly associated with SF_{cr} (Table 2, Model 3). These results indicate the possibility that the decreased SF_{cr} that indicates increased tubular Cr reabsorption may be caused by increased P_{glo} via increased R_e . In our previous study [8], P_{glo} and R_e were considered to be significantly associated with C_{in} (measured GFR). Increased GFR may be related to increases in intratubular urinary Cr, possibly leading to increased reabsorption of urinary Cr, and possibly further leading to a decrease in $SF_{cr} < 0$. It has been reported that angiotensin II activation increases R_e , leading to glomerular hypertension and hyperfiltration [41, 42]. Thus, from the current findings, it may be possible that relatively greater activation of intrarenal RAS may occur in subjects with $SF_{cr} < 0$, and that it is associated with tubular Cr reabsorption via increased P_{glo} and R_e in healthy subjects, although some undetermined mechanisms may be further present.

In general, serum Cr is excreted into urine via both glomerular filtration and tubular secretion [37, 43]. Thus, the secretion of Cr from the tubuli causes C_{cr} to overestimate GFR. In fact, it has been reported in mice and humans that organic anion transport contributed to Cr secretion in the tubuli [44]. It has been reported that cimetidine blocks the organic anion transport to improve the reliability of C_{cr} as a marker of GFR in human [45]. However, previous data support the existence of tubular Cr reabsorption in experimental and human studies, although to date tubular Cr reabsorption has been reported, to be present under relatively unusual physiological conditions [13–15]. Matos et al. [15] reported that Cr is reabsorbed along the tubuli in newborn rabbits and suggested that the mechanism responsible for tubular Cr reabsorption is slow urinary flow along the tubuli in the newborn state and increased back-leak of Cr along the immature tubular structures. Alt et al. [13] also reported net tubular Cr reabsorption in fetal piglets. Coulthard et al. [14] also reported that a net reabsorption of Cr in the tubuli is present in both in fetuses and neonates of pigs. These experimental data suggest the real presence of Cr reabsorption in immature tubuli [15]. On the other hand, Shen et al. [16] reported that the specific organic anion transporter 2 might contribute to Cr reabsorption in human renal proximal tubuli. Hibma et al. [46] reported that the possibility of tubular Cr reabsorption could not be excluded in a human study [46]. Sjöström et al. [17] observed tubular Cr reabsorption in dehydrated human adults. They proposed that the increased dissociation between C_{cr} and C_{in} in subjects with a decline in GFR is due to a decrease in tubular Cr reabsorption but not to an increase in tubular Cr secretion. In the present study, we showed that, in subjects with decreased C_{in} , that is, decreased GFR, of post-donation, SF_{cr}

has become lower to be <0 (Fig. 4), than the pre-donation C_{in} . Our results suggest that as C_{in} (GFR) decreased, SF_{cr} increased, supporting decreased reabsorption or increased secretion. Thus, the data of the observation by Sjöström et al. [17] appear to be consistent with our findings that showed the decrease in GFR (C_{in}) in those with $SF_{cr} < 0$ 1 year after kidney donation, although their subjects were not similar to our healthy subjects. Thus, from the current findings and reports by others, we consider that tubular Cr reabsorption exists in some healthy subjects, although the physiological meaning and mechanism underlying this phenomenon remains unclear. In addition, the phenomenon that $SF_{cr} < 0$ in some of the healthy subjects may be caused by increased tubular Cr reabsorption via increased intrarenal, hemodynamic, physical pressure, such as increased P_{glo} and R_e .

There are some limitations to the present study. First, the study was performed in a relatively small number of Japanese subjects, and a large-scale study is needed to confirm the relationship between SF_{cr} and intrarenal hemodynamic changes. However, our study examined 80 healthy subjects utilizing a relatively complicated but precise method of determining C_{in} with timed urine and blood sampling and was performed in a single institution. Variation of the methods between several institutions was avoided in the present single-institution study which could be considered to be an advantage of the present study. Second, we were not able to directly measure R_a , R_e , and P_{glo} in the present study. It is not possible to directly measure these parameters in human studies, compared with animal experiments. Thus, in the present study, we used Gomez's formulae to assess intrarenal hemodynamics, as reported previously by others [29, 31, 32, 47] and ourselves [24, 26, 34, 35]. Third, this was a cross-sectional, clinical study that disproved the established theory in which tubular creatinine reabsorption has not been considered deliberately. Further validation and experimental studies are needed to explore the physiological meaning and mechanism(s) underlying tubular Cr reabsorption. Fourth, to accurately assess Cr handling in the tubules, it might have been desirable to measure C_{cr} , C_{in} , and C_{PAH} simultaneously. It is also known that 24-h C_{cr} is affected by some physical conditions, such as voiding troubles and collection errors. However, as the specialized departments that precisely examine the renal function when determining the suitability of kidney donations, we were able to avoid these errors as much as possible, as mentioned above. As the present study used an opt-in protocol, we measured C_{in} and C_{PAH} simultaneously; as part of the opt-out, retrospective, and cross-sectional observation of the present study, we collected the data of C_{cr} from the medical records and charts of the study subjects, along with routine, clinical, medical data obtained from the electronic charts of all subjects. Accordingly, in the present study, which included both opt-in and opt-out protocols, we did not measure C_{cr} using timed urine samples (90-min urine), simultaneously at the time of measuring C_{in} and C_{PAH} . However, as mentioned, the C_{cr} of each subject was examined very accurately and carefully, as described above. Finally, our study examined Japanese healthy subjects. Further studies are needed to address Cr handling in tubuli among subjects of other ethnic backgrounds and in subjects with various kidney diseases.

In conclusion, we demonstrated the presence of Cr reabsorption in renal tubuli in 31.3% of our healthy subjects. Our study suggests that intrarenal hemodynamics, as expressed by increased P_{glo} and R_e , are associated with Cr reabsorption in renal tubuli. We propose that one of the mechanisms responsible for the increase in the rate of urinary Cr secretion with declined GFR is a decrease in tubular Cr reabsorption.

Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at which the studies were conducted, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was

approved by the Ethics Committee of Osaka City University Graduate School of Medicine (#3955) for opt-out study. The study protocol was (originally) approved for the related studies published elsewhere [24, 26, 34–36] by the Ethics Committee of Osaka City University Graduate School of Medicine (#3955), and written informed consent was obtained from all subjects. The present study protocol was performed with an opt-out option, as was explained in instructions on the website of the hospital (This study is an opt out study of extended follow-up care).

Conflict of Interest Statement

The authors declare that there are no conflicts of interests 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 sectors.

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Author Contributions

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

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