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Original Article

ORIGINAL ARTICLE

Renal functional reserve in living kidney donors

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ABSTRACT

Background. Factors associated with renal functional reserve (RFR)—presence or absence—are not completely clear and remain underinvestigated.

Methods. In this observational study, we evaluated the characteristics associated with the presence or absence of RFR in healthy subjects—living kidney donors—before donation. Renal function was assessed with measured glomerular filtration rate (GFR)—the clearance of iohexol—and RFR with the infusion of amino acids. An increase in GFR >10% after amino acid infusion indicated the presence of RFR. We defined *a priori* three groups of donors: without, with and using RFR. Subjects using RFR had a baseline GFR >100 ml/min but without an increase in GFR after stimulation.

Results. Subjects without RFR were predominantly women (81%) with the lowest body surface area (BSA; 1.71 ± 0.17 m^2) and basal GFR (85 ± 9 ml/min). Subjects with RFR were also mostly women (85%), with a higher BSA (1.83 ± 0.18 m^2; $P = .039$) and basal GFR (93 ± 15 ml/min). Subjects using RFR were mostly males (56%) and had the highest BSA (1.94 ± 0.18 m^2; $P < .0001$ versus without RFR; $P =$ ns versus with RFR), basal GFR (123 ± 20 ml/min; $P < .0001$ versus subjects without RFR; $P < .0001$ versus with RFR) and weight (82.1 ± 13.2 kg; $P < .0001$ versus without RFR; $P =$ ns versus with RFR).

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Conclusions. The presence or absence of RFR was associated to differences in gender, weight, BMI, BSA and basal GFR. Women with small BSA may have lower GFR and no RFR, indicating possibly a lower renal endowment even in the normal range of renal function. The use of RFR may indicate a response to a higher metabolic demand in subjects with higher renal endowment.

GRAPHICAL ABSTRACT

Clinical Kidney Journal

Renal functional reserve in living kidney donors

This observational study evaluates the characteristics associated with the presence or absence of renal functional reserve (RFR) in healthy subjects – living kidney donors – before donation.

Methods

Living kidney donors

RFR test with amino acid infusion

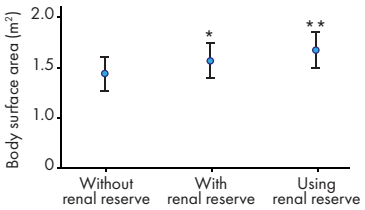
Change in measured glomerular filtration rate (GFR) – iohexol

3 groups:
- Without RFR
- With RFR
- Using RFR

Results

	Without RFR (n=26)	With RFR (n=27)	Using RFR (n=27)
Females (n-%)	21 (81)*	23 (85)*	12 (44)
BSA (m^2)	1.71 ± 0.17*	1.83 ± 0.18	1.94 ± 0.18*
Basal mGFR (ml/min)	85 ± 9*	93 ± 15*	123 ± 20
Stimulated mGFR (ml/min)	88 ± 8*	111 ± 16	115 ± 12*

Without RFR: predominantly women with the lowest BSA and basal GFR
With RFR: mostly women, with a higher BSA and basal GFR
Using RFR: mostly males, with the highest BSA and basal GFR, and weight



Conclusion: The presence or absence of RFR was associated with differences in gender, weight, BMI, BSA, and basal GFR. Women with small BSA may have lower GFR and no RFR – possibly for lower renal endowment – even in the normal range of renal function. The use of RFR may indicate a response to a higher metabolic demand in subjects with higher renal endowment.

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Keywords: glomerular filtration rate, kidney transplantation, living donor, renal endowment, renal functional reserve

KEY LEARNING POINTS

What was known:

- Renal functional reserve (RFR) is the capacity to increase glomerular filtration rate (GFR) under physiological or pathological stimuli. Physiological stimuli include pregnancy and protein or amino acid load; pathological stimuli include a significant loss of renal mass and conditions associated with hyperfiltration, such as early diabetes and obesity.
- The mechanisms behind RFR are not fully known. Physiological stimuli induce a similar increase in GFR and renal plasma flow (RPF) without major changes in filtration fraction (FF). Pathological stimuli lead to an increase in GFR greater than RPF, causing an elevation in FF and glomerular hypertension.
- The determinants and clinical relevance of RFR—presence or absence—are not completely clear. The lack of RFR could be associated with reduced renal mass or endowment and may determine a worse response to injuries. The presence of RFR may indicate larger renal mass and better capacity to overcome damage.

This study adds:

- The presence or absence of RFR was associated to differences in gender, weight, body mass index, body surface area (BSA) and basal GFR.
- We evaluated RFR with gold-standard methods: the plasma clearance of iohexol to measure GFR and the infusion of amino acids to test RFR. Measured GFR avoids the unpredictable error of estimated GFR and was not adjusted by BSA to avoid the problems of over- or underestimation of renal function.
- We established *a priori* three groups of subjects: without, with and using RFR. Otherwise, we would have included in the same group subjects without RFR and low GFR and those with high GFR and using RFR, two groups that are clearly different.

Potential impact:

- Measuring RFR may inform on the kidney's ability to adapt to physiological or pathological stimuli. Therefore, it may represent an extra check on renal health beyond the traditional tests of renal function.
- In the context of living kidney transplantation, measuring RFR could help estimate the risk of renal function decline and response to renal damage over time.

INTRODUCTION

Renal functional reserve (RFR) is the capacity to increase glomerular filtration rate (GFR) under certain stimuli [1]. The latter could be endogenous, exogenous, physiological or pathological. The main physiological endogenous stimulus is pregnancy, while the physiological exogenous stimuli are oral protein loading and intravenous infusion of amino acids or dopamine. Pathological stimuli include a significant loss of renal mass, such as nephrectomy, and conditions associated with hyperfiltration, such as early diabetes and obesity [2].

The mechanisms behind RFR are not fully known. Physiological stimuli induce a similar increase in GFR and renal plasma flow (RPF) resulting from the reduction in both afferent and efferent glomerular arteriolar resistances. This leads to an increase in GFR without major changes in filtration fraction (FF) [3]. In the hormonal milieu of pregnancy, an increase in GFR up to 50% occurs for adaptations to volume expansion and systemic vasodilation [4]. Amino acids increase sodium proximal tubular reabsorption, activating the tubuloglomerular feedback (TGF), and stimulate endocrine and paracrine factors, including glucagon, nitric oxide (NO) and prostaglandins (PGs) [3]. Pathological stimuli lead to an increase in GFR greater than RPF by decreasing pre-glomerular and/or increasing post-glomerular resistances. It follows an elevation in FF and glomerular hypertension [5]. Hyperfiltration in diabetes and obesity is caused by an imbalance of mediators regulating the arteriolar tone and by TGF activation following increased sodium proximal tubular reabsorption [5]. Another possible explanation to the activation of renal reserve is that the resting kidney has a population of 'dormant nephrons' that normally contribute little or nothing to filtration. Under stress, these nephrons are called into play, leading to an increase in GFR and RPF [6]. However, this attractive hypothesis is not supported by evidence, particularly in humans.

RFR can be assessed by measuring GFR before and after an amino acid infusion or oral loading [7]. Notably, RFR may not always be easy to detect: in situations of hyperfiltration, such as pregnancy, obesity or early diabetes, the increase in GFR after stimulation may not be found [8].

The determinants and clinical relevance of RFR—presence or absence—are not completely clear. On the one hand, the lack of RFR could be associated with low renal endowment or reduced renal mass due to various causes or inadequate haemodynamic reaction to stimuli. This may determine a worse kidney response to injuries. On the other hand, the presence of RFR may indicate larger nephron mass as well as better capacity to overcome kidney damage [2]. Therefore, RFR assessment may have a prognostic value in clinical practice, informing about the kidney's ability to adapt to physiological or pathological stimuli. However, the factors associated with the presence or absence of RFR and its clinical meaning remain underinvestigated [3, 9].

In this study, we evaluated the clinical characteristics, phenotypes and factors associated with the presence or absence of RFR in healthy subjects—i.e. living kidney donors. Renal function was evaluated with measured GFR (mGFR)—the clearance of iohexol—and RFR with the infusion of amino acids.

MATERIALS AND METHODS

Study design

This observational study was designed to analyse the characteristics associated with the presence or absence of RFR in healthy subjects—living kidney donors—before donation. The present analysis is part of the ongoing clinical trial evaluating the

impact of RFR on the evolution of renal function in donors and recipients (EudraCT number: 2018-001710-15). This work focused on the basal characteristic of the donors. The protocol was approved by the Ethics Committee of the Hospital Universitario de Canarias (HUC).

Living kidney donors

Inclusion criteria were age >18 years; eligibility for kidney donation according to the clinical practice of our centre; clinical stability at the moment of the examination, defined as the absence of acute episodes that may influence renal function, such as hypovolaemia, use of non-steroidal anti-inflammatory drugs, severe infectious diseases, acute cardiovascular disease or acute kidney injury.

Subjects were excluded if ineligible for kidney donation, including the presence of diabetes, body mass index (BMI) ≥35 kg/m^2 and a history of cardiovascular events; unable to understand the protocol; had severe psychiatric illness; or allergic to one or more of the amino acids present in the solution or to iodine or contrast media.

Procedures and measurements

Before transplantation, we collected baseline data for all consecutive living kidney donors evaluated in the Living Donor Unit of the HUC.

Variables

General variables included age, gender and smoking status.

Concomitant conditions included arterial hypertension (systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or the use of medications); impaired fasting glucose (fasting glucose 100–125 mg/dl) and/or an impaired glucose tolerance test (glucose level 2 hours after an oral glucose overload of 140–199 mg/dl) and/or a glycated haemoglobin (HbA1c) of 5.6–6.4% [10]; and dyslipidaemia (total cholesterol ≥250 mg/dl and/or low-density lipoprotein (LDL) ≥130 mg/dl and/or triglycerides ≥200 mg/dl and/or the use of medications).

Anthropometric measures included weight, height, BMI and body surface area (BSA), calculated by the DuBois and DuBois formula [11].

Analytics included haemogram, creatinine, total cholesterol, LDL, high-density lipoprotein (HDL), triglycerides (TGs), uric acid, fasting glucose and HbA1c. Urinalysis included proteinuria, albuminuria and creatinine clearance performed on a 24-hour sample.

An oral glucose tolerance test (OGTT) was performed as part of the clinical evaluation of donors. At fasting, donors received an oral load of 75 g of glucose, then blood samples for glucose were taken before and 2 hours after the load [10].

All possible donors in the Canary Islands undergo mGFR with the plasma clearance of iohexol using dried blood spots (DBSs) as has been described previously [12, 13]. In brief, 5 ml of the iohexol solution (Omnipaque 300, GE HealthCare, Madrid, Spain) are injected intravenously in a forearm vein over 2 minutes. Then, 120 minutes after the injection, capillary blood (10 μl) is taken by finger prick, collected by a capillary pipette and deposited on filter paper at 120, 150, 180, 210 and 240 minutes. Iohexol was measured in DBSs as previously described by high-performance liquid chromatography [12, 13] and iohexol clearance (ml/min) was calculated according to a one-compartment model and then corrected by the formula proposed by Bröchner-Mortensen [14]. GFR was unadjusted to analyse the impact of weight changes on mGFR without the interference of the adjustment for BSA. For this particular study, two determinations of mGFR were performed, before and after stimulation with intravenous amino acids to test RFR.

A RFR test was performed 5–7 days after the baseline mGFR. We followed the protocol described by Livi *et al.* [15]. In brief, with an 8-hour fast, a Nephrotect (Fresenius Kabi, Bad Homburg, Germany) solution (totally amino acids 100 g/l) was injected at an infusion rate of 4.16 ml/min for 2 hours and 30 minutes (total infusion 600 ml). Then, 30 minutes after completing amino acid infusion, the procedure for mGFR was performed as indicated above. Finally, RFR calculation was performed as the absolute or percentage variation of the plasma clearance of iohexol before and after amino acid overload. Before tests (mGFR or RFR), donors were instructed to avoid high-intensity exercise and a diet rich in proteins and fats.

All donors undergo renal ultrasound and computed tomography scans to evaluate renal morphology, arteries and veins and kidney length.

Statistical analysis

Definition of RFR

This is an exploratory analysis in which we wanted to evaluate the clinical characteristics of the subjects with and without RFR. Accordingly, we had to define RFR. Although RFR indicates an increase of GFR after stimulation, the magnitude of the change in GFR to establish its presence or absence is not established by consensus. Thus, to define the presence or absence of RFR, we had to select a threshold of the variation in mGFR after stimulation with amino acids. Every time a determination is repeated, intra-individual variability of the method must be taken into consideration. In our laboratory, the intra-individual variability of mGFR using the clearance of iohexol is ≈3% (Sergio Luis Lima, personal communication). Thus, *a priori*, to be sure that the change in GFR was not associated with variations in the method, we defined the presence of RFR as an increase in GFR at least three times larger than this 3% variability (i.e. >10% of increment in mGFR). Accordingly, subjects with an mGFR change after amino acid stimulation <10% were considered as having no RFR. However, it is known that there are some conditions where RFR can be in use, as in subjects with metabolic syndrome, obesity and diabetes, where diverse stimuli promote hyperfiltration. Thus it is possible that in some cases the lack of response to amino acids indicates not the absence of RFR, but its use due to factors active before the stimulation [8]. Theoretically, this group should have higher levels of GFR than those without RFR due to other causes. Then, in an attempt to individualize this subgroup of subjects, we established a threshold of baseline mGFR <100 ml/min or ≥100 ml/min for subjects without RFR. According to these definitions, subjects were classified as those without RFR (group A): baseline GFR <100 ml/min without major changes (<10%) in GFR after stimulation; those with RFR (group B), with changes (>10%) in GFR after stimulation; and those without RFR but using it (group C): baseline GFR ≥100 ml/min without major changes (<10%) in GFR after stimulation.

Results are expressed as mean ± SD and binary variables as numbers (%). Comparison between groups was performed with analysis of variance or chi-squared tests as determined by the characteristic of the variable. For analysis we used SPSS version

Table 1: Basal characteristics of subjects without, with and using RFR.

Characteristics	Total (N = 80)	Without RFR (group A) (n = 26)	With RFR (group B) (n = 27)	Using RFR (group C) (n = 27)
Age (years), mean ± SD	49 ± 9	53 ± 8	48 ± 7	48 ± 10
Female, n (%)	56 (70)	21 (81)[a]	23 (85)[b]	12 (44)
Body height (cm), mean ± SD	167 ± 9	164 ± 9[c]	165 ± 8	170 ± 9
Body weight (kg), mean ± SD	74.6 ± 14.2	65.3 ± 11.8[d]	75.9 ± 12.7	82.1 ± 13.2[e]
BMI (kg/m^2), mean ± SD	26.79 ± 4.25	24.26 ± 4.24[f]	27.74 ± 3.64	28.27 ± 3.83[g]
BSA (m^2), mean ± SD	1.83 ± 0.2	1.71 ± 0.17[h]	1.83 ± 0.18	1.94 ± 0.18[i]
Smoking habit, n (%)				
Never	48 (66)	15 (58)	16 (59)	17 (63)
Former smoker	12 (15)	4 (15)	3 (11)	5 (18.5)
Active smoker	20 (25)	7 (27)	8 (30)	5 (18.5)
Concomitant conditions, n (%)				
Obesity (BMI ≥27)	40 (50)	5 (19)[j]	18 (67)	17 (63)[k]
Arterial hypertension	11 (14)	1 (4)[l]	2 (7)	8 (30)
Dyslipidaemia	29 (36)	12 (46)	8 (30)	9 (33)
Prediabetes	20 (25)	4 (15)	7 (26)	9 (33)
Analytics, mean ± SD				
Total cholesterol (mg/dl)	185 ± 32	187 ± 27	182 ± 36	184 ± 34
LDL (mg/dl)	107 ± 26	111 ± 23	102 ± 25	107 ± 30
HDL (mg/dl)	55 ± 14	59 ± 12	57 ± 15	51 ± 13
Triglycerides (mg/dl)	105 ± 52	98 ± 44	91 ± 37[m]	125 ± 65
Uric acid (mg/dl)	4.3 ± 1.6	4 ± 1.3	4.5 ± 0.9	4.3 ± 2.3
Fasting glucose (mg/dl)	91 ± 7	90 ± 5	90 ± 6	92 ± 10
OGTT (mg/dl)	105 ± 24	102 ± 21	100 ± 25	112 ± 24
HbA1c (%)	5.35 ± 0.33	5.31 ± 0.26	5.44 ± 0.35	5.32 ± 0.35
Renal function				
Serum creatinine (mg/dl)	0.79 ± 0.16	0.79 ± 0.13	0.78 ± 0.16	0.80 ± 0.18
Albuminuria (mg/24 h)	6.5 ± 6.2	7 ± 8	6 ± 4	7 ± 5
Basal mGFR (ml/min)	101 ± 22	85 ± 9[n]	93 ± 15[o]	123 ± 20
Stimulated mGFR (ml/min)	105 ± 17	88 ± 8[p]	111 ± 16	115 ± 12[q]
Kidney size by CT (cm), mean ± SD				
Left kidney length	10.8 ± 0.9	10.4 ± 0.8[r]	10.6 ± 0.6[s]	11.4 ± 0.8
Right kidney length	10.6 ± 0.9	10.2 ± 0.7[t]	10.5 ± 0.8[u]	11.2 ± 1

Statistics significance: [a]group A versus C, $P = .015$; [b]group B versus C, $P = .004$; [c]group A versus C, $P = .031$; [d]group A versus B, $P < .001$; [e]group C versus A, $P < .0001$; [f]group A versus B, $P = .005$; [g]group C versus A, $P = .001$; [h]group A versus B, $P = .039$; [i]group C versus A, $P < .0001$; [j]group A versus B, $P = .001$; [k]group C versus A, $P = .003$; [l]group A versus C, $P = .024$; [m]group B versus C, $P = .049$; [n]group A versus C, $P < .0001$; [o]group B versus C, $P < .0001$; [p]group A versus B, $P < .0001$; [q]group A versus C, $P < .0001$; [r]group A versus C, $P < .0001$; [s]group B versus C, $P < .0001$; [t]group A versus C, $P < .0001$; [u]group B versus C, $P = .008$.

25.0 (IBM, Armonk, NY, USA). *P*-values of .05 were considered statistically significant.

Sensitivity analysis

We evaluated the variables associated with RFR using a multinomial logistic regression analysis where the outcome was the absence, presence or use of RFR. In the model (univariable and multivariable) we included those variables with a proven association with RFR: age, baseline GFR (before stimulation), kidney length, weight, BMI and BSA. The last three variables were not included simultaneously. One variable was introduced every 8–10 events.

All data and information were stored in a secure online database (RedCap) designed ad hoc for the study, using the web application provided by the RedCap Consortium (http://www.project-redcap.org/). Based on Spanish law for data protection, all data were anonymized and the identification of subjects was stored and was not accessible from the internet.

RESULTS

A total of 80 subjects met the inclusion criteria. The mean age was 49 ± 9 years and most were women (70%). Weight and BMI averaged 74.6 ± 14.2 kg and 26.8 ± 4.3 kg/m^2, respectively, and BSA was 1.83 ± 0.2 m^2. A total of 11 (14%) subjects had hypertension and 20 (25%) had prediabetes. The average basal mGFR was 101 ± 22 ml/min (Table 1). A total of 26 (32%) subjects had no RFR, 27 (34%) had RFR and 27 (34%) were using RFR (Fig. 1A).

Group A—donors without RFR

Basal mGFR was 85 ± 9 ml/min with no relevant changes after amino acid load. Right and left kidney lengths were 10.2 ± 0.7 and 10.4 ± 0.8 cm, respectively. These subjects were mostly females (81%), 53 ± 8 years of age, with lower BMI (24.3 ± 4.2 kg/m^2) and BSA (1.71 ± 0.17 m^2) than the other two groups (Fig. 1B). One patient (4%) had hypertension, 4 (15%) had prediabetes and 12 (46%) dyslipidaemia.

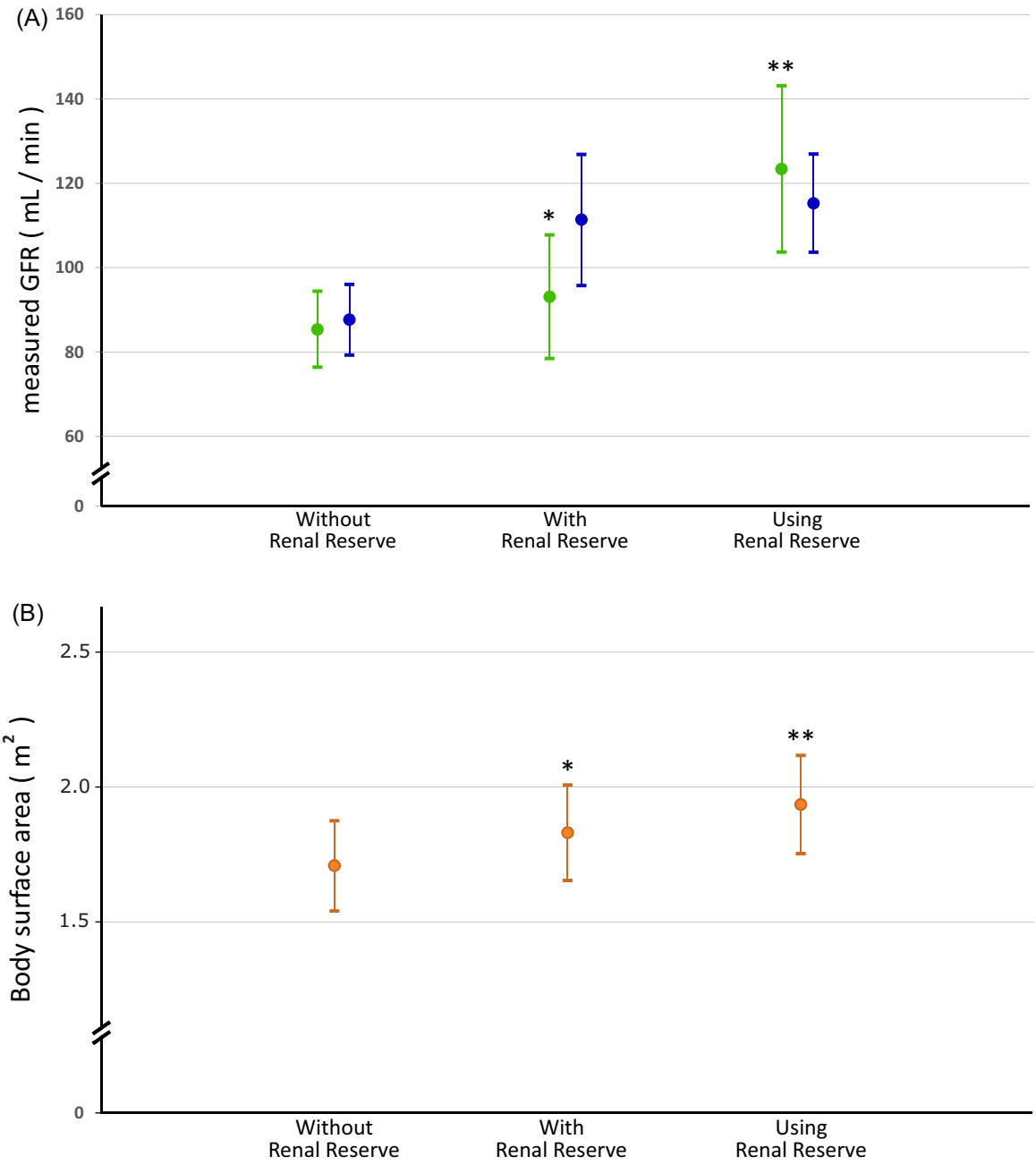

Figure 1: **(A)** Measured GFR at baseline (green dots) and after the induction of renal functional reserve (blue dots) and **(B)** BSA in subjects without, with or using RFR. *Baseline GFR in subjects with RFR versus subjects using RFR ($P < .0001$). **Baseline GFR in subjects using RFR versus subjects without RFR ($P < .0001$). *BSA in subjects with RFR versus subjects without RFR ($P = .039$). **BSA in subjects using RFR versus subjects without RFR ($P < .0001$).

Group B—donors with RFR

Basal mGFR was 93 ± 15 ml/min and it increased to an average of 111 ± 16 ml/min after amino acid overload. Right and left kidney lengths were 10.5 ± 0.8 and 10.6 ± 0.6 cm, respectively. Age was 48 ± 7 years and the donors were predominantly female (85%). Compared with group A, they had higher BMI (27.7 ± 3.6 kg/m^2; $P = .005$) and BSA (1.83 ± 0.18 m^2; $P = .039$) (Fig. 1B). Two subjects had arterial hypertension (7%), seven (26%) prediabetes and eight (30%) had dyslipidaemia.

Group C—donors using RFR

Basal mGFR was 123 ± 20 ml/min. After amino acid overload, the GFR decreased in some cases, leading to a mean of 115 ± 12 ml/min. Right and left kidney lengths were 11.2 ± 1 and 11.4 ± 0.8 cm, respectively. Donors were 48 ± 10 years of age, with an almost balanced gender distribution (females 44%). BMI and BSA were 28.3 ± 3.8 kg/m^2 and 1.94 ± 0.18 m^2, respectively. A third of donors presented with arterial hypertension (30%), dyslipidaemia (33%) and prediabetes (33%). Compared with groups

Table 2: Multinomial logistic regression: subjects without RFR versus those with RFR or those using RFR.

Subjects		β	P-value	OR	95% CI
With RFR	Basal mGFR	0.05	0.08	1.06	0.99–1.12
	BMI	0.18	0.02	1.20	1.03–1.40
Using RFR	Basal mGFR	0.22	0.0001	1.24	1.12–1.38
	BMI	0.02	0.885	1.02	0.81–1.28

BMI: Body mass index

A and B, this group had the highest basal mGFR, kidney size, BMI and BSA (Fig. 1B). Basal mGFR and kidney size were significatively higher than in both group A and B. BMI and BSA were significatively higher than in group A ($P = .001$ and $P < .0001$, respectively) and comparable for group B ($P =$ ns for both). The prevalence of females was less marked compared with the other two groups.

Sensitivity analysis

In the multinomial logistic regression analysis (Table 2), the variables associated with RFR were the following: (a) comparing donors with RFR versus those without RFR: baseline (pre-stimulation) mGFR, with borderline significance {odds ratio [OR] 1.06 [95% confidence interval (CI) 0.99–1.12], $P = .08$} and BMI [OR 1.20 (95% CI 1.03–1.40), $P = .02$]; (b) comparing those using RFR versus those without RFR: baseline (pre-stimulation) mGFR: [OR 1.24 (95% CI 1.12–1.38), $P < .0001$] and BMI was not significant. Replacing BMI by weight or BSA led to similar results, although BSA induced more instability in the model. Other variables were not significant (data not shown).

DISCUSSION

We analysed the characteristics of healthy potential kidney donors regarding the presence or absence of RFR before donation. Subjects without RFR were predominantly females with lower BSA and mGFR. Those with RFR were also mostly women, but with higher BSA and mGFR. Finally, subjects apparently using RFR were those with the largest BSA and mGFR, with a comparable distribution of gender.

To evaluate RFR, we used two gold standard methods: the plasma clearance of iohexol to measure GFR [13] and the intravenous infusion of mixed amino acids to test RFR [15]. By using mGFR we avoid the unpredictable error of estimated GFR by formulas [16]. Furthermore, mGFR was not adjusted by BSA to avoid the problems associated with interpretation of renal function as a function of BSA or as a function of weight and height [17]. The adjustment by BSA and its reference to 1.73 m^2 (supposedly a representative BSA) frequently leads to major changes in GFR: in obese subjects, GFR is decreased and in lean subjects it is increased, both artificially [17–19]. Moreover, the adjustment by BSA eliminates gender differences in renal function [17].

Our main finding was that the absence or presence of RFR was associated with differences in gender, weight, BMI, BSA and basal GFR. The explanation of this phenomenon is complex. In group A (donors without RFR), the majority of the donors were female with lower BSA, height and weight than in the other groups. Interestingly, the GFR was low, although still above the threshold for donation. Several epidemiological studies have reported a direct relationship between height and total kidney volume or length [20, 21]. Short subjects tend to have smaller kidneys and lower renal mass, whereas tall subjects have larger kidneys and higher renal mass. Thus it is plausible that this group has lower renal mass compared with groups B and C, although sufficient to ensure a normal GFR. Another aspect to consider is the fact that most of the donors in this group are women (81%). Previous studies observed that women may have smaller kidneys and lower GFR than men [22, 23]. For example, Johnson *et al.* [24] observed in >200 living donors that women have lower renal parenchymal volume than men [24]. Also, women may have a lower number of glomeruli than men, approximately 9–12% [22, 25]. So, considered as a group, women may have lower renal endowment and GFR than men. It is plausible that the lack of RFR in this group is the combination of two factors related with lower renal endowment: short height and female gender. However, it must be considered that these donors had levels of GFR above the threshold for donations, which may indicate that the low renal endowment could be borderline, enough to result in good GFR levels but not enough to increase GFR after stimuli.

In group B (donors with RFR), most of donors were women with comparable characteristics with group A except for a higher BSA due to a moderate increment in weight (not in height). Why two groups, comparable in many variables that can affect renal function, differ in the presence or absence of RFR is intriguing. It could be argued that women in this group have larger renal endowment than those in group A because they had a greater BSA and height. Renal endowment is highly variable. The total amount of nephrons with which an individual is born may range from 200 000–300 000 to 1.4–1.8 million units per kidney, even within the healthy population [26]. We may speculate that this group has a higher renal endowment compared with group A (in line with a greater height and BSA), which allowed the response to the amino acid stimulus rather than the group without RFR. According to this hypothesis, the baseline GFR was higher than in group A, although not statistically significant.

Finally, group C (donors using RFR) consisted of a more balanced number of men and women, with the largest BSA and GFR compared with the previous groups. However, the subjects in this group did not exhibit RFR and, in many cases, a reduction in GFR after stimulation was observed. This finding is intriguing. The decrease in GFR after stimuli to evaluate RFR has been described before in subjects with high GFR [8]. Since the level of GFR could be considered unexpectedly high, we may think that these subjects were hyperfiltering, a condition where RFR cannot be easily evaluated [8]. This is why we considered that the subjects in this group were indeed using their RFR. One of the main causes of hyperfiltration is the increase in metabolic demand associated with weight [5, 27]. This group had the highest BMI and BSA and the greatest kidney size. Taken together, these observations would explain both the presence of sufficient renal endowment and the concomitant state of hyperfiltration induced by the use of RFR. Another possible explanation for the decrease in GFR after stimulation could be vascular instability due to hypertension and the use of antihypertensive drugs.

As indicated above, the different responses of GFR to the stimuli of amino acids may be due to different levels of renal endowment even in healthy subjects with good health and renal function. The capacity of the response of GFR to metabolic demand is influenced by nephron mass. Unfortunately, up to now there have been no simple methods to evaluate nephron endowment and so our hypothesis needs further testing when these methods become available. Also, we consider that the division of subjects into three groups was relevant. Without this, we would have included in the same group subjects without RFR and low GFR and those with high GFR and using RFR, two groups that are clearly different. Also, the differences between gender, BSA and

GFR would have been even. In conclusion, we believe it is crucial to make such a distinction, as it has clinical implications comparable to having or not having RFR. This discrimination has not been addressed in previous studies [9].

Living kidney donation could be considered a clinical model to study the impact of different stimuli on two comparable kidneys. In both the donor and the recipient, it is helpful to understand how the kidney responds to a halving of the renal mass after nephrectomy; to changes in metabolic demand, which may increase or decrease; and to different types of damage. In the specific case of the recipient, allograft rejection, infection, nephrotoxicity and recurrent or de novo renal disease may occur. In this scenario, the evaluation of RFR may have a clinical role. Measuring RFR may inform about the kidney's ability to adapt to physiological or pathological stimuli. In the context of living kidney transplantation, which consists of a nephrectomy that halves the nephron number of a subject, this could help in estimating the risk of renal function decline and response to renal damage over time. The absence of RFR could indicate in donors limitations to compensate for the loss of renal mass. In the recipient, the graft may struggle to adapt to a higher metabolic demand, which typically occurs when the recipient has a higher weight and BSA than the donor [28, 29]. Finally, both in donors and recipients, the lack of RFR may indicate an impaired capacity to overcome eventual injuries. The presence of RFR, instead, is a good basis for the kidney to better adapt to new situations, both in the donor and the recipient, and to respond to injury. In donors who are using their RFR, it would be advisable to intervene in order to reduce the factors that induce hyperfiltration, being not only a risk for long-term deterioration of renal function [30–32], but also a cardiovascular risk factor [33]. In conclusion, the measurement of RFR may provide an extra check on renal health beyond the traditional tests of renal function. However, these hypotheses must be tested in long follow-up studies, with repeated measurements over time of both GFR and RFR [9].

The study has limitations that must be addressed. First, it is an exploratory analysis with a limited number of cases. Thus this is a hypothesis-generating study that must be tested in larger cohorts. Second, the cut-off points used to group subjects into those using RFR are arbitrary, as are most of the cut-offs used with GFR. Thus our results must be tested and confirmed in larger series with diverse thresholds for GFR. Third, the method of estimation of renal mass can be questionable since the kidney length is less precise than total kidney volume. However, kidney length has a well-established correlation with patient height [20, 21], which provides a reasonable approximation of renal size [34]. Fourth, we did not have birthweight data, which would have given us additional insights into the presence or absence of RFR. Also, renal plasma flow would have provided more insights in the interpretation of the results. Finally, the lack of follow-up requires further research, which is ongoing and shall illustrate the effect of pre-donation RFR in long-term outcomes.

We found that the presence or absence of RFR was associated with differences in gender, BSA and basal GFR. Renal endowment may play a role in this. The determination of RFR in living kidney donors could help estimate the kidney's ability to adapt to physiological or pathological stimuli. This may help to estimate the risk of renal function decline and the response to kidney damage over time.

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AUTHORS' CONTRIBUTIONS

A.G.R. and E.P. developed the original idea and designed the protocol of the study. Y.R.V., M.S.R., E.M.I., A.A.B., A.M., E.C.M., S.G., F.G.C., A.F.H., L.P.T., D.H.M., B.E. and A.G.R. were responsible for patient recruitment and data collection. C.A.S. was responsible for data quality checks. A.G.R., E.P., C.A.S. and E.R. wrote the first version of the manuscript. S.L.L., L.D.M., C.C. and F.G. performed the clearance of iohexol and the renal functional reserve. F.G.R. designed the database for the study.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared upon reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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