Higher body mass index is associated with higher fractional creatinine excretion in healthy subjects

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Abstract

Background. Accurate glomerular filtration rate (GFR) measurement in normal to high range is important for epidemiological studies and workup for kidney donation. Creatinine-based equations perform poorly in this GFR range. Creatinine clearance (CrCl) provides a substitute, provided urine is collected accurately and tubular creatinine handling can be accounted for. The latter is poorly characterized in the normal GFR range.

Methods. Therefore, we studied performance of CrCl, fractional creatinine excretion (FE⁰) and its determinants in 226 potential kidney donors (47% males, mean 53 ± 10 years). GFR was assessed as ¹²⁵I-iothalamate clearance, simultaneously with 2-h CrCl and 24-h CrCl.

Results. Mean GFR was 101 ± 18, 2-h CrCl 110 ± 20 and 24-h CrCl 106 ± 29 mL/min/1.73 m². Mean bias of 24 h CrCl was 7.4 [inter-quartile range -6.7 to 20.0] mL/min/1.73 m², precision (R²) 0.39 and 30% accuracy 82%. Mean FE⁰ was 110 ± 11%. FE⁰ correlated with body mass index (BMI) (r = 0.34, P < 0.001). Consequently, bias of 24-h CrCl increased from 2.7 (inter-quartile range -6.5 to 16.7) to 8.6 (inter-quartile range -5.8 to 20.5) and 12.6 (inter-quartile range 7.0 to 25.4) mL/min in subjects with BMI <25, 25–30 and >30 kg/m², respectively (P < 0.05). On multivariate analysis, BMI and gender were predictors of FE⁰.

Conclusions. CrCl systematically overestimates GFR in healthy subjects. The overestimation significantly correlates with BMI, with higher FE⁰ in subjects with higher BMI. The impact of BMI on tubular creatinine secretion can be accounted for, when using CrCl for GFR assessment in the normal to high range, by the following formula: GFR = 24-h CrCl − (22.75 + 0.76 × BMI − 0.29 × mean arterial pressure (−6.11 if female).

Keywords: BMI; creatinine clearance; fractional creatinine excretion; GFR; renal function estimation

Introduction

Extensive experience is available on the measurement of renal function in subjects with renal function impairment [1, 2]. Recently, measurement of renal function in subjects without renal disease has become a focus of interest for early detection of renal disease in the general population [3], and screening of prospective kidney donors [4]. In addition, elevated glomerular filtration rate (GFR) has been identified as an early manifestation of a metabolic risk profile [5–7], re-emphasizing the need for well-validated renal function estimates for populations with renal function in the normal or higher range.

The gold standard for GFR assessment is by the clearance of specific tracers, such as inulin and ¹²⁵I-iothalamate [2], but these are not suited for routine measurements. Creatinine-based renal function equations are therefore recommended in several guidelines (i.e. KDOQI [8]), as these are simple and cheap, and thus well-suited to the outpatient setting [9, 10]. However, these equations were empirically developed in populations with renal function impairment, and their performance is modest to poor in healthy populations [11–14].

Creatinine clearance (CrCl) calculated from 24-h urine collection is not recommended currently as alternative [8], for two main reasons: inaccuracy due to inaccurate 24-h urine collection (i.e. the non-systematic error) and considerable overestimation of GFR by CrCl in subjects with renal function impairment, due to tubular secretion of creatinine [8, 15]. As tubular secretion is assumed to be relatively insignificant in subjects with normal to high GFR...
[8, 15], 24-h CrCl could be a suitable alternative for renal function measurement in healthy populations, provided that adequate 24-h urine collection is ensured by a dedicated setting with proper instructions for urine collection. Although various studies [16–18] have looked into the predictive value of 24-h CrCl for GFR and one interesting study by Baxmann et al. [19] investigated the determinants of serum and urinary creatinine levels, specific analyses of the determinants of bias in 24-h CrCl versus GFR in populations without renal function impairment have not been performed.

In the current study, therefore, we studied performance of 24-h CrCl in subjects without renal disease, as compared to clearance of 125I-iothalamate as gold standard (measured GFR) and analysed for determinants of the systematic error, as the latter could potentially be corrected for. To be able to assess the contribution of tubular creatinine secretion in a possible systematic error in 24-h CrCl, we assessed fractional excretion of creatinine (FEcrea) from simultaneous measurement of GFR and CrCl over a 2-h period (2 h CrCl) to refrain from diurnal factors [20] and collection errors.

**Materials and methods**

**Study population**

Two hundred and fifty subjects screened via GFR measurement as kidney donor between March 2006 and March 2009 were considered for inclusion in the study. Inclusion criteria were acceptance for the kidney donation programme, measured GFR >80 mL/min, measurement of 24-h CrCl, no history of diabetes and no proteinuria.

**Collection of blood and urine samples**

All collections of blood and urine were made on the day of renal function measurement. In the morning when patients arrived after overnight fasting, blood was drawn for measurement of serum creatinine, albumin and basic haematological parameters. Height, weight and blood pressure were recorded and renal haemodynamic measurements started, which are described in more detail below. Blood pressure was measured by Dinamap® after patients were in a semi-supine position for at least 15 min. Mean arterial pressure (MAP) was then calculated as [1/3 × systolic blood pressure + 2/3 × diastolic blood pressure]. After 1.5 h of continuous tracer infusion, i.e. when steady state was reached, blood was drawn and urine was collected, to make sure all urine collected thereafter was collected during steady state. Blood was drawn every hour thereafter for 4 h and urine was collected every 2 h for 4 h, while continuing infusion of 125I-Iothalamate and 131I-Hippuran, thus allowing for measurement of GFR and 2 h CrCl over two 2-h periods.

**Renal haemodynamic measurement**

GFR was measured by constant infusion of 125I-Iothalamate, with correction for voiding errors by simultaneous measurement of the clearance of 131I-Hippuran as described previously [21, 22]. At 08.00 h, a priming solution of 0.4 mL/kg body weight (BW) was administered plus an extra 0.6 MBq 125I-Iothalamate to ensure steady state of the plasma tracers within the time frame of the measurement.

Thereafter, continuous infusion was started with four MBq 131I-Hippuran and three MBq 131I-Iothalamate/100 mL saline. After a stabilization period of 1.5 h, two 2-h clearance periods followed. GFR was measured during the second 2-h clearance period (i.e. when steady state was achieved most accurately) as the urinary clearance of 125I-Iothalamate (U/V-P), where U = urinary concentration of 125I-Iothalamate, V = volume of urine collected over a certain period (i.e. 2 h) and P = plasma concentration of 125I-Iothalamate and corrected for voiding errors by multiplying U/V-P by the ratio of plasma clearance of 131I-Hippuran to urinary clearance of 131I-Hippuran. This correction method is based on the fact that, during steady state, the plasma clearance of 131I-Hippuran equals its urinary clearance when urine collection is perfect. Thus, the voiding error can be calculated from the ratio of urinary clearance and plasma clearance of 131I-Hippuran. This measured GFR measurement has a day-to-day coefficient of variation of 2.5% [23].

**Creatinine clearance**

Creatinine was measured with the Roche enzymatic creatinine assay, which is isotope dilution mass spectrometry-traceable. Serum creatinine samples were obtained during the measurement of 125I-Iothalamate GFR. All subjects collected a single 24-h urine sample the day preceding 125I-Iothalamate GFR assessment, being out of hospital. Twenty-four-hour CrCl was calculated as U/V-P, where U represents the concentration of creatinine in urine, V represents the volume of the 24-h urine sample and P represents the concentration of creatinine in serum.

Two-hour CrCl was simultaneously assessed with measured GFR. During the measurement of 125I-Iothalamate GFR, creatinine was measured in a 2-h urine portion also used for 125I-Iothalamate measurement. CrCl was subsequently calculated according to U/V-P and corrected for the voiding error analogous to the clearance of 125I-Iothalamate, allowing for precise CrCl calculation. From the same sample, FEcrea was also calculated as (UPcrea*100)/UPtot.

The MDRD equation for estimated GFR (eGFR) was calculated as follows: 1.154 × age0.203 × 0.742 if female) [23], where [crea] is serum creatinine in mg/dL.

The CKD-EPI equation was calculated as follows: 141 × min ([crea]/κ)1.1 × max ([crea]/κ)1.209 × 0.993 0.411 if female, where [crea] is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, λ = 0.203 for females and 0.198 for males, min indicates the minimum of [crea]/κ or one and max indicates the maximum of [crea]/κ or one [24].

The Cockcroft-Gault equation was calculated as follows: (140 – age) × weight/([crea] × 0.85 if female, where [crea] is serum creatinine in mg/dL [25].

The Inulin-Based eGFR equation was calculated as follows: (155 – age) × weight/([crea] × 8.84) × 0.85 if female, where [crea] is serum creatinine in mg/dL [26].

For comparison in bias between 2-h CrCl, 24-h CrCl, CKD-EPI, MDRD, Cockcroft-Gault, Inulin-Based eGFR and GFR, data corrected for body surface area (BSA) are given, calculated according to the Dubois’ formula (weight0.425 × length0.725 × 0.007184) [27]. Data were expressed as mL/min/1.73 m².

**Data analysis**

Data were expressed as mean ± SD when normally distributed or as median (inter-quartile range) when skewed. Pearson’s correlation coefficients were calculated for univariate correlations. Predictive performance of 2-h CrCl, 24-h CrCl, MDRD and CKD-EPI were assessed according to the method proposed by Bostom et al. [28], which expresses predictive performance of a measurement as bias, precision and accuracy. Bias is the mean prediction error and calculated as X(CrCl-GFR)/N, where X(CrCl-GFR) = predicted – true value and N = sample size.

Precision is a value for the degree of spread and expressed as Pearson’s correlation quotient (R²). Accuracy is expressed as % of observations within, respectively, 10 and 30% of true GFR. For the comparison of 2-h CrCl, 24 h CrCl and GFR, we used the method proposed by Bland and Altman [29].

Univariate and multivariate modelling was performed to determine significant predictors of systematic error in 2-h and 24-h CrCl. Univariate analysis included body dimension parameters, gender, age, MAP and 24 h creatinine excretion (reflecting muscle mass), all factors known to influence GFR/CrCl. Additional multivariate analyses were performed with multiple models, all parameters used in univariate analysis with P < 0.1 were included. Different models with these parameters were then built for each body dimension parameter [length, weight, body mass index (BMI) and BSA] to determine the best fitting model. Based on these models, formulas were constructed with variables that were significant upon multivariate analyses to adjust bias in CrCl for these variables.

Finally, strata were formed for variables associated (P < 0.1) with bias in both 2-h and 24-h CrCl. A table was constructed with these strata to compare trends in biases in different eGFR formulas and renal function over different strata.

**Statistical analyses**

were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL).

**Results**

Of 250 subjects eligible for the study, 226 subjects met the criteria for inclusion and were included in the current study.
Twelve subjects had GFR <80 mL/min and for 12 subjects, 2-h CrCl data were not available. All subjects were of Caucasian origin. Seven subjects had BMI <20 kg/m²; however, in all of these subjects, creatinine excretion was above values recommended for their respective body weight, indicating cachexia or malnutrition was not present in these subjects [30].

Characteristics and measured variables of the population are given in Table 1, showing that our population was middle aged with a slight preponderance of women, and, by default, normal renal function. Mean FE creat as calculated from the simultaneous clearances of 125I-iothalamate and creatinine was 110 ± 11%, indicating 10% of CrCl was accounted for by tubular secretion.

In Table 2, the predictive performance of 2-h CrCl, 24-h CrCl and eGFR for measured GFR is given, showing the mean prediction error (bias), the degree of spread (mean prediction error, the degree of spread (bias) MDSD and CKD-EPI with smaller bias and better accuracy of 58% and 75%, respectively). Cockcroft-Gault and IB-eGFR performed significantly better than 30% accuracy of 58% and 75%, respectively). Cockcroft-Gault and IB-eGFR underestimated measured GFR to a lesser extent (P < 0.001). Bias was higher for 2-h CrCl than for 24-h CrCl (mean 11.2 ± 13.3 mL/min/1.73 m² versus mean 6.8 ± 19.2 mL/min/1.73 m² respectively, P < 0.017), but precision was best for 2-h CrCl with $R^2$ of 0.76 and 30% accuracy of 96%. The bias in 2-h CrCl indicates a systematic error that cannot be due to collection errors, as voiding errors were corrected for by ratio of $^{131}$I-Hippuran serum concentration to urine clearance. For 24-h CrCl, precision and accuracy were poorer ($R^2 = 0.39; 30\%$ accuracy 82%). MDRD and CKD-EPI performed poorly with high bias (mean $-15.6$ mL/min/1.73 m² and $-24.2 \pm 19.5$ mL/min/1.73 m² respectively, $P < 0.001$), but precision was for 24-h CrCl and 2-h CrCl and patient characteristics are given. Univariate determinants of the bias of 24-h CrCl were body weight, BMI and FE creat. Univariate determinants for bias of 2-h CrCl were gender, body weight, BMI, BSA, 24-h urinary creatinine excretion and FE creat and univariate determinants for FE creat were gender, body weight, BMI and BSA.

Multivariate linear regression models were then constructed. The best fitting regression model with FE creat as dependent variable ($r = 0.380$) included the variables age, gender, MAP, 24-h urinary creatinine excretion, serum creatinine and BMI. In this model, only male gender ($\beta = 0.220, P = 0.024$) and BMI ($\beta = 0.343, P < 0.001$) were significant independent determinants of FE creat. Models with BW or BSA instead of BMI had a lower $r (r = 0.354$ and $r = 0.306$, respectively).

For bias in 24 h CrCl, the best-fitting model was constructed with gender, BMI and MAP ($r = 0.249, P = 0.007$), which provided the following formula:

\[
\text{Bias in 24 h CrCl} = 22.75 + (0.76 \times \text{BMI}) - (0.29 \times \text{MAP}) - (6.11 \text{ if female})
\]

A more simplified version with just BMI and gender ($r = 0.195, P = 0.025$) would give the following:

\[
\text{Bias in 24 h CrCl} = -7.08 + (0.78 \times \text{BMI}) - (4.36 \text{ if female})
\]

Correcting 24 h CrCl for these factors would thus give the following formula:

\[
\text{Corrected 24 h CrCl} = \text{Measured 24 h CrCl} - \text{Bias in 24 h CrCl}
\]

### Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total, N = 226</th>
<th>Males, n = 106</th>
<th>Females, n = 120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (%)</td>
<td>47/53</td>
<td>49/57</td>
<td>46/63</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.8 ± 10.1</td>
<td>52.9 ± 10.1</td>
<td>52.8 ± 10.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>80.1 ± 14.2</td>
<td>86.3 ± 12.9</td>
<td>74.6 ± 12.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 ± 0.09</td>
<td>1.80 ± 0.08</td>
<td>1.69 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 3.7</td>
<td>26.5 ± 3.3</td>
<td>25.9 ± 4.0</td>
</tr>
<tr>
<td>Creatinine excretion (g/day)</td>
<td>1.48 ± 0.48</td>
<td>1.81 ± 0.40</td>
<td>1.19 ± 0.33</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>94.1 ± 11.0</td>
<td>96.5 ± 9.8</td>
<td>92.0 ± 11.6</td>
</tr>
<tr>
<td>Measured GFR (mL/min)</td>
<td>113 ± 22</td>
<td>122 ± 21</td>
<td>105 ± 21</td>
</tr>
<tr>
<td>GFR/BSA (mL/min/1.73 m²)</td>
<td>101 ± 18</td>
<td>103 ± 17</td>
<td>99 ± 18</td>
</tr>
<tr>
<td>ERPF (mL/min)</td>
<td>380 ± 101</td>
<td>411 ± 97</td>
<td>369 ± 100</td>
</tr>
<tr>
<td>ERPF/BSA (mL/min/1.73 m²)</td>
<td>346 ± 83</td>
<td>346 ± 80</td>
<td>345 ± 86</td>
</tr>
<tr>
<td>CrCl/2 h (mL/min)</td>
<td>125 ± 27</td>
<td>136 ± 25</td>
<td>114 ± 25</td>
</tr>
<tr>
<td>CrCl/2 h/BSA (mL/min/1.73 m²)</td>
<td>110 ± 20</td>
<td>114 ± 19</td>
<td>107 ± 21</td>
</tr>
<tr>
<td>CrCl/24 h (mL/min)</td>
<td>120 ± 34</td>
<td>134 ± 29</td>
<td>107 ± 34</td>
</tr>
<tr>
<td>CrCl/24 h/BSA (mL/min/1.73 m²)</td>
<td>106 ± 29</td>
<td>113 ± 24</td>
<td>101 ± 31</td>
</tr>
<tr>
<td>MDRD (mL/min/1.73 m²)</td>
<td>83 ± 16</td>
<td>85 ± 17</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>CKD-EPI (mL/min/1.73 m²)</td>
<td>89 ± 15</td>
<td>92 ± 15</td>
<td>79 ± 15</td>
</tr>
<tr>
<td>FE creat (%)</td>
<td>110 ± 11</td>
<td>112 ± 11</td>
<td>109 ± 11</td>
</tr>
</tbody>
</table>
The quantitative impact of BMI on bias of CrCl and on Fe creat, respectively, is illustrated in Figure 3, providing scatter plots of BMI versus bias and Fe creat. To facilitate translation of the impact of different factors on renal function and biases, Table 4 provides data on measured GFR, Fe creat and biases of CrCl and different eGFR formulas for strata of gender, BMI and creatinine excretion. It shows higher bias in men as compared to women, irrespective of the formula used (all P < 0.05). GFR is higher in overweight and obese subjects (P < 0.05 for trend), but Fe creat is higher with higher BMI as well (P < 0.05 for trend), leading to higher bias in CrCl in overweight and obese subjects (P < 0.05). Bias in eGFR formulas that do not
Higher BMI increases tubular creatinine excretion

Table 3. Univariate linear regression analyses

<table>
<thead>
<tr>
<th></th>
<th>Bias 24 h CrCl</th>
<th></th>
<th>Bias 2 h CrCl</th>
<th></th>
<th>FEcreat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>P</td>
<td>B</td>
<td>P</td>
<td>B</td>
</tr>
<tr>
<td>Age</td>
<td>−0.01 (−0.27 to 0.27)</td>
<td>0.990</td>
<td>−0.09 (−0.27 to 0.08)</td>
<td>0.299</td>
<td>0.02 (−0.13 to 0.17)</td>
</tr>
<tr>
<td>Gender</td>
<td>−4.79 (−10.17 to 0.60)</td>
<td>0.081</td>
<td>−4.90 (−8.36 to −1.44)</td>
<td>0.006</td>
<td>−2.96 (−5.88 to 0.03)</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.20 (0.00–0.39)</td>
<td>0.048</td>
<td>0.35 (0.23–0.40)</td>
<td>&lt;0.001</td>
<td>0.24 (0.14–0.34)</td>
</tr>
<tr>
<td>Height</td>
<td>0.04 (−0.28 to 0.35)</td>
<td>0.826</td>
<td>0.20 (−0.01 to 0.39)</td>
<td>0.052</td>
<td>0.06 (−0.11 to 0.23)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.83 (0.09–1.56)</td>
<td>0.028</td>
<td>1.31 (0.87–1.76)</td>
<td>&lt;0.001</td>
<td>1.03 (0.65–1.41)</td>
</tr>
<tr>
<td>BSA</td>
<td>10.7 (−3.2 to 24.5)</td>
<td>0.131</td>
<td>21.6 (13.2–29.9)</td>
<td>&lt;0.001</td>
<td>13.6 (6.4–20.8)</td>
</tr>
<tr>
<td>MAP</td>
<td>−0.21 (−0.46 to 0.04)</td>
<td>0.097</td>
<td>0.00 (−0.16 to 0.16)</td>
<td>0.997</td>
<td>0.01 (−0.13 to 0.14)</td>
</tr>
<tr>
<td>24 h CrExcr</td>
<td>n/a^b</td>
<td>&lt;0.001</td>
<td>1.16 (1.12–1.19)</td>
<td>&lt;0.001</td>
<td>n/a^b</td>
</tr>
<tr>
<td>FEcreat (%)</td>
<td>0.62 (0.38–0.85)</td>
<td>&lt;0.001</td>
<td>1.57 (1.09–0.25)</td>
<td>0.003</td>
<td>2.79 (−0.34 to 5.93)</td>
</tr>
</tbody>
</table>

^a Univariate linear regressions analyses for baseline characteristics and, respectively, bias in 24-h creatinine clearance, bias in 2-h CrCl and FEcreat. 24-h CrExcr, 24-h creatinine excretion.

^b Not available due to arithmetic relationship.

Fig. 3. (A) Bias in 2-h CrCl plotted against BMI, (B) bias in 24-h CrCl plotted against BMI and (C) FEcreat plotted against BMI.

take body weight into account, i.e. MDRD and CKD-EPI, becomes larger with higher BMI, with underestimation of measured GFR larger in obese subjects (P < 0.05). Remarkably, biases of Cockcroft-Gault and IB-eGFR formula (data not shown), both formulas that take body weight into account, are larger in higher BMI categories (P < 0.05) as well. For creatinine excretion, as surrogate for muscle mass and protein intake, the trends are similar, with GFR higher in the groups with creatinine excretion (P < 0.05), but higher FEcreat with more creatinine excretion (P < 0.05), leading to higher biases in CrCl when creatinine excretion becomes larger (P < 0.05). Underestimation of GFR by MDRD and CKD-EPI (both P < 0.05) was higher when creatinine excretion was higher. However, bias in Cockcroft-Gault and IB-eGFR remained constant among different strata of creatinine excretion.

Discussion

In this study in healthy kidney donors, CrCl systematically overestimated GFR. The overestimation was significantly and independently associated with BMI, ranging from −6% in lean subjects to −15% in obese subjects. FEcreat indicated net tubular secretion of creatinine in these healthy subjects. Remarkably, a higher BMI was an independent determinant of higher FEcreat. Thus, the tubular handling of creatinine and the impact of BMI on tubular creatinine handling need to be accounted for in the interpretation of creatinine-based renal function as an estimate of GFR.

In line with other studies in healthy kidney donors before donation [16, 18], CrCl systematically overestimated GFR in our study. The interpretation of 24-h CrCl results can be hampered, due to urine collection errors and the diurnal rhythm of GFR. The strength of the current study is the simultaneous assessment of GFR and CrCl, eliminating the impact of collection errors and diurnal factors, and allowing for assessment of net tubular secretion of creatinine, as given by FEcreat. The mean FEcreat of 110% demonstrates the presence of net tubular secretion of creatinine in these healthy subjects. This is in line with older reports in a small number of chronic kidney disease patients [15]; our data are the first to show a BMI-dependent overestimation of GFR by CrCl in a large cohort of healthy subjects.

The larger number of subjects in our study allowed for the analysis of determinants of FEcreat. Interestingly, higher BMI was independently associated with higher FEcreat. Male gender was also independently associated with higher FEcreat. Higher FEcreat in male subjects has been reported in rodents [31, 32] previously, and although there have been a few studies on gender-defined differences in creatinine secretion in humans [33, 34], our study is the first to demonstrate higher FEcreat in male human subjects.

The mechanism of the association of higher FEcreat with higher BMI cannot be ascertained from our study. Data
from the literature support an association between creatinine supply (i.e. creatinine levels in peritubular capillaries) and tubular secretion of creatinine. First, the presence of net creatinine secretion in subjects with impaired renal function and accordingly elevated creatinine levels is well established [15, 35]. In healthy subjects, recent studies demonstrated that infusion of exogenous creatinine leads to increases in FEcre at up to 200% immediately after infusion [36, 37]. Thus, apparently, in healthy subjects, tubular secretion rate of creatinine increases with increased supply of creatinine. As male gender and BMI are both associated with larger muscle mass and hence creatinine supply, as also apparent from 24-h creatinine excretion, our data are consistent with the assumption that creatinine supply determines FEcre. This was also shown in a previous study [19], where urinary creatinine excretion correlated strongly with body weight, and even stronger with lean body mass, which is mainly determined by muscle mass. Unfortunately, lean body mass was not measured in our study, which would have allowed for better understanding of the correlation between FEcre and BMI, i.e. whether it is related to muscle mass or to weight excess.

However, the supply hypothesis does not explain why BMI was the main determinant of FEcre, rather than BSA or 24-h creatinine excretion. Differences in signal-to-noise ratio for the different parameters could be involved, but alternatively, specific effects of weight excess on tubular function could be postulated that deserve further investigation. It has been shown, for example, that not only glomerular hyperfiltration [38] but also tubular changes are present in obese subjects. So far, the functional significance of those tubular changes has not been addressed. Our current data may reflect the functional correlate of tubular changes associated with weight excess.

In conditions of compensatory renal hypertrophy, increased activity of tubular organic anion transporters (i.e. the transporters secreting creatinine) has been shown [39]. It would be of interest to investigate whether such tubular changes are also present in conditions of weight excess. Tubular creatinine transport may also be up-regulated via increased urinary acidity. Obesity is associated with lower urinary pH [40], which increases production of NH3 from glutamine, a process with net gain of ATP [41]. While this ATP is generally thought to be used for increased tubular sodium transport [42]. It might also be used to increase transport of creatinine via the organic anion transporter system.

For clinical application the systematic error in 24-h CrCl warrants attention. The average overestimation was smaller than for the simultaneously assessed CrCl, probably due to the lower nocturnal renal function, that is incorporated in 24-h CrCl. Nevertheless, a higher BMI was also significantly associated to a higher bias in 24-h CrCl [20]. Interestingly, BMI is also a well-established determinant of the systematic errors in creatinine-based equations [43], although this was not the case in our population. Whereas the overestimation was only 4 mL/min in lean subjects, it amounted to 7 and 12 mL/min in overweight and obese subjects, respectively. This systematic error will have to be taken into account when CrCl is used to evaluate renal function in subjects with weight excess.

Increasing evidence supports an association between early metabolic abnormalities and elevated CrCl [7, 44], assessed from creatinine-based renal function estimates in epidemiological studies in the general population as well as transplant recipients. Generally, the elevated CrCl in those studies is interpreted as hyperfiltration, which is supported by data obtained with true GFR measurements [45, 46], but our data show that a tubular component is likely to be involved in the elevated CrCl as well. It would be highly interesting to explore whether accounting for these different components of increased CrCl can improve its prognostic impact.

The group of Herrera and Rodriguez-Iiturbe [47] showed that healthy subjects increased their tubular creatinine secretion after a protein-rich meal about 4-fold, whereas CKD patients were unable to increase their tubular creatinine secretion. In another study, they showed [37] that the relative contribution of tubular creatinine secretion to CrCl is higher in kidney donors than in healthy controls. Based on these data, it could be hypothesized that tubular creatinine secretion might be a compensatory mechanism that is
Higher BMI increases tubular creatinine excretion

up-regulated when functional kidney mass is reduced but might be lost when the functional number of nephrons reaches a critical point. Measurement of tubular creatinine secretion might therefore reflect the functional number of nephrons and as such, further research into the predictive role of tubular creatinine secretion may be of interest for predicting renal function decline. While true GFR measurement has been advocated as the gold standard for renal function measurement and predicting disease progression, it may, however, well be that renal status is best reflected by a combination of a glomerular and a tubular component.

Another clinical implication of our findings relates to the calculation of fractional clearances. Our findings suggest that the calculation of a fractional clearance as clearance of a substance divided by clearance of creatinine is biased in a BMI-dependent way. This could bear impact on interpretation of, for example, FE<sub>Na</sub> <sup>[48]</sup> or albumin/creatinine ratio <sup>[49]</sup>. Therefore, considering the interaction between weight excess and renal sodium handling <sup>[46]</sup>, accounting for this bias would be highly recommendable to further unravel the relationship between BMI, tubular sodium handling and renal function decline.

The predictive performance of MDRD and CKD-EPI formulas in our population was in line with reports in the literature on populations with normal or slightly impaired renal function <sup>[11–14]</sup>. Our data indicate that, in a dedicated setting where subjects are motivated to accurately collect 24-h urine, 24-h CrCl is better suited than MDRD and CKD-EPI formulas for estimation of GFR when in the normal to higher range and that performance may be improved by correcting for BMI and gender influences. Performance of Cockcroft-Gault and iB-eGFR formulas that include body weight was markedly better than performance of MDRD and CKD-EPI and they may therefore provide a reasonable alternative in the normal to high GFR range when 24 h CrCl is not available. Whether our findings hold true in subjects with lower GFR would be of interest, further validation studies in larger cohorts are needed to confirm our findings in both normal and lower GFR range.

Several limitations of our study should be considered. First, we used a single outpatient collection of 24-h urine. Inaccurate urine collection is considered the most important threat to the validity of 24-h urine. The use of two or three 24-h urine samples to improve the validity of 24-h CrCl has been proposed <sup>[50]</sup>, so possibly the predictive performance of 24-h CrCl could be improved. However, the finding of the BMI dependency of the bias was robust and replicated in 2-h CrCl that is independent of collection errors.

Second, we had no information on food, and particularly meat, intake, which may have been involved in some of the associations we observed. As shown, dietary protein intake is a determinant of renal function <sup>[51]</sup>; furthermore, correlations exist between protein intake, muscle mass and creatinine excretion <sup>[19]</sup>. As information on body composition and hence muscle mass was unavailable, these correlations could not be studied. Furthermore, due to data on body composition not being available, subjects with lower BMI may have suffered from cachexia or malnutrition, but creatinine excretion was in the normal range in all subjects, indicating proper nutritional status. Although this lack of information does not refute our main finding, i.e. the consistent association between higher BMI and higher FE<sub>crea</sub>, it was not possible to dissect whether our finding is mainly due to weight excess or to muscle mass as well.

In conclusion, in this population of healthy kidney donors, tubular secretion of creatinine was determined by BMI, leading to BMI-dependent bias of CrCl. Accounting for the impact of BMI on tubular creatinine handling may improve feasibility of 24-h CrCl for renal function assessment in subjects with renal function in the normal or upper range and in studies addressing the renal phenotype of obesity.

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