

# Estimating Extracellular Fluid Volume in Healthy Individuals: Evaluation of Existing Formulae and Development of a New Equation



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**Introduction:** Several clinical settings require an accurate estimation of the physiologically expected extracellular fluid volume (ECFV). We aimed to analyze the performances of existing ECFV-estimating equations and to develop a new equation.

**Methods:** The performances of 11 ECFV-estimating equations were analyzed in 228 healthy kidney donor candidates (Bichat Hospital, Paris, France) who underwent ECFV measurement using the distribution volume of <sup>51</sup>Cr-labeled EDTA (<sup>51</sup>Cr-EDTA). An equation was developed using a penalized linear modeling approach (elastic net regression) and externally (Tenon Hospital, Paris, France,  $N = 142$ ) validated.

**Results:** Participants from Bichat (mean age  $45.2 \pm 12.0$  years, 43.0% men) and Tenon ( $47.8 \pm 10.3$  years, 29.6% men) hospitals had a mean measured ECFV of  $15.4 \pm 2.8$  l and  $15.1 \pm 2.1$  l, respectively. Available ECFV-estimating formulae have highly variable precision and accuracy. The new equation incorporating body weight, height, sex, and age had better precision and accuracy than all other equations in the external validation cohort, with a median bias of  $-0.20$  (95% CI:  $-0.35$  to  $-0.05$ ) l versus  $-2.63$  ( $-2.87$  to  $-2.42$ ) l to  $-0.57$  ( $-0.83$  to  $-0.40$ ) l and  $0.21$  ( $0.12$  to  $0.43$ ) l to  $2.89$  ( $2.65$  to  $3.11$ ) l, for underestimating and overestimating equations, respectively, an interquartile range for the bias of  $0.88$  ( $0.70$  to  $1.08$ ) l versus  $0.91$  ( $0.71$  to  $1.20$ ) l to  $1.93$  ( $1.67$  to  $2.25$ ) l, and an accuracy within 10% of 90.9% ( $83.8$  to  $94.4$ ) versus 88.0% ( $81.0$  to  $92.3$ ) to 8.5% ( $4.2$  to  $13.4$ ). These results were consistent across subgroups defined by sex, body mass index (BMI), body surface area (BSA), age, and ethnicity.

**Conclusion:** We developed and validated a new equation to estimate the individual reference value of ECFV, which is easily usable in clinical practice. Further validation in cohorts including individuals of extreme age and corpulence remains needed.

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KEYWORDS: <sup>51</sup>Cr-EDTA; equation estimation; extracellular fluid volume; isotope; reference value

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Extracellular fluid volume (ECFV) is tightly regulated by the kidneys, through the modulation of urinary sodium excretion. In healthy individuals,

ECFV varies markedly with anthropometric parameters and is therefore not readily predictable. In patients, measured ECFV may be higher (overhydration) or lower (dehydration) than the theoretical (physiologically expected) value. Therefore, individual estimation of the theoretical ECFV is of major clinical importance to quantify the magnitude of ECFV deviation from the normal condition. Indeed, if different tools, including bioelectrical impedance spectroscopy, have been

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developed to measure ECFV,<sup>1</sup> evaluating the degree of overhydration or dehydration requires accurate estimation of the physiologically expected individual extracellular volume. In addition, in the last decades, new simplified techniques of glomerular filtration rate (GFR) measurement based on single-sample plasma clearance raised attention on the importance of theoretical ECFV evaluation.<sup>2</sup> Finally, several authors have suggested that GFR should be expressed scaled to ECFV rather than to BSA, because this might be more physiologically and clinically relevant for the assessment of renal function.<sup>3–9</sup> Indeed, the ratio GFR/ECFV indicates the fraction of the ECFV that passes the glomerular membranes as an ultrafiltrate of plasma per unit time and thus indicates how often “that which is to be regulated” (i.e., the ECFV) comes into contact with the “regulator” (i.e., the kidneys).<sup>3,4</sup> An accurate prediction of the theoretical ECFV in a given individual is therefore important in many clinical settings.

The historical gold standard for ECFV measurement was established as the volume of distribution of bromide, determined from the total remaining quantity of the tracer divided by its concentration after an equilibrium period.<sup>1,8,10,11</sup> Other tracers have been developed, of which the radioactive <sup>51</sup>Cr-labelled ethylenediaminetetraacetic acid (<sup>51</sup>Cr-EDTA) has been found to yield the most accurate estimation of ECFV, in line with its distribution in the extracellular compartment, which is even more strict than that of bromide.<sup>8,12,13</sup> Nevertheless, such direct measurement of ECFV using isotope dilution requires urine sampling, which is cumbersome in clinical practice, so that other measurement methods have been developed from the analysis of the complete plasma disappearance curve after a single injection of tracers used for GFR measurement, ECFV being calculated as the product of GFR by the mean transit time.<sup>3,14,15</sup> Still, establishing the full plasma disappearance curve including the early phase remains quite cumbersome, so that simplified techniques based on the late disappearance curve (mono-compartment model), and using various correction factors, are preferred in clinical practice.<sup>3,5,13,16–19</sup>

Several equations have been developed to estimate theoretical ECFV from anthropometric parameters.<sup>2,6,9,11,12,19–21</sup> However, these equations were developed in small samples,<sup>2,9,12</sup> in specific patient populations,<sup>2,6</sup> or in mixed populations of both children and adults.<sup>6,9</sup> More importantly, no large-scale study used the above-mentioned gold standard ECFV measurement methods.<sup>1,8,10,11</sup> In addition, to our knowledge, none of these equations have been externally validated.

The aims of our study were, first, to evaluate the performances and the validity of all available ECFV-estimating formulae against a reference measurement and, second, to develop and validate a new equation for the estimation of theoretical ECFV in healthy adults.

## METHODS

### Study Populations

Data from healthy adults referred for GFR measurement before a potential live kidney donation were used (i) to validate the published formulae and for development and internal validation of the new ECFV-estimating equation (Bichat Hospital, Paris, France, March 2007–September 2018,  $N = 411$ ) and (ii) for external validation of the newly developed equation (Tenon Hospital, Paris, France, January 2006–February 2019,  $N = 261$ ).

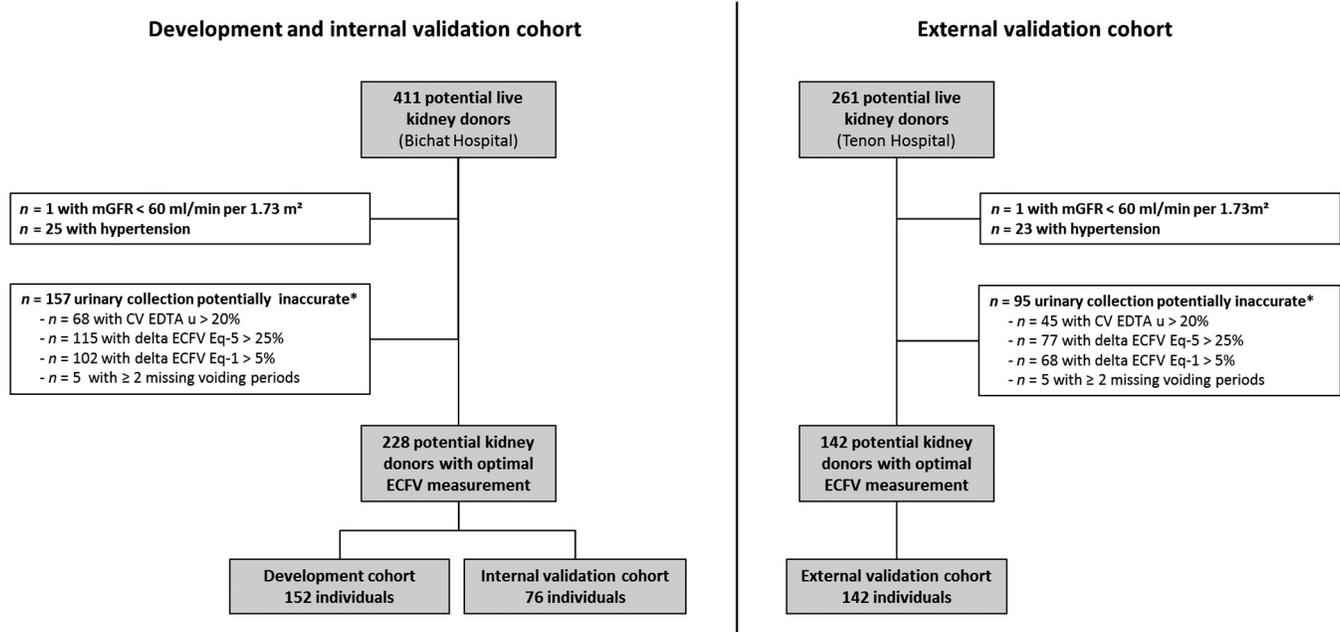
### Data Collection

Anthropometric data were measured in all participants. Routine laboratory markers were also collected. In both cohorts, GFR was measured from the renal clearance of <sup>51</sup>Cr-EDTA.<sup>22,23</sup> As <sup>51</sup>Cr-EDTA diffusion is restricted to the extracellular compartment, ECFV was measured during the same procedure, as the distribution volume of the tracer.<sup>23,24</sup> After a bolus i.v. injection of 1.8 to 3.5 megabecquerels (MBq) of <sup>51</sup>Cr-EDTA (GE Healthcare, Vélizy, France), patients were asked to void after allowing 90 minutes for equilibration of the tracer in the ECFV and every 30 minutes thereafter until 270 minutes after the injection. Blood samples were drawn in the contralateral arm at midpoint of each 30-minute urine period, and urinary clearance was calculated from the average of the 6 urinary clearances in these 30-minute periods. The equation of the late plasma disappearance curve was determined from the regression of plasma concentration as a function of time and was used to extrapolate the plasma concentration of the tracer at each voiding time. ECFV was calculated at each voiding time as the ratio of the remaining quantity (i.e., the injected minus the cumulative excreted quantity) over the extrapolated plasma concentration of <sup>51</sup>Cr-EDTA<sup>23</sup> and expressed in liters. Activity of urinary and plasma samples was measured with the Wallac Wizard 3<sup>00</sup> 1480 (PerkinElmer) gamma counter.

$$ECFV(\text{liters})_{(t)} = \frac{Q_{\text{injected}} - Q_{\text{excreted}(t)}}{\text{plasma } ^{51}\text{CrEDTA concentration}_{(t)}}$$

### Selection of Participants

Individuals with measured GFR < 60 ml/min per 1.73 m<sup>2</sup> or with treated hypertension were excluded from the study. Moreover, although direct measurement of the distribution volume of <sup>51</sup>Cr-EDTA is a



**Figure 1.** Flowchart. \*Participants may have overlapping causes of inaccurate urine collection. CV, coefficient of variation; ECFV, extracellular fluid volume; Eq, equilibrium; mGFR, measured glomerular filtration rate.

reference method for ECFV evaluation, any inaccuracy in voiding completeness compromises the accuracy of the calculated excreted quantity (hence distribution volume) of the tracer. As the reliability of the gold standard measurement was crucial in our study, very stringent selection criteria were used to ascertain the validity of ECFV measurement (Supplementary Figure S1). Data sets from all participants were reviewed by 2 independent experts (ALF and EVP). Individuals with any sign of inaccurate urinary collection, defined by  $\geq 2$  missing voiding periods and/or an intra-subject coefficient of variation of the 6 (or 5) fractionated urinary clearances of the tracer  $> 20\%$ ,<sup>25</sup> were excluded from the analyses. In addition, as any urine loss during the procedure leads to cumulative errors in ECFV, although the overall steadiness of consecutive ECFV measurement was used to screen for regular and complete voiding, for this study, the reference ECFV value was considered as the minimum of the first 2 measurements (after equilibrium and after the first 30-minute period), the second one being lower and more accurate than the first when voiding is incomplete at equilibrium. To ensure that no urine was lost during any of these 2 voiding periods, any increase between the first and the second ECFV values  $> 5\%$  was also an exclusion criterion. Finally, as the combination of urine loss and incomplete voiding at equilibrium could not be detected by this 5% increase criteria (both errors compensating each other at the second void), a subsequent increase between the reference value and the last ECFV value  $> 25\%$  which could not be explained by a subsequent urine loss after

the second void (as analyzed by the corresponding fractionated urinary clearance data) was interpreted as a urine loss during equilibrium and the corresponding data set was also excluded from the present analysis. Importantly, participants may have overlapping causes of inaccurate urine collection. This thorough screening process left a total of 228 subjects (Bichat cohort) with fully validated data sets. The same procedure was applied to the external validation cohort, leaving 142 participants (Tenon cohort) with valid sets of data for the present study (Figure 1).

## Statistical Analyses

### Evaluation of ECFV-Estimating Equations

ECFV-estimating equations evaluated in this study are reported in Table 1. The “20% of body weight” formula, frequently indicated as an approximation of ECFV in physiology textbooks,<sup>26</sup> was also tested. Their performances were evaluated using the following main parameters: bias (difference between estimated and measured ECFV), precision (interquartile range of the bias), and two metrics of accuracy (root mean square error and percentage of estimated values within 10% of measured ECFV) (Supplementary Method). The 95% CIs were calculated using 10,000 bias-corrected and accelerated bootstrap iterations.<sup>27</sup> Performances of the equations were also graphically analyzed by plotting predicted versus measured ECFV and using the Bland-Altman representation.<sup>28</sup>

### Development of a New ECFV-Estimating Equation

Subjects from the Bichat database were randomly divided into 2 of 3 for the development sample ( $n =$

**Table 1.** Equations used to estimate the theoretical ECFV

Author, journal, ref	Yr	n	Tracer	Gold standard	Population	Formula
Moore et al. <sup>11</sup>	1963	17 males 17 females	<sup>84</sup> Br-iodide	$\frac{Q(^{84}\text{Br}_{\text{injected}} - Q^{84}\text{Br}_{\text{excreted}})}{\text{plasma } ^{84}\text{Br concentration}}$	Healthy population	Males: ECFV = 7.35 + 0.135 × weight Females: ECFV = 5.27 + 0.134 × weight
Brøchner-Mortensen et al., <i>Scand J Clin Lab Invest.</i> <sup>12</sup>	1982	84	<sup>51</sup> Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Healthy population Age: 18–70 yr	Males: Log <sub>10</sub> ECFV = 0.0026 × weight + 3.9510 Females: Log <sub>10</sub> ECFV = 0.0030 × weight + 3.8657 Males: Log <sub>10</sub> ECFV = 0.1957 × BSA Dubois + 3.7667 Females: Log <sub>10</sub> ECFV = 0.2669 × BSA Dubois + 3.6102
Granerus et al., <i>Swedish Soc Radiol Proc.</i> <sup>21</sup>	1985	—	<sup>51</sup> Cr-EDTA	Plasma disappearance curve (mono-compartment model)	—	Males: ECFV = (166 × weight) + 2490 Females: ECFV = (95 × weight) + 6170
Christensen et al., <i>Clin Physiol.</i> <sup>2</sup>	1986	45	<sup>99m</sup> Tc-DTPA	Plasma disappearance curve (bi-compartment model)	Age: 30–79 yr Cancer GFR: 39–126 ml/min	ECFV = (8116.6 × BSA Dubois – 28.2)/1000
Bird et al., <i>J Nucl Med.</i> <sup>6</sup>	2003	411	<sup>51</sup> Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Age: 1–87 yr Nephropathy Cancer	ECFV = weight <sup>(0.6469)</sup> × height <sup>(0.7236)</sup> × 0.02154
Silva et al., <i>Physiol Meas.</i> <sup>20</sup>	2007	1538	<sup>2</sup> H <sub>2</sub> O and <sup>40</sup> K	$\frac{152 \times \text{TBW} - \text{TBK}}{148}$	Age: 18–98 yr Multiethnic healthy population	Males: ECFV = –12.424 + (0.191 × weight) + (0.0957 × height) + (0.025 × age) Females: ECFV = –4.027 + (0.167 × weight) + (0.05987 × height)
Peters et al., <i>Nucl Med Commun</i> <sup>9</sup>	2011	170 (69 children + 101 adults)	<sup>51</sup> Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Children: nephropathy (age: 0.5–13 yr) Adults: healthy kidney donors (age: 19–76 yr)	ECFV = 6.08 × BSA Haycock <sup>1,26</sup>
Peters et al., <i>Nephrol Dial Transplant.</i> <sup>19</sup>	2012	1878	<sup>51</sup> Cr-EDTA/ <sup>99m</sup> Tc-DTPA	Plasma disappearance curve (mono-compartment model)	Healthy kidney donors Age: 19–77 yr	Males: ECFV = 5.01 + 0.124 × weight Females: ECFV = 4.28 + 0.116 × weight Males: ECFV = (–2.47 + 8.76 × BSA Haycock) Females: ECFV = (–1.96 + 8.05 × BSA Haycock)

<sup>51</sup>Cr-EDTA, <sup>51</sup>Cr-labelled ethylenediaminetetraacetic acid; BrV, bromide volume; BSA, body surface area; <sup>99m</sup>Tc-DTPA, <sup>99m</sup>Tc-labelled diethylenetriaminepentaacetic acid; ECFV, extracellular fluid volume; PV, plasma volume; Q, quantity; RCV, red blood cell volume; ref, reference; TBK, total body potassium; TBW, total body water.

In the Moore formula, as bromide enters into the red blood cell to a significant degree, a correction of the BrV of distribution for red blood cell (RCV) bromide and PV was carried out by the authors as follows:  $\frac{\text{BrV} - \text{PV} - 0.6\text{RCV}}{1.11} + 0.92 \text{PV}$ .

In the Silva formula, ECFV was deducted from total body water (calculated as the distribution volume of deuterium, <sup>2</sup>H<sub>2</sub>O) and total body potassium. BSA was estimated using the Dubois or Haycock formula; ECFV (expressed in l or ml according to formulae); Q, quantity; TBK mmol; TBW kg. Dubois formula: BSA [m<sup>2</sup>] = 0.007184 × height [cm]<sup>0.725</sup> × weight [kg]<sup>0.4255</sup>; Haycock formula: BSA [m<sup>2</sup>] = weight [kg]<sup>0.5378</sup> × height [cm]<sup>0.3964</sup> × 0.024265.

152) and 1 of 3 for the internal validation sample ( $n = 76$ ). Equation development process is detailed in the [Supplementary Method](#). Assumption of normality of ECFV was verified. Although this assumption was roughly acceptable to study ECFV linearly, a Box-Cox transformation<sup>29</sup> was also applied on ECFV (function *boxcox* of the R package *MASS*), leading to a natural logarithm transformation of ECFV. Relationships between both ECFV (linear) and log-transformed ECFV and predictors were studied ([Supplementary Method](#)). Least-square linear regression was used to relate measured ECFV to clinical and biological characteristics of healthy individuals. ECFV-related variables were defined *a priori* and included body weight, height, age, sex, ethnicity, fasting urinary sodium excretion, and fractional excretions of sodium, uric acid, and urea. Nonlinear relationship between each continuous predictor and ECFV was explored. Then, a combination of clinical guidance and stepwise forward approach was used to select covariates in the adjusted model. Improvement in model performance through addition of new covariates in multivariable linear regression model was evaluated using the Akaike Information Criterion.<sup>30</sup> Adjusted  $R^2$ , root mean square error, and absolute bias were also evaluated. Models 1 to 4 (and models 1-log to 4-log) were developed by sequentially adding body weight, sex, height, and age. Models 5 (and 5-log) and 6 (and 6-log) were developed with the same covariates of models 3 (and 3-log) and 4 (and 4-log) but using elastic net regression method<sup>31</sup> (R package *glmnet*) with 5-fold cross-validation, to improve the quality of the prediction ([Supplementary Method](#)).

### Internal Validation

The most accurate models (models 6 and 6-log) were evaluated in the internal validation data set. Equation obtained from the development cohort was applied in the total population of the internal validation cohort, but also according to subgroups defined by sex, age (<40, 40–60, >60 years), ethnicity (European vs. African origin), BMI (<20, 20–30, >30 kg/m<sup>2</sup>), and BSA (<1.73, 1.73–2, >2 m<sup>2</sup>). Performances of the predictive models were evaluated graphically and using the same metrics as described previously. Calibration was studied by plotting predicted versus measured ECFV for each quintile of predicted ECFV. Magnitude of the deviation was compared across quintiles using a linear regression model, with bias and quintiles entered as the dependent and independent variables, respectively (the lower the  $R^2$  and the higher the  $P$  value, the better the prediction model). Finally, development and internal validation data sets were combined to derive the final coefficients using a penalized elastic net regression.

### External Validation of the New ECFV-Estimating Equation

The new ECFV-estimating equation was externally validated in the Tenon cohort ( $N = 142$ ), using the same graphical representation and metrics as for the internal validation. Finally, the new equation was compared with the other formulae.

There were no missing data for any of the covariates used for the development and the internal and external validation of the new equation. All statistical analyses were conducted using R 3.6 software (<https://cran.r-project.org/>). The transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) statement<sup>32</sup> was followed for reporting the development and validation of the multivariable prediction model ([Supplementary Method](#)).

### Consent and Ethics

All patients gave their written consent for scientific use of anonymous data. The study was approved by the Local Ethics Committee (Institutional Review Board 00006477, project number 14-051, *Hôpitaux Universitaires Paris-Nord Val de Seine, Assistance Publique-Hôpitaux de Paris*).

## RESULTS

### Characteristics of the Study Populations

In the 228 participants of the development and internal validation cohorts ([Figure 1](#) and [Table 2](#)), mean age was  $45.2 \pm 12.0$  years, 43.0% were men, and 14.2% were of African origin. Mean BMI was  $25.9 \pm 4.6$  kg/m<sup>2</sup>. Mean measured GFR was  $90 \pm 15$  ml/min per 1.73 m<sup>2</sup>. Mean measured ECFV was  $17.0 \pm 2.6$  and  $13.7 \pm 2.1$  l in males and females, respectively ([Supplementary Figure S2](#)). The 142 participants of the external validation cohort were older, more often females, and had a lower measured GFR ([Table 2](#)). Overall characteristics of the patients included in the analyses did not differ from those who were excluded because of irregular voiding potentially compromising the validity of ECFV measurement ([Supplementary Table S1](#)).

### Relationship Between ECFV and Anthropometric Parameters

ECFV was highly correlated with body weight ( $r = 0.85$ ) and BSA ( $r = 0.86$ ) and was on average  $21.3 \pm 2.1$  and  $21.0 \pm 2.4\%$  of body weight in males and females, respectively. For the lowest and highest values of BMI, ECFV represented >20% and <20% of body weight, respectively, and this finding was similar in males and females ([Figure 2](#)).

### Performances of ECFV-Estimating Equations

Bias, precision, and accuracy of the ECFV estimation formulae are presented in [Figure 3](#) and [Supplementary](#)

**Table 2.** Clinical characteristics of the study populations

Characteristics	Development and internal validation cohorts (Bichat) N = 228	External validation cohort (Tenon) N = 142	P value	Development data set (Bichat) n = 152	Internal validation data set (Bichat) n = 76	P value
<b>Anthropometric characteristics</b>						
Age (yr)	45.2 ± 12.0	47.8 ± 10.3	0.03	44.9 ± 12.0	45.8 ± 12.0	0.60
Age (%)			0.03			0.77
<40 yr	35 (24.6)			59 (38.8)	26 (34.2)	
40–60 yr	90 (63.4)			76 (50.0)	40 (52.6)	
>60 yr	17 (12.0)			17 (11.2)	10 (13.2)	
Sex (males, %)	98 (43.0)	42 (29.6)	0.01	62 (40.8)	36 (47.4)	0.42
Ethnicity (African origin, %)	32 (14.2)	23 (20.9)	0.16	21 (14.0)	11 (14.7)	1.00
Body weight (kg)	73.5 ± 14.4	71.2 ± 12.6	0.13	73.4 ± 14.6	73.6 ± 14.1	0.93
Height (cm)	168.4 ± 9.8	165.0 ± 8.2	0.001	168.5 ± 10.5	168.4 ± 8.2	0.98
Body mass index (kg/m <sup>2</sup> )	25.9 ± 4.6	26.2 ± 4.5	0.51	25.9 ± 4.9	25.8 ± 3.9	0.94
Body mass index (%)			0.92			0.58
<20 kg/m <sup>2</sup>	21 (9.2)	12 (8.5)		15 (9.9)	6 (7.9)	
20–30 kg/m <sup>2</sup>	164 (71.9)	101 (71.1)		106 (69.7)	58 (76.3)	
>30 kg/m <sup>2</sup>	43 (18.9)	29 (20.4)		31 (20.4)	12 (15.8)	
Body surface area (DuBois) <sup>a</sup>	1.83 ± 0.20	1.78 ± 0.17	0.01	1.83 ± 0.21	1.83 ± 0.20	0.93
Body surface area (Haycock) <sup>b</sup>	1.86 ± 0.22	1.81 ± 0.19	0.05	1.86 ± 0.22	1.86 ± 0.22	0.91
<b>Biological parameters</b>						
mGFR (ml/min per 1.73 m <sup>2</sup> )	90 ± 15	85 ± 14	0.001	90 ± 15	92 ± 16	0.39
Measured ECFV (l)	15.4 ± 2.8	15.1 ± 2.1	0.33	15.4 ± 2.8	15.5 ± 2.7	0.81
<b>Estimated ECFV (l)</b>						
Moore formula	16.0 ± 2.6	15.5 ± 2.2	0.03	16.0 ± 2.6	16.2 ± 2.6	0.65
Brøchner-Mortensen formula (weight)	13.0 ± 1.8	12.5 ± 1.5	0.02	12.9 ± 1.8	13.1 ± 1.8	0.63
Brøchner-Mortensen formula (BSA)	12.9 ± 1.7	12.4 ± 1.4	0.01	12.9 ± 1.7	12.9 ± 1.6	0.75
Granerus formula	14.0 ± 2.4	13.5 ± 1.9	0.02	14.0 ± 2.3	14.1 ± 2.4	0.69
Christensen formula	14.9 ± 1.7	14.4 ± 1.4	0.01	14.8 ± 1.7	14.9 ± 1.6	0.93
Bird formula	14.2 ± 2.2	13.4 ± 1.8	0.02	14.1 ± 2.2	14.2 ± 2.1	0.93
Silva formula	18.7 ± 3.2	18.0 ± 2.6	0.02	18.7 ± 3.2	18.8 ± 3.2	0.90
Peters formula (BSA 1)	14.0 ± 2.2	13.6 ± 1.9	0.04	14.0 ± 2.3	14.0 ± 2.2	0.92
Peters formula (BSA 2)	13.4 ± 2.1	12.9 ± 1.7	0.02	13.4 ± 2.1	13.5 ± 2.1	0.79
Peters formula (weight)	13.4 ± 2.1	12.9 ± 1.8	0.03	13.7 ± 2.1	13.5 ± 2.1	0.71
20% Body weight	14.7 ± 2.9	14.3 ± 2.5	0.13	14.7 ± 2.9	14.7 ± 2.	0.93

BSA, body surface area; ECFV, extracellular fluid volume, mGFR, measured glomerular filtration rate.

<sup>a</sup>DuBois formula: BSA [m<sup>2</sup>] = 0.007184 × height [cm]<sup>0.725</sup> × weight [kg]<sup>0.4255</sup>.

<sup>b</sup>Haycock formula: BSA [m<sup>2</sup>] = weight [kg]<sup>0.5378</sup> × Height [cm]<sup>0.3964</sup> × 0.024265.

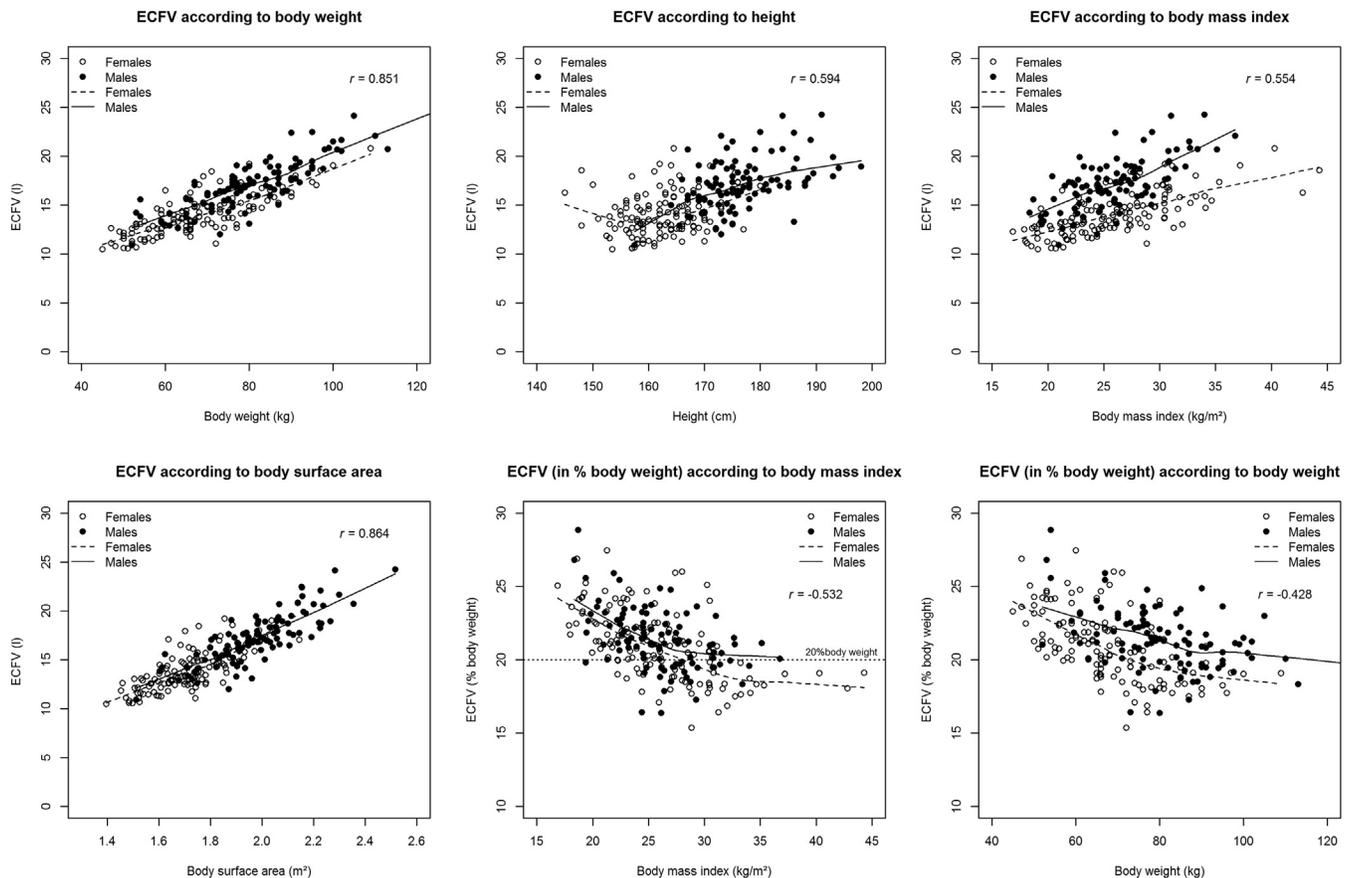
Continuous data are expressed in mean ± SD and categorical data are expressed in n (%). BSA estimated using DuBois or Haycock formula.

**Table S2.** Median bias of the Christensen formula (−0.47 l, 95% CI [−0.69 to −0.19]) was lower than that of the other formulae. Interquartile range for the difference was close to 2 l for all the equations. The best accuracies within 10% were obtained with the Moore (65.8 [58.8 to 71.5]), Christensen (66.7 [60.1 to 71.9]), and 20% body weight (62.3 [55.3 to 68.0]) formulae. Bland and Altman graphs (Figure 3) revealed that the Moore and 20% body weight formulae were more accurate across the whole ECFV range, whereas for most other formulae, underestimation increased (negative bias) as ECFV increases (Figure 3).

### Development of the New ECFV-Estimating Equation

A new equation relating measured ECFV to clinical and biological characteristics of healthy individuals was developed. In univariable analysis, body weight was the strongest predictor of ECFV. Height and age better fitted the data with quadratic and cubic

transformations, respectively, compared with no (linear) or spline transformation. Nevertheless, in multivariable analysis, none of the fractional polynomial or spline transformations of the predictors provided a better fit to ECFV (and log-ECFV) compared with a linear model. The  $\beta$ -coefficient for the covariates, statistics for goodness-of-fit, and prediction performance for successive equation modeling in both ECFV and log-ECFV are reported in Supplementary Table S3. In sequential models predicting ECFV and log-ECFV, the adjusted R<sup>2</sup>, Akaike Information Criterion, and root mean square error improved with the inclusion of body weight, sex, height, and age. None of the tested interactions were significant. Models 6 and 6-log (i.e., fully adjusted models predicting ECFV and log-ECFV, respectively, using elastic net regularization method) were considered for the internal validation step.



**Figure 2.** Functional relationship between measured ECFV and anthropometric parameters, according to sex (Bichat cohort,  $N = 228$ ). Smoothed regression lines were computed using the nonparametric LOESS method and are represented as solid (for males) and dashed (for females) black lines. Pearson correlation coefficient  $r$  was also calculated for the total population. ECFV, extracellular fluid volume; LOESS, LOcally Estimated Scatterplot Smoothing.

### Internal Validation

As equations to estimate both ECFV and log-ECFV from predictors gave similar performances in the internal validation data set (Supplementary Table S4), the simplest model (model 6) was chosen. Prediction and accuracy were consistent across subgroups defined by sex, BMI, BSA, age, and ethnicity (Figure 4 and Supplementary Figure S3). Final coefficients of the selected model 6 were derived from pooled development and internal validation data sets, so that the final equation is:

$$ECF \text{ (liters)} = \alpha + 0.1393 \times \text{weight [kg]} + 0.0455 \\ \times \text{height [cm]} + 0.0125 \times \text{age [years]}$$

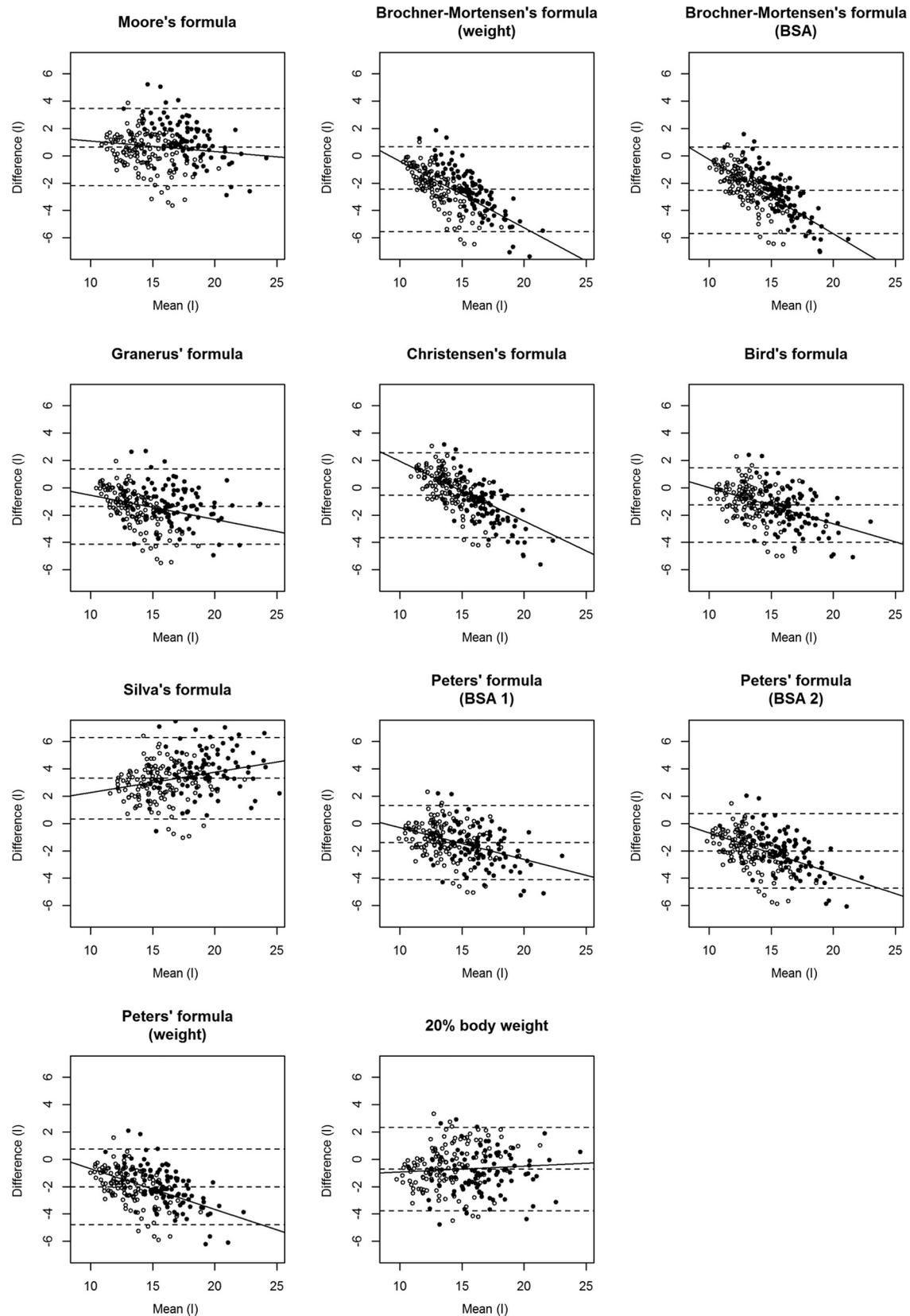
With  $\alpha = -2.6631$  for males and  $-3.3407$  for females

The multiplication factor for sex is incorporated into the intercept, which results in different intercepts for each sex.

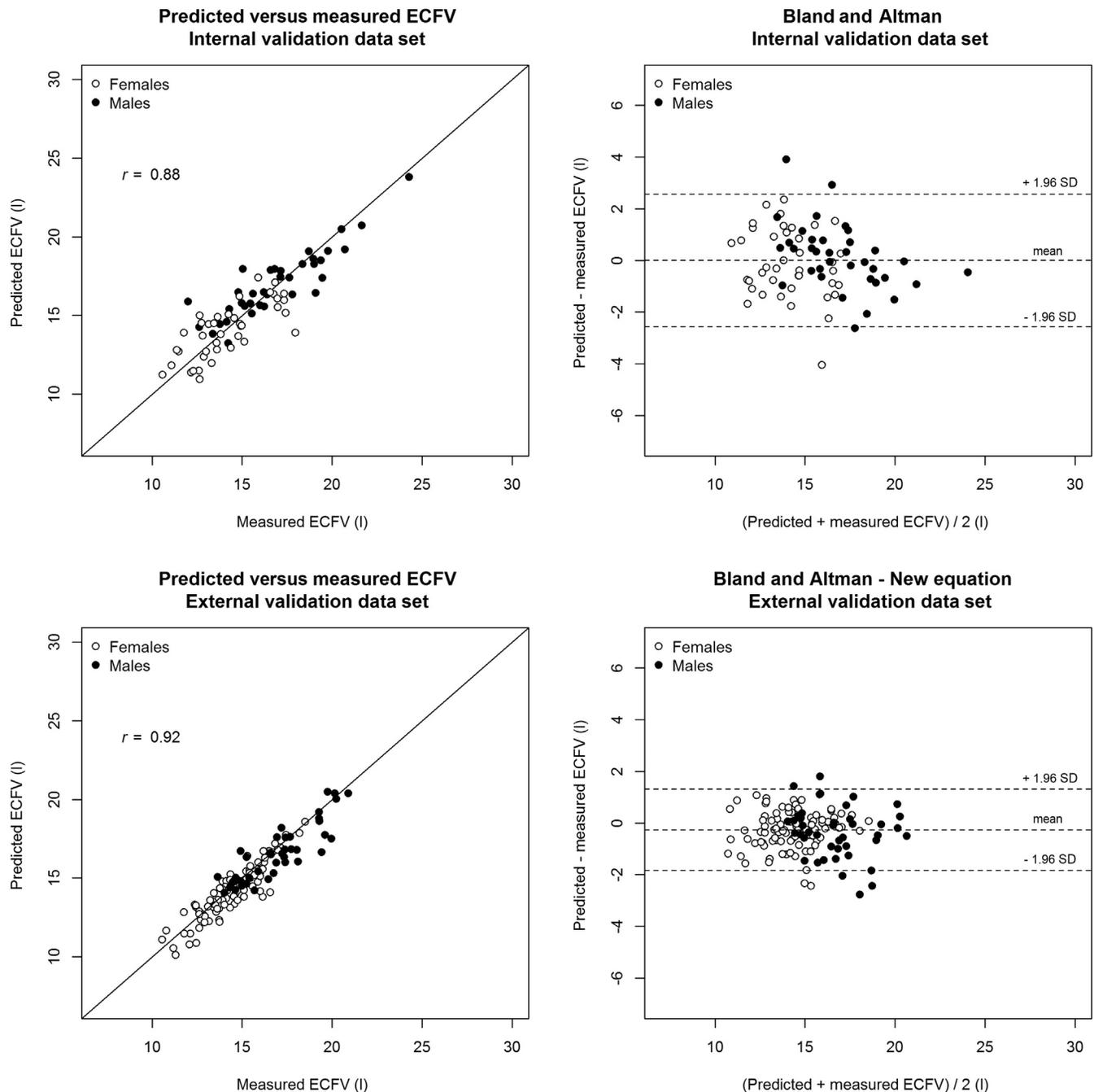
### External Validation of the New ECFV-Estimating Equation

Figure 4 and Supplementary Figure S4 reveal the predicted versus measured ECFV in the external validation

cohort. Metrics for performances of the new equation revealed a median bias of  $-0.20$  l ( $-0.35$  to  $-0.05$ ), a median absolute bias of  $0.49$  l ( $0.38$  to  $0.60$ ), an interquartile range for the difference of  $0.88$  l ( $0.70$  to  $1.08$ ), a mean absolute percentage error of  $4.19\%$  ( $3.65$  to  $4.82$ ), root mean square error of  $0.056$  ( $0.050$  to  $0.064$ ), and percentage of estimated values within  $10\%$  of  $90.9\%$  ( $83.8$  to  $94.4$ ) (Supplementary Table S5). Prediction and accuracy were consistent across subgroups (Figure 4 and Supplementary Figure S5). Compared with all other formulae, the new ECFV-estimating equation displayed the best performances in the external validation cohort (Figure 5a-d, Supplementary Figure S6, and Supplementary Table S5). Although overall performances of the Moore formula were close to those of the new equation in the external validation cohort (Figure 5a-d), the Moore equation suffered from an overestimation of ECFV in males: median bias of  $1.046$  l ( $6.4\%$ ) versus  $-0.37$  l ( $-2.3\%$ ) in Moore and new equation, respectively (Supplementary Figure S7). In addition, because body weight and gender are the only parameters in the Moore formula, its performances across the range of BMI were not as accurate as ours.



**Figure 3.** Bland-Altman graphical representations of the estimating equation published in the literature versus measured ECFV (Bichat cohort,  $N = 228$ ). For each ECFV-estimating equation, the difference (estimated – measured ECFV) is plotted versus mean ( $(\text{estimated} + \text{measured ECFV}) / 2$ ). Mean bias, upper and lower limits of agreement (mean bias  $\pm 1.96 \times$  SD of bias) are represented by the dashed lines. Regression line is represented by the solid black line. BSA, body surface area; ECFV, extracellular fluid volume.

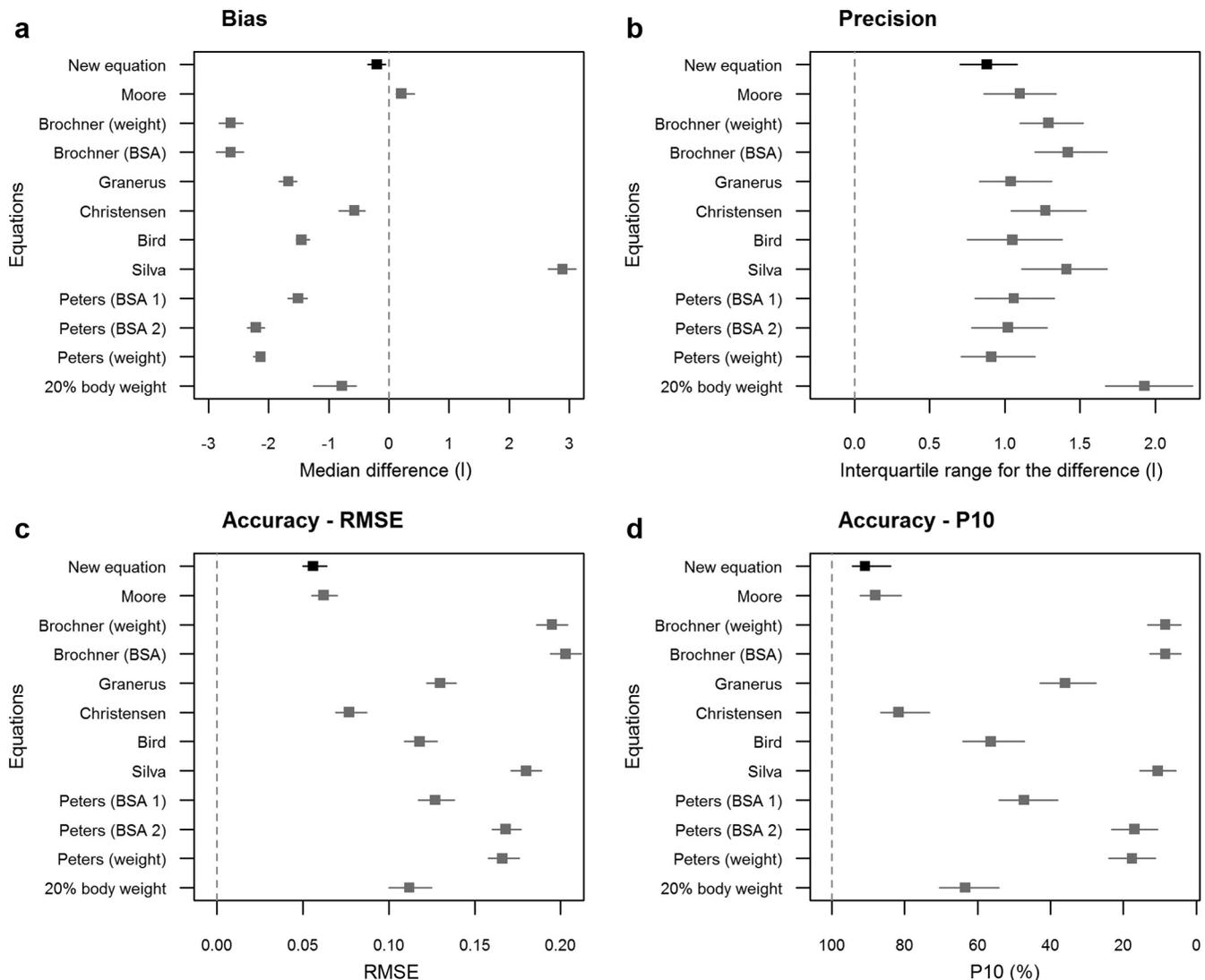


**Figure 4.** Predicted versus measured ECFV in internal (Bichat cohort,  $n = 76$ ) and external (Tenon cohort,  $N = 142$ ) validation cohorts. Values of ECFV predicted by the model 6 and the new developed equation were plotted against the measured values of ECFV, in the internal (Bichat cohort,  $n = 76$ ) and external (Tenon cohort,  $n = 142$ ) validation cohorts, respectively. In Bland-Altman plots, mean bias, upper and lower limits of agreement (mean bias  $\pm 1.96 \times$  SD of bias) are represented by the dashed lines. ECFV, extracellular fluid volume.

## DISCUSSION

Our study conducted in healthy individuals with a very thorough screening of ECFV measurement using isotope dilution showed that the precision and accuracy of the ECFV-estimating equations previously published were highly variable and their suitability for routine clinical practice was questionable for most of them. This could be explained at least in part by the fact that they were often developed in small sample

size,<sup>2,9,12</sup> in specific patient populations,<sup>2,6</sup> or in mixed populations of children and adults,<sup>6,9</sup> or without distinction between body composition of males and females.<sup>2,6,9</sup> In addition, these equations were not validated in external cohorts.<sup>2,9,11,12,19–21</sup> Moreover, in most cases, the benchmark used to develop the equations was not a gold standard measurement of ECFV.<sup>1,8,10,11,33</sup> Indeed, in one study, ECFV was deduced from total body water and intracellular fluid volume evaluated by total body potassium.<sup>20</sup> In all



**Figure 5.** Comparison of the performances of the published and the new ECFV-estimating equations, in the external validation cohort (Tenon cohort,  $N = 142$ ). The newly developed ECFV-estimating equation referred to as “new equation,” is represented in black, and the other equations are represented in gray. (a) Bias was defined as the median difference between estimated and measured ECFV. (b) Precision was evaluated using the IQR of the bias. Accuracy was evaluated using (c) the root mean square error and (d) the P10 of the measured value. Horizontal bars represent 95% CIs computed using 10,000 BCa bootstrap iterations. BCa, bias-corrected and accelerated; BSA, body surface area; ECFV, extracellular fluid volume; IQR, interquartile range; P10, percentage of estimates that were within 10%; RMSE, root mean square error.

other studies, except one<sup>11</sup> in which reference values of ECFV relied on a direct measurement method—quite similar to ours—in 34 subjects, and another<sup>2</sup> based on the plasma disappearance curve of a radioactive tracer using a 2-compartment model in 45 subjects, ECFV was derived from the late plasma disappearance curve of an exogenous tracer with various mathematical corrections meant to better estimate the “true” ECFV. Interestingly, the only equation<sup>11</sup> based on direct ECFV measurement from bromide dilution yielded the best performances. Nevertheless, this formula suffered from an overestimation of ECFV in males and was not as accurate as ours across the range of BMI, because it

only integrates body weight and sex. Finally, the accuracy of ECFV assessment is directly affected by the tracer used, and it has been found that the distribution volume of <sup>51</sup>Cr-EDTA yields a closer approximation of the ECFV than that of other radioactive and nonradioactive tracers, and even that than of bromide.<sup>8,12–14</sup> Indeed, although it is a historical gold standard for ECFV measurement, bromide may overestimate ECFV owing to a minor leakage in the intracellular space,<sup>11,12</sup> so that a correction factor is used in bromide dilution formulae. A limitation of the isotope dilution method compared with plasma decay-derived methods is that complete and accurate bladder voiding

is mandatory, which we ensured at the cost of reducing our study population after applying very strict selection criteria.

We developed and validated a set of prediction models for ECFV estimation among healthy individuals. Our final model performed better than all other formulae, although it relied on the same simple anthropometric markers. Indeed, the addition of other biological parameters and ethnicity did not improve the model performances. The reliability of our equation can be explained, at least in part, because our reference ECFV value was a direct measurement using isotope dilution in a large population with very stringent criteria to ascertain its technical validity as explained previously, but also because a robust statistical method was used to build prediction models. We found that body weight and BSA were the strongest predictors of ECFV and that mean ECFV was  $21.1\% \pm 2.3\%$  of body weight. Accordingly, Ladegaard-Pedersen *et al.*<sup>34</sup> revealed that the distribution volume of  $^{51}\text{Cr-EDTA}$  was on average 21.8% of body weight, and using the same tracer, Brøchner-Mortensen<sup>12</sup> revealed that ECFV represented 19.5% and 18.8% body weight in males and females, respectively. Nevertheless, when ECFV is expressed as a fraction of body weight, a major limitation is that body composition (i.e., lean vs. fat body mass) is not taken into account.<sup>12</sup> Consequently, even if the intersubject comparability of ECFV is better when ECFV is compared as a fraction of BSA than as a fraction of body weight, we chose to include body weight and height separately (instead of BSA or BMI) in the model for a better flexibility in the computation of the coefficients, and thus a better fit of the models. As expected, our results revealed that ECFV was higher in males than in females,<sup>12</sup> but the relationship between sex and ECFV was not affected by body weight, height, or age (i.e., *P* values for interactions between sex, body weight, height, and age were not significant). As previously observed by Silva *et al.*,<sup>20</sup> we did not find a significant association between ECFV and ethnicity. Accuracy of our equation was robust across subgroups.

This new equation, which provides the individual reference (normal) value of ECFV, has important implications for both clinical practice and research. Indeed, several pathologic conditions lead to disturbances of sodium homeostasis and abnormality or modification in fluid distribution. The assessment of the magnitude of overhydration (or dehydration) remains a clinical challenge, as a given measured ECFV may correspond to a marked overhydration in some patients, or to a marked dehydration in others, depending on age, sex, and anthropometric parameters

(and therefore on the individual theoretical ECFV value). Our new equation will help appreciate how ECFV may deviate from the normal condition, and thus help optimizing patient management. Indeed, to evaluate the extent of overhydration (or dehydration), the following two pieces of information are needed: first, measured ECFV of the patient (using bedside bioelectrical impedance spectroscopy or even isotope dilution), and second, the individual reference value, the magnitude of over- or dehydration being calculated as the difference between the measured and the reference value of ECFV. Interestingly, although limits of agreements of ECFV measurement using bioelectrical impedance spectroscopy compared with isotope dilution are quite large,<sup>35</sup> the bias between both methods is on average close to 0, so that our equation can be expected to provide appropriate reference values for ECFV measured with bioelectrical impedance spectroscopy.

In addition, to compare hydration status at the population level, ECFV needs to be “normalized” or “indexed” to take into account the variability of ECFV associated with anthropometric parameters. Expressing ECFV as the ratio of measured over individual theoretical ECFV, using our equation, would be helpful in clinical research. Another important clinical application of our results is single-sample GFR measurement, which requires an accurate estimation of theoretical ECFV.<sup>2</sup> Finally, our equation could be used to express GFR scaled to ECFV, rather than scaled to BSA. Indeed, it has been found that assessment of renal function based on GFR indexed to ECFV is more clinically relevant<sup>3–8,33</sup> because ECFV is the compartment filtered by the kidneys. GFR/ECFV reflects the percentage of the ECFV cleared per unit of time.<sup>8,33</sup> Of note, the inverse ratio, ECFV/GFR, reflects the time needed for the kidneys to clear the complete ECFV, the so-called concept of mean transit time or, in other words, the mean residence time of the filtration marker in the ECFV before filtration.<sup>3,14,15</sup> Finally, in line with these considerations, although GFR scaled to BSA differs between males and females, this difference is ironed out when GFR is scaled to ECFV.<sup>8</sup>

Strengths of this study include its design, with separate databases for development and validation of the new equation, a prespecified rigorous statistical analytical plan, and use of the penalized elastic net regression to limit overfitting. Nonetheless, we acknowledge some limitations. The stringent selection criteria diminished the number of subjects included in these analyses. This, however, allowed establishing the validity of the reference measurement better than any previous study, and the number of included individuals was still well above that of most of these

studies. In addition, even if the precision and accuracy of our equation was consistent across subgroups, the equation should be used with caution in patients with extreme values of BMI or anthropometric characteristics and in elderly patients because the present study included few such individuals. Likewise, our equation should not be used in children as only adult patients were included in our study populations. Finally, regarding ethnicity, only data on African origin (required for GFR estimation) was available in the data set; other ethnicities such as Asian origin were not specified. Nevertheless, ethnicity defined as African origin or not did not improve the model performances.

In conclusion, our results showed that precision and accuracy of the previously published ECFV-estimating equations were highly variable. We developed and validated a new ECFV-predicting equation easily usable and which might prove a useful tool for clinical practice and research. External validation in other cohorts including individuals of extreme age and BMI remains needed.

## DISCLOSURE

All the authors declared no competing interests.

## ACKNOWLEDGMENTS

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## DATA STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

## AUTHOR CONTRIBUTIONS

ALF, EVP, MF, and GG designed the study. ALF and EVP reviewed the data sets from all participants. ALF and OL performed the statistical analyses. ALF, EVP, MF, and GG interpreted the data. ALF and EVP drafted the manuscript. All authors made critical revision of the manuscript for important intellectual content.

## SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

**Supplementary Methods.**

**Table S1.** Comparison of the characteristics of the study population with those of the population excluded from the study.

**Table S2.** Performances of the published formulae used to estimate ECFV compared with measured ECFV (Bichat cohort).

**Table S3.** Beta-coefficients and goodness-of-fit of the sequential models in the development data set.

**Table S4.** Comparison of the model performances in the internal and external validation data sets.

**Table S5.** Performances of the published and the new ECFV-estimating equations in the external validation cohort (Tenon cohort).

**Figure S1.** Exclusion criteria for the selection of patients with a valid ECFV measurement.

**Figure S2.** Distribution of measured extracellular fluid volume according to sex (Bichat Cohort).

**Figure S3.** Predicted versus measured extracellular fluid volume according to subgroups in the internal validation cohort (model 6) (Bichat cohort).

**Figure S4.** Calibration of the new equation in the external validation cohort (Tenon cohort).

**Figure S5.** Predicted versus measured extracellular fluid volume according to subgroups in the external validation cohort (new equation) (Tenon cohort).

**Figure S6.** Bland-Altman graphical representations of the published and new ECFV-estimating equations versus measured ECFV (Tenon cohort).

**Figure S7.** Comparison of the Moore formula and the new equation, according to sex, weight and body mass index (Tenon cohort).

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