CHAPTER 5

Extracellular Water: Reference values for Adults

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ABSTRACT

Extracellular water (ECW) is a large and clinically important body compartment that varies widely in volume both in health and disease. Interpretation of ECW measurements in the clinical setting requires consideration of potential influencing factors such as age, race, sex and other variables that moderate fluid status. Normative values are currently lacking in a large, ethnically diverse healthy group of subjects. The aim of the current study was to develop conditional quantile equations for ECW based on weight, height, age, sex, and race using a large (n=1538, 854 females and 684 males) healthy adult multi-ethnic (African American [AA], Asian, Caucasian, Hispanic) sample. ECW was derived from total body water (TBW) and potassium (TBK) measured by isotope dilution and whole-body ^{40}K counting, respectively. Quantile regression methods were used to identify five percentile levels (10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}). Weight was a significant variable at each quantile in both males and females. Weight, height, height^2, height^3, age, age^2, age^3, race, and interactions were variably significant across the five selected quantiles. These regression equations provide ECW quantile reference values based on analysis of a large multi-ethnic adult population and should prove useful in evaluating the relationship between a subject's or group's measured ECW and a well-characterized reference population.

"Make everything as simple as possible, but not simpler."
Albert Einstein

INTRODUCTION

Water is the most abundant component of body mass in most healthy adults (1). Water is distributed into two main compartments, intracellular (ICW) and extracellular (ECW). Extracellular water is a large and clinically important body compartment that varies widely in volume both in health and disease (2-7).

The relative size of body water compartments varies with age. According to Forbes and colleagues (8), extracellular fluid (ECF) volume exceeds intracellular fluid (ICF) volume in the fetus, and the ECF/ICF ratio progressively falls during infancy and childhood to the point where ICF volume accounts for the major proportion of total body fluid. Aging reverses this trend and the ECF/ICF ratio reverts towards the infantile status. A recent cross-sectional study of a large multiethnic sample of healthy adults confirmed the age-related variation in fluid distribution, notably a larger ECW/ICW (*E/I)* with greater age after controlling for other influencing factors (9).

Appropriate interpretation of ECW in the clinical setting requires consideration of potential factors that moderate fluid distribution, including sex, race, age, weight, and height. Normative values are unavailable.

The aim of the current study was to develop equations for the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles of ECW, conditioned on relevant moderator variables, that can serve as reference values when evaluating ECW in individuals or groups with clinical conditions.

METHODS

Subjects and Protocol

Subjects were a convenience sample of healthy adults of diverse race/ethnicity and age participating in other unrelated investigations. Additional details of the study group are provided in references (10, 11). Data from these subjects were also used in an earlier report on fluid distribution in adults (9).

Subjects with a history of high blood pressure and/or under medication treatment for high blood pressure were excluded from study. Body composition studies were carried out over a one-day period once a screening medical examination established the subject's healthy status. Extracellular water was derived from a combination of total body potassium (TBK) and total body water (TBW) measurements. The protocol was approved by the Institutional Review Board of St. Luke's-Roosevelt Hospital.

Body Composition Measurements

Total Body Water

Total body water was measured by deuterium ($^{2}H_2O$, L) or tritium ($^{3}H_2O$, L) dilution with coefficients of variation (CV) of 1.5% and 2.0%, respectively. The water dilution volumes were then adjusted to mass estimates as described by Schoeller (12). Specifically, both dilution volumes were converted to water mass assuming an average body temperature of 36°C. The tritium dilution space was also adjusted for proton exchange by assuming actual water volume is 96% of the measured isotope distribution volume.

Total Body Potassium (TBK)

Total body potassium was estimated from the measured 1.46 MeV γ-ray decay of naturally occurring ^{40}K as TBK = $^{40}K/0.000118$ (13). The subject's ^{40}K was determined by counting for 9 minutes in a 4Π whole-body counter (13). The raw count is corrected for body mass as described in Pierson et al. (14).This system has a between-measurement CV of 1.5%.

Extracellular water was then calculated using Wang's equations that assume stable intracellular and extracellular potassium concentrations of 152 and 4 mmol/kg H_2O, respectively (1, 15):

$$ECW = (152xTBW - TBK)/148 \quad (1)$$

TBK and TBW in (1) are expressed in mmol and kg, respectively.

Statistical Methods

Group characteristics are presented as means and standard deviations. Statistical analyses were carried out using SPSS *v*12.0 (SPSS Inc., 2003). $P<0.05$ was considered significant except for multiple comparisons where Scheffé's adjustment was made if equal variances were assumed or Dunnett's T3's adjustment if equal variances were not assumed.

Independent sample t-tests were used to compare ECW values between males and females. One-way ANOVA was used to compare ECW across race groups within gender. Quantile regression was carried out by programming an appropriate loss function in the nonlinear regression option of SPSS. The significance of each variable in the quantile regression was tested by the logistic regression method recently described in Redden et al. (16). We tested the statistical significance of age, height, weight, race, weight^2, height^2, age^2, weight^3, height^3, age^3, and interactions in equations for the 10th, 25th, 50th, 75th, and 90th percentiles.

RESULTS

Subject Characteristics

The subjects were 854 females and 684 males who completed the study protocol. The race distribution (F/M) was 193/137 African American, 135/117 Asian, 357/259 Caucasian, and 169/171 Hispanic. The subject characteristics for the model development sample are presented in **Table 5.1**.

Table 5.1. Subject characteristics.

	Males				
	AA	**Asian**	**Caucasian**	**Hispanic**	**Total**
N	137	117	259	171	684
Age (y)	48.7±17.9	50.0±18.1	50.8±18.6	44.6±15.8	48.7±17.9
Weight (kg)	80.1±13.5	69.0±8.9	78.0±11.3	76.8±12.7	76.6±12. 3
Height (m)	1.75±0.07	1.71±0.06	1.76±0.07	1.70±0.08	1.73±0.08
BMI (kg/m²)	26.1±3.8	23.7±2.6	25.3±3.0	26.5±3.5	25.5±3.4
ECW (kg)	20.8±3.8	18.1±3.0 [a]	20.5±3.6	20.0±3.9	20.0±3.7[b]
	Females				
	AA	**Asian**	**Caucasian**	**Hispanic**	**Total**
N	193	135	357	169	854
Age (y)	53.0±16.8	48.9±17.5	53.0±18.8	49.2±15.7	51.6±17.6
Weight (kg)	73.0±12.8	55.3±7.9	62.7±10.4	66.4±10.3	64.6±12.0
Height (m)	1.62±0.07	1.57±0.06	1.63±0.07	1.56±0.06	1.61±0.07
BMI (kg/m²)	27.7±4.5	22.3±2.8	23.7±3.8	27.2±4.1	25.1±4.4
ECW (kg)	18.4±3.1[c]	14.6±2.2[c]	16.0±2.6	16.5±2.8	16.4±3.0

Abbreviations: AA, African American; N, number of subjects; BMI, body mass index; ECW, extracellular water.
[a] Asian males differed from other ethnic male groups ($p < 0.001$); [b] Males differed from females ($p < 0.001$); [c] Asian and Afro-American females differed from all ethnic female group ($p < 0.001$).

Subjects ranged in weight from a low of 40.5 kg to a high of 122.8 kg with an overall BMI (mean ± SD) and range of 25.3 ± 4.0 kg/m^2 and 18.5-39.4 kg/m^2, respectively. The mean age of the whole group was 50.3 ± 17.8 yrs with a range of 18-98 yrs.

Model Development

Males

Weight was a highly significant variable in the regression for men at all five quantile levels ($p<0.001$; **Table 5.2**).

Age^3 for the 25th and 50th percentiles and age^2 for the 90th percentile were significantly associated with ECW ($p=0.011$, $p=0.009$ and $p=0.007$, respectively), after adjusting for weight. For the 75th and 90th percentiles height^3 was also significantly associated with ECW ($p=0.015$ and $p=0.032$, respectively), after adjusting for weight and age^3 by AA (75th percentile) and weight and age^2 (90th percentile) while for the 50th percentile a significant association of height^2 with ECW ($p=0.003$) was found after adjusting for weight and age^3. For the 75th percentile an age^3 by AA interaction was significantly associated with ECW ($p=0.015$) after adjusting for weight, while the 25th percentile equation only includes an age^3 by Asian ($p=0.012$) interaction term, after adjusting for body weight and age^3. This positive relationship with age seen at the 75th percentile in AA males indicates that this quantile is shifted upward (more ECW) in older AA compared to their counterparts in other ethnic groups.

Table 5.2. Coefficients for ECW Quantiles in males.

Quantile	Model	Coefficients[a]	Log-Likelihood Ratio Statistic	Difference	P-value
0.10	Intercept Only	4.3836	442.95	---------	--------
	Weight	0.1607	317.23	125.72 [b]	<0.001
0.25	Intercept Only	2.3948	769.27	---------	--------
	Weight	0.2032	566.23	203.04 [b]	<0.001
	Weight, Age^3	0.0000026	559.79	6.437 [c]	0.011
	Weight, Age^3, Age^3 x Asian [e]	-0.0000023	554.25	5.548 [c]	0.012
0.50	Intercept Only	-3.8858	948.23	---------	--------
	Weight	0.1969	690.22	258.01 [b]	<0.001
	Weight, Age^3	0.000003	683.44	6.785 [c]	0.009
	Weight, Age^3, $Height^2$	2.7585	674.36	9.080 [c]	0.003
0.75	Intercept Only	1.2900	769.27	---------	--------
	Weight	0.2120	561.90	207.37 [b]	<0.001
	Weight, Age^3 x AA [d]	0.000003	552.01	9.886 [c]	0.002
	Weight, Age^3 x AA [d], $Height^3$	0.7649	546.03	5.976 [c]	0.015
0.90	Intercept Only	-0.4296	447.34	--------	--------
	Weight	0.2568	298.23	149.12 [b]	<0.001
	Weight, Age^2	0.00036	290.99	7.241[c]	0.007
	Weight,Age^2 $Height^3$	0.5840	286.39	4.596	0.032

Abbreviations: AA, African American.
[a] Coefficient for the significant variable which has been added in each row. Note that for each quantile the entire model must be used (i.e., including the intercept and all the predictor variables).
[b] Difference between log-likelihood ratio statistic for intercept only model and log-likelihood ratio statistic including weight as a predictor.
[c] Difference between log-likelihood ratio statistic for independent variable(s) on the row above and log-likelihood ratio statistic for variables in this row.
[d] AA = 1 ; non-AA = 0
[e] Asian = 1 ; non-Asian = 0

Females

Both weight (p<0.001) was a significant variable in the quantile regression for the 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles (**Table 5.3**).

After adjusting for body weight, height^3 was significantly associated with ECW ($p<0.01$) across the quantiles except for the 50th percentile where height was a significant predictor of ECW ($p<0.001$). At the 25th and 50th percentiles the age by AA interaction was significantly related to ECW ($p=0.008$ and $p<0.001$, respectively), after adjusting for body weight and height^3 (25th percentile) and body weight and height (50th percentile), as indicated in **Table 5.3**. Also, an interaction between age^3 and AA was related with ECW at the 75th percentile while an age^2 by AA interaction term was associated with ECW at the 90th percentile ($p=0.020$ and $p=0.003$, respectively), after adjusting for weight and height^3. This positive coefficient for age in AA females indicates that older AA females have a greater amount of ECW at these quantiles than their counterparts in other race groups. For the 90th percentile an age by Asian interaction was negatively related to ECW ($p=0.041$), after adjusting for body weight, height^3 and age^3 x AA.

Table 5.3. Coefficients for ECW quantiles in females.

Quantile	Model	Coefficients[a]	Log-Likelihood Ratio Statistic	Difference	P-value
0.10	Intercept Only	0.4157	553.48	-----------	--------
	Weight	0.1423	385.67	167.81 [b]	<0.001
	Weight, $Height^3$	1.0545	378.09	7.581 [c]	0.006
0.25	Intercept Only	1.5323	959.37	-----------	--------
	Weight	0.1589	683.70	275.67 [b]	<0.001
	Weight, $Height^3$	0.7917	661.93	21.77 [c]	<0.001
	Weight, $Height^3$, Age x AA [d]	0.0108	654.89	7.045 [c]	0.008
0.50	Intercept Only	-3.3572	1183.90	-----------	--------
	Weight	0.1571	845.04	338.86 [b]	<0.001
	Weight, Height	5.8547	816.22	28.82 [c]	<0.001
	Weight, Height, Age x AA [d]	0.0110	800.36	15.86 [c]	<0.001
0.75	Intercept Only	1.4017	959.37	-----------	--------
	Weight	0.1781	659.76	299.61 [b]	<0.001
	Weight, $Height^3$	1.1232	631.12	28.64 [c]	<0.001
	Weight, $Height^3$, Age^3 x AA [d]	0.000002	625.73	5.395 [c]	0.020
0.90	Intercept Only	1.0479	557.87	-----------	--------
	Weight	0.1899	307.25	250.62 [b]	<0.001
	Weight, $Height^3$	1.2341	283.42	23.83 [c]	0.004
	Weight, $Height^3$, Age^2 x AA [d]	0.00028	274.38	9.041 [c]	0.003
	Weight, $Height^3$, Age^2 x AA[d], Age^3 x Asian [e]	0.0000028	270.22	4.160 [c]	0.041

Abbreviations: AA, African American, Cauc, Caucasian.
[a] Coefficient for the significant variable which has been added in each row. Note that for each quantile the entire model must be used (i.e., including the intercept and all the predictor variables).
[b] Difference between log-likelihood ratio statistic for intercept only model and log-likelihood ratio statistic including weight as a predictor.
[c] Difference between log-likelihood ratio statistic for independent variable(s) on the row above and log-likelihood ratio statistic for variables in this row.
[d] AA = 1 ; non-AA = 0
[e] Asian = 1 ; non-Asian = 0

DISCUSSION

We used quantile regression and a recently developed statistical technique for testing the significance of conditioning variables (16) to generate equations for the 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles of ECW in a large, healthy, ethnically-diverse, adult sample of men and women. At each quantile, ECW was strongly affected by weight in both males and females. Weight, height, $height^2$, $height^3$, age, age^2, age^3, race, and their interactions were variably associated with ECW at the five selected quantiles.

Previous studies of ECW tended to have relatively small sample sizes with limited ethnic variation (17-22). Moore (18) published the first ECW reference values for adult males and females based on body weight alone. Compared to our new models, Moore's equations underestimate ECW in males and females by ~2.3 kg and ~2.4 kg for the 50^{th} percentile. However, Moore evaluated a limited number of subjects and bromide dilution was used as the ECW reference method. In contrast, we calculated ECW from TBW and TBK measurements in a large subject pool that included four race groups. Our models show that, in addition to body weight, the subject's age, height, race, and interactions of these variables, may all be independently related to ECW. Additionally, the ECW space estimated by bromide dilution may differ slightly from that provided by our TBW-TBK method (23).

Several ECW prediction equations have been developed by means of the bioelectrical impedance (BIA) method (24-26), although this is an indirect approach that must be calibrated against some other reference method. The utility of BIA equations as a means of generating reference values is therefore limited.

Our findings show that the ECW quantile regression lines are not uniform across race groups. A significant relation between age and AA race was observed at certain quantiles such that after adjusting ECW for weight, or weight and height, there was a positive association of age and ECW compared to their counterparts in other race groups. This observation is consistent with our recent study (9) based on the same database that reveals AA males and females have a larger mean ECW volume with greater age compared to other race groups, even after adjusting for body composition measures. Also, the negative association between age and Asian found at the 25th percentile in the male sample is in line with this recently published study that showed Asians as the only race group presenting no relation with age after controlling for body composition variables (9).

Study Limitations

A convenience sample was used to generate these reference values and it is possible that our subjects, though many in number, are not representative of other populations.

As noted, we applied TBW and TBK to estimate ECW, and other methods may estimate a slightly different ECW volume even in the same subjects (23). An advantage of the TBW-TBK method is that it based on a physiological model applicable in healthy adults. Nevertheless, our reference values should be carefully interpreted when other methods such as bromide dilution or radio-labeled sulfate are used to estimate ECW.

CONCLUSION

The current study applied quantile regression methods to a large and diverse subject pool to derive quantile values for ECW, which may be used for reference comparisons. The ECW compartment varies widely in health and is sensitive to underlying acute and chronic diseases. Accordingly, the newly developed quantile equations should prove useful in the clinical and research setting.

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