Development of predictive equations for total body water using the deuterium-dilution method as the gold standard in a population of asymptomatic HIV-positive Zulu women in South Africa

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Keywords: HIV, women, total body water, lean body mass, anthropometry, predictive equation, bioelectrical impedance analysis, deuterium dilution, FTIR

Abstract

Objectives: The study aimed to derive predictive equations for total body water determinations with bioelectrical impedance and anthropometric measurements in a population of asymptomatic human immunodeficiency virus (HIV) -positive Zulu women.

Design: Cross-sectional data from within an ongoing prospective study that observed the effect of infant feeding mode on maternal and child health in a cohort of asymptomatic HIV-positive women were used for this objective.

Setting: A community health centre in Durban.

Subjects: Asymptomatic HIV-infected women who were not eligible for antiretroviral therapy. They were resident in the area and were of Zulu ethnicity.

Outcome measures: Development of predictive equations for total body water.

Results: Success was achieved in developing predictive equations for total body water using bioelectrical impedance analysis and anthropometric measurements that were specific to the HIV-positive female Zulu population. These equations were developed using the total body water that was obtained from deuterium-dilution method as a gold standard.

Conclusion: These predictive equations are likely to be more valid for the HIV-positive female African populations of similar build than the current predictive equations that derive from the Western population.

Ⓟ **Peer reviewed. (Submitted: 2012-02-02. Accepted: 2012-07-30.) © SAJCN** **S Afr J Clin Nutr 2012;25(4):186-191**

Introduction

Human immunodeficiency virus (HIV) -associated wasting has been well documented. Kotler et al observed that body cell mass was a more sensitive prognostic indicator than body weight alone in their analyses of 43 studies of HIV-infected individuals that were conducted at their laboratory.[1] In a study that analysed predictors of the long-term survival of HIV-infected individuals, lean body mass was found to be a significant independent predictor of survival, rather than body cell mass.[2]

Various techniques are available to assess and monitor body composition. Until recently, most body composition studies in developing countries were limited to the use of anthropometric techniques. Bioelectrical impedance analysis (BIA) has been used, where affordable. Impedance is proportional to total body water. It is measured by the difference in the voltage between electrodes attached to the wrist and ankle. Impedance can be measured at different frequencies to enable the development of prediction equations for total body water. Most BIA machines are equipped with an empirical equation that is built into the system that derives total body water and lean body mass using the impedance measurements, and the age, sex, weight and height values that are fed into the machine. These equations have been derived in Western populations against a reference method. Prediction formulas for body composition tend to be population-specific.[3,4] Previously obtained BIA results, based on white subjects, might be inappropriate in black populations and need to be retested. Dioum et al showed that BIA prediction equations for total body water differed between African American and white subjects, but the reasons for this remain unclear.[5]

Simple, valid methods that are reproducible are required to assess body composition in HIV-infected African women. Of the different available reference methods for body composition assessment, stable isotope measurements are now considered to be more practical and acceptable in terms of minimal discomfort to subjects.[6,7] Deuterium oxide (^2H_2O) and oxygen-18 are two stable isotopes that have been safely used. However, ^2H_2O, which is relatively cheaper, is more frequently used in body composition studies.

Papathakis et al showed that body composition estimates obtained by BIA, together with anthropometry, were comparable to the deuterium-dilution method, and that body mass index (BMI) and mid-upper-arm circumference (MUAC) were useful in predicting fat mass in HIV-infected and uninfected women.[8] However, BIA and MUAC were not found to be reliable measures when predicting lean body mass.[8] Therefore, in order to elucidate the impact of nutritional interventions and practices on body composition, it is preferable to use the gold standard, namely the deuterium-dilution method, to standardise the BIA measurements. Therefore, the objective was to formulate predictive population-specific equations for the calculation of lean body mass through bioelectrical impedance measurements and anthropometric measurements that were standardised against the deuterium-dilution method.

Method

The study sample consisted of postpartum HIV-positive women who participated in an ongoing study that observed the impact of infant feeding mode on maternal health at a primary healthcare clinic in Durban. This centre serves the community of Cato Manor, which comprises people of Zulu ethnicity. HIV-positive mothers who were eligible for antiretroviral therapy (World Health Organization stages 3 and 4, CD4 count ≤ 200 cells/mm^3) and not resident in the area were excluded from the study. Ethics approval for the study was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (H081/05). Mothers were enrolled after written informed consent had been obtained.

As the primary study was a longitudinal study, and anthropometric and body composition measurements were conducted at two weeks post-delivery and three monthly thereafter up to nine months, for the purpose of this analysis, the measurements that were taken between six and nine months postpartum were used. This was because it was believed that the body composition would have become stabilised by that time. Measurements of women who were still lactating were excluded from this analysis. It was ensured that only participants who had stopped breastfeeding for at least three months prior to this visit were included. Furthermore, it was shown in our primary study that there were no significant differences between the body composition of breastfeeding and formula-feeding mothers in our cohort.[9] Body composition measurements were only taken on the visit after ensuring that the subject was haemodynamically stable, in other words experiencing no oedema, diarrhoea or vomiting.

Anthropometry measurements

Anthropometric measurements that were taken from the mothers included weight, height, MUAC and triceps skinfold (TSF) thickness. Weight was measured using an electronic scale (Seca 882) and recorded to the nearest 0.1 kg while the subject wore minimal clothing.[10] Height was measured using a stadiometer (Seca 225) and recorded to the nearest 0.1 cm using standardised methodology.[10] MUAC was measured using a flexible, inelastic tape measure (Seca) to the nearest 0.1 cm. The measurements were taken while the arm hung freely at the subject's side.[11] Lange callipers were used to measure TSF thickness, which was calculated to the nearest millimetre. Both TSF and MUAC measurements were taken on the right side of the body.[12] BMI was calculated by dividing the weight of the subject by her height squared (kg/m^2).

To reduce inter-observer variability and to enhance reliability, all anthropometric measurements were taken by either the principal investigator or the dietitian, using standardised techniques and the same equipment.[13] The weight, height, MUAC and TSF measurements were taken in duplicate and the mean of the two measurements was used. To enhance precision, if the two measurements differed by more than 0.1 kg for weight, or by more than 0.5 cm for height, MUAC and TSF thickness, the measurements were repeated.

Bioelectrical impedance analysis method

A quad-frequency analyser (Bodystat QuadScan 4000 Hydration Body Composition Monitoring Unit, Isle of Man, British Isles) was used for the BIA. The BIA measurement was taken mid-morning after patients had fasted for four hours.[14] All subjects were measured at approximately the same time at each visit to rule out any diurnal variations. All metal objects were removed from the subject before performing the analyses. To enhance reliability, all the assessments were performed using standardised procedures and electrode placement, by either the principal investigator or the dietitian. The analyser was calibrated before each analysis using the calibrator supplied by the manufacturer (Bodystat QuadScan 4000). All measurements were taken in duplicate and a mean value was calculated. Measurements were repeated if there was a difference of more than five units in the impedance measurements.

Deuterium-dilution methodology to determine body composition

^2H_2O is water in which 99.8-99.9% of the hydrogen atoms are in the form of deuterium. No side-effects have been noted with the amount that is used for dilution techniques.[15,16] The principle of isotope dilution is that when a known quantity of a labelled compound is added to a biological system and mixes with that pool, after a few hours, the enrichment reaches a plateau. The dilution of the labelled compound by the endogenous unlabelled compound gives the size of the pool. The pool, also called volume of distribution (V_D), is larger than the total body water because of nonaqueous exchange. Nonaqueous exchange is a process by which the isotopes in body water exchange with hydrogen atoms in other body components, such as protein and fat. V_D has been estimated to be 1.041 times total body water. Therefore, total body water (kg) is obtained by dividing V_D by 1.041. Total body water contains both intracellular and extracellular fluid. It usually represents 50-60% of body weight in lean adults, and less than 40% in obese adults.

The natural abundance of deuterium is 0.015%. Therefore, it is essential to obtain a baseline saliva sample to calculate the background. Enrichment is the concentration of deuterium above the baseline sample. The Fourier transform infrared spectroscopy (FTIR) instrument subtracts the background from the reading that is obtained from the post-dose sample. The software that is used was developed by the Medical Research Council Collaborative Centre for Human Nutrition Research, United Kingdom, and can be downloaded from its website (www.mrc-hnr.cam.ac.uk). The fat-free mass or lean body mass is approximately 73.2% of the total body water. Therefore, the lean body mass can be calculated from the total body water. The fat mass is the difference between body weight and lean body mass.

Collection of saliva samples and administration of deuterium

A standardised operating procedure, based on International Atomic Energy Agency (IAEA) guidelines, was developed and followed for collection of the samples.[17] A baseline saliva sample was collected from subjects who had fasted for an hour before the visit. Once the sample was collected, the subject was given a preweighed dose of $^{2}H_2O$ to drink. Subsequent saliva samples were collected four hours after ingestion of the deuterium dose. Participants were requested not to eat or drink anything during the four-hour period. All samples were stored in a -20°C freezer. Samples were then batched and analysed using the FTIR instrument. The instrument was housed in the School of Chemistry, University of KwaZulu-Natal.

Fourier transform infrared spectroscopy methodology

The underlying principle of infrared spectroscopy is that when infrared radiation is passed through a sample, while a portion is absorbed by the sample, the remainder is transmitted. Each substance has its own unique spectrum which represents its molecular absorption and transmission. This principle is used to determine the composition of various specimens and other analyses. Shimadzu's IRsolution® software was used for analysis.

Data preparation

All data were entered into an MS Access® (MS Office Access 2003) database that was created for the study. The data were rechecked for any missing data and entry errors.

Statistical methods

Analysis was conducted using SPSS® 15.0. The total body water that was obtained through deuterium dilution as the gold standard was used to develop regression population-specific equations for our population. A spreadsheet that contained all anthropometric measurements, values of total body water obtained from the BIA and FTIR, as well as impedance values obtained from the BIA at 5, 50, 100 and 200 kHz, was imported into SPSS®. A univariate analysis was carried out for the continuous variables to check for normality of distribution. Following this, a bivariate analysis was conducted to check for any associations between the total body water obtained through FTIR and all the other measurements. The dataset was then randomly partitioned into two portions: one was labelled the development sample, and the other, the validation sample. The development sample was used to develop the equation and the validation sample to cross-validate it.

Using stepwise linear regression on the development sample, including the variables with the strongest Pearson's correlation coefficient, a model was obtained. The model was then applied to the validation sample and an adjusted predicted value for total body water was obtained. A paired t-test was carried out between total body water obtained from the FTIR and the adjusted predicted value obtained from the model. This was done to estimate the bias, deviation of the bias and 95% confidence intervals (CIs). Obtained values from the two assays were compared using a Bland-Altman plot, by plotting the difference between the values against the mean of the values. The same methodology was used to form an equation using the total body water obtained from the BIA and from the anthropometric measurements alone. The total body water obtained from the built-in equations of the BIA was also compared to the total body water obtained through the deuterium-dilution method via FTIR. We also calculated the total body water in our population using a published equation developed in an African population in Senegal.[18] The predicted values for total body water obtained from the different measurements were then compared using one-way analysis of variance and the post hoc Bonferroni test.

Results

During the period December 2006-July 2008, 204 subjects were enrolled in the study and a subsample of 144 was included in this analysis. The dataset of 144 subjects was randomly split into a

Table I: Descriptive statistics of the development and validation samples

Variable	Development sample, n = 73		Validation sample, n = 71	
	Mean	SD	Mean	SD
Age (years)*	26	23-31	27	23-30
Weight (kg)	67.41	12.79	67.41	12.36
Height (m)	1.57	0.06	1.57	0.05
MUAC (cm)	29.63	4.06	29.77	4.04
TSF (mm)	20.87	7.20	21.83	7.07
BMI (kg/m^2)	27.24	5.33	27.39	5.05
Total body water (FTIR) (l)	30.98	3.88	31.01	3.89
Lean body mass (FTIR) (kg)	42.44	5.32	42.49	5.34
Fat mass (FTIR) (kg)	24.98	9.69	24.92	9.02
Total body water (BIA) (l)	31.31	3.21	31.49	3.19
Height^2 (m^2)	2.48	3.21	31.49	3.19
Impedance 5 kHz (R_5)	689.20	85.72	676.58	85.23
Impedance 50 kHz (R_{50})	606.08	76.13	594.22	72.86
Impedance 100 kHz (R_{100})	572.74	72.36	562.01	70.70
Impedance 200 kHz (R_{200})	544.21	69.71	534.11	67.90
Height^2/R_5	0.0037	0.0005	0.0037	0.0005
Height^2/R_{50}	0.0042	0.0006	0.0042	0.0006
Height^2/R_{100}	0.0044	0.0006	0.0045	0.0006
Height^2/R_{200}	0.0046	0.0007	0.0047	0.0006

* Median with interquartile range presented

BIA: bioelectrical impedance analysis, BMI: body mass index, FTIR: Fourier transform infrared spectroscopy, MUAC: mid-upper-arm circumference, SD: standard deviation, TSF: triceps skinfold development sample consisting of measurements taken from 73 subjects and a validation sample consisting of measurements taken from 71 subjects (see Table I).

On assessing the correlations between total body water (FTIR), in other words, total body water obtained through the deuterium-dilution method via FTIR and age, anthropometric measurements, BIA values and total body water obtained through the built-in equation of the BIA, it was found that all measurements, except age, were significantly associated with total body water (FTIR) (see Table II).

Table II: Pearson's coefficients for association with total body water (Fourier transform infrared spectroscopy) for the various measurements

Measurement	Pearson's correlation coefficient	p-value
Age (years)	0.159	0.057
Weight (kg)	0.738	< 0.001
Height (m)	0.376	< 0.001
MUAC (cm)	0.615	< 0.001
TSF (mm)	0.454	< 0.001
BMI (kg/m^2)	0.585	< 0.001
Total body water (BIA) (l)	0.894	< 0.001
Height^2 (m^2)	0.376	< 0.001
Impedance 5 kHz (R_5)	0.682*	< 0.001
Impedance 50 kHz (R_{50})	0.718*	< 0.001
Impedance 100 kHz (R_{100})	0.718*	< 0.001
Impedance 200 kHz (R_{200})	0.711*	< 0.001
Height^2/R_5	0.811	< 0.001
Height^2/R_{50}	0.849	< 0.001
Height^2/R_{100}	0.850	< 0.001
Height^2/R_{200}	0.849	< 0.001

* Negative correlation

BIA: bioelectrical impedance analysis, BMI: body mass index, MUAC: mid-upper-arm circumference, TSF: triceps skinfold

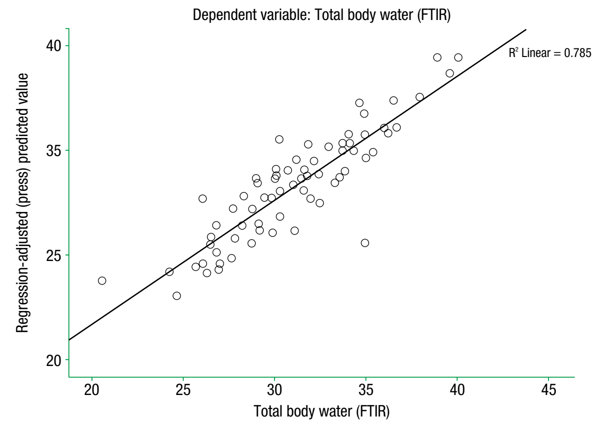

Figure 1: Scatter plot between total body water (l) estimated according to the deuterium-dilution method and predicted total body water (l) estimated from the equation, using total body water obtained from the bioelectrical impedance analysis device

Population-specific predictive equation for calculation of total body water using total body weight values obtained through BIA

Total body water (BIA), in other words, total body water obtained through the in-built equations of the BIA, was significantly correlated to total body water (FTIR) (see Table II). On using stepwise linear regression analysis, the model using total body water (BIA) alone had the best fit with a R^2 of 0.79 (standard error 1.77) (see Figure 1). The validated equation for estimating the total body water using total body water (BIA) is Total body water = 1.089 x total body water BIA - 3.273. The difference between the predicted total body water and total body water (FTIR) was computed and plotted against the mean of the predicted total body water and total body water (FTIR) in a Bland-Altman plot (see Figure 2). The bias was calculated to be 0.002, 95% CI -0.43 and 0.43, p-value = 0.992.

Population-specific predictive equation for calculation of total body water using BIA

Using BIA and anthropometric measurements, it was found that the weight and height squared over impedance values at 5, 50, 100 and 200 kHz were most significantly associated with the total body water values obtained through the FTIR (see Table II). Through stepwise regression, the model with weight and height^2 (H^2)/R_{100} was the model with the best fit, with R^2 of 0.776. The validated equation for calculation of total body water using BIA was therefore Total body water = 0.105 x W + 4 076.28 x H^2/R_{100} + 5.763 (where W = weight in kg, H = height in metres and R_{100} = impedance at 100 kHz). The difference between the mean of the adjusted predicted value and the total body water (FTIR) were computed and plotted in a Bland-Altman plot. The bias was calculated to be -0.003, 95% CI between -0.44 and 0.43, p-value = 0.988.

Population-specific predictive equation for calculation of total body water using anthropometric measures only

Using stepwise linear regression, the model using weight, height and TSF had the best fit with an R^2 of 0.651. The validated equation to

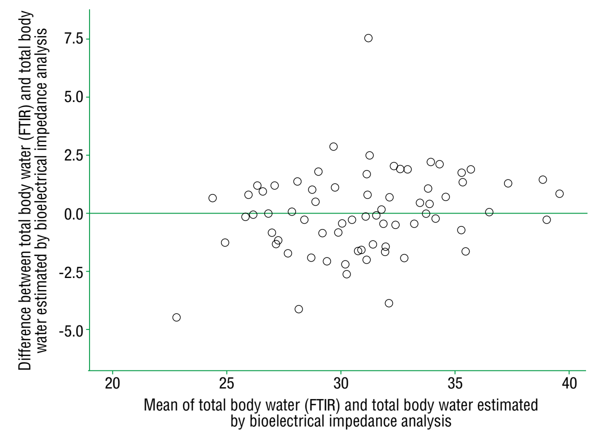

Figure 2: Bland-Altman plot comparing the method of estimating total body water (l) using the prediction equation with total body water (bioelectrical impedance analysis) (l) to the total body water (l), estimated according to the deuterium-dilution method

calculate the total body water using weight, height and TSF obtained was Total body water = 0.277 x W + 6.056 x H^2 - 0.130 x TSF + 0.210. The bias for the equation was -0.003, 95% CI between -0.57 and 0.56, p-value = 0.992. The Bland-Altman plot was plotted using the difference between the mean of the adjusted predicted value and the total body water from the FTIR. The bias was calculated to be -0.003, 95% CI between -0.57 and 0.56, p-value = 0.992.

We estimated total body water using MUAC and weight with an R^2 of 0.564. The validated equation to calculate total body water using weight and MUAC obtained was Total body water = 0.278 x W – 0.136 x MUAC + 16.29. The Bland-Altman plot was plotted between the mean and the difference between total body water (FTIR) and the predicted total body water. The bias was calculated to be -0.01, 95% CI between -0.64 and 0.62, p-value = 0.975.

On including height in the model, R^2 improved to 0.629. The validated equation to calculate total body water using weight, height and MUAC was Total body water = 0.185 x W + 6.667 x H^2 + 0.114 x MUAC – 1.333. The Bland-Altman plot was plotted. The bias was calculated to be -0.016, 95% CI between -0.59 and 0.56, p-value = 0.957.

On using weight and height alone, R^2 obtained was 0.632. The validated equation to calculate total body water using weight and height was Total body water = 0.220 x W + 6.169 x H^2 + 0.945. The Bland-Altman plot was plotted as described. The bias was calculated to be -0.015, 95% CI between -0.59 and 0.56, p-value = 0.958.

Assessment of published predictive equation

We applied the published equation Total body water = 0.3788 x H^2/R_{50} + 0.2348 x W + 0.8152 to our data set.[16] The obtained total body water values were significantly lower than the total body water (FTIR). Bias was estimated to be -0.48, 95% CI -0.90 and -0.05, p-value = 0.027.

Comparison of the different methods of estimating total body weight

We then compared the total body water estimated by the different measures (predictive equations 1-7) to each other (see Table III).

- *Equation 1:* Total body water = 1.089 x total body water BIA - 3.273 (using total body water estimated by the BIA)
- *Equation 2:* Total body water = 0.105 x W + 4076.28 x H^2/R_{100} + 5.763 (using height, weight and impedance at 100 kHz)
- *Equation 3:* Total body water = 0.277 x W + 6.056 x H^2 - 0.130 x TSF + 0.210 (using weight, height and TSF)
- *Equation 4:* Total body water = 0.278 x W – 0.136 x MUAC + 16.29 (using weight and MUAC)
- *Equation 5:* Total body water = 0.185 x W + 6.667 x H^2 + 0.114 x MUAC – 1.333 (using height and MUAC)
- *Equation 6:* Total body water = 0.220 x W + 6.169 x H^2 + 0.945 (using height and weight)
- *Equation 7:* Total body water = 0.3788 x H^2/R_{50} + 0.2348 x W + 0.8152 (published equation in literature).

There were no significant differences in the estimation of total body water between any of the methods, except for the published equation that significantly estimated a lower total body water when compared to the deuterium-dilution method and the others.

Discussion

Lean body mass and therefore total body water estimations are becoming essential and part of the overall management of patients, especially in the HIV-positive population. Lipodystrophy is a well-established side-effect of some of the antiretroviral drugs, as well as a metabolic complication of the disease itself. Therefore, it is essential for healthcare workers in resource-limited settings to have access to simple, easy-to-use methods of estimating lean body mass in order to follow a more holistic approach to patient management.

Generic BIA equations have been in use in South Africa to calculate lean body mass. However, these are often not representative of the population that is being studied, as they are generated from Western populations. Therefore, it is important that South African population-specific predictive equations are used.

The values for total body water obtained from the deuterium-dilution method (the gold standard) were used. By performing stepwise linear regressions, five predictive equations were formed. These were, firstly, for use with BIA values; secondly, for use with BIA devices that generate a total body water value; and thirdly for use in the field using weight and height only; weight, height and TSF; weight and MUAC; and weight, height and MUAC.

Table III: Comparison of the different methods of estimating total body water (FTIR)

Method of estimation	Mean difference	Standard error	Significance	95% CI of the difference	
				Lower-bound	Upper-bound
BIA	-0.476	0.211	0.027	-0.898	-0.055
Adjusted BIA	0.002	0.214	0.992	-0.426	0.430
Weight, height and TSF	-0.003	0.285	0.992	-0.570	0.564
Weight, height and MUAC	-0.016	0.291	0.957	-0.595	0.564
Weight and height	-0.015	0.288	0.958	-0.589	0.559
Weight, height, and impedance	-0.002	0.213	0.991	-0.427	0.422
Weight and MUAC	-0.01	0.314	0.975	-0.637	0.617
Published equation	14.370	0.302	< 0.001	13.767	14.973

The total body water obtained through the built-in equations in the BIA had a very strong correlation with the total body water obtained from the deuterium-dilution method. All the methods, namely anthropometry, impedance values, and total body water estimated using BIA, were able to produce similar values of total body water that were statistically not different from each other, or from total body water (FTIR).

The prediction equation developed in Senegal for the HIV-positive population there was not valid for our population. This could perhaps be due to a lower mean BMI among the Senegalese population sample. Our population had a high mean BMI and although HIV positive, these subjects were clinically stable and not yet on antiretroviral therapy.

Therefore, these equations are population-specific and would be valid in the asymptomatic HIV-positive female Zulu population. They are preferable to the Western equations, even for other African female populations with a similar builds. It would be useful for long-term follow-up to evaluate lipodystrophy in women of similar build who are initiated on antiretroviral therapy.

Acknowledgements

We would like to thank our patients for their participation in the study, the staff of the MTCT-Plus Initiative, the staff of Umkhumbane Clinic, the staff of the Family Clinic, King Edward Hospital and the departments of paediatrics, obstetrics and gynaecology at King Edward Hospital. We extend our thanks to the Ethekwini Municipality Health Department and the nutrition directorate of the Provincial Department of Health for their support in executing the study at their facilities. We thank Tonya Esterhuizen for supervising the statistical analyses and Francesca Esposito for helping with the anthropometric and BIA measurements. We thank Prof Mauro Valencia for expert advice on prediction equations. We would like to thank IAEA for donating the equipment used in the study, and Drs Christine Slater, Lena Davidsson, Najjat Mokhtar and Neil Jarvis of the IAEA for technical advice and training in body composition methodology. The Hasso Plattner Trust fund provided running costs for the study.

Declaration

The MTCT-Plus Initiative provided funding for the programme, staff and antiretroviral drugs.

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