Research article

Poor haemoglobin-haematocrit agreement in apparently healthy adult population; a cross-sectional study in Cape Coast Metropolis, Ghana

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ABSTRACT

Background: This study estimated total body water (TBW), daily water intake (DWI) and haemoglobin-haematocrit relationship in adults in a tropical environment where active lifestyles could precipitate plasma volume contraction.

Methods: This cross-sectional study recruited 170 participants, and was carried out between February 2018 and May 2018 at University of Cape Coast. Semi-structured questionnaires were used to obtain demographic data and DWI. Five ml of venous blood sample was drawn for full blood count, haemoglobin variant determination, serum sodium and potassium levels. TBW was estimated using Chumlea's anthropometric equation. Statistical significance was set at $p < 0.05$ under two-tail assumption.

Results: Whereas 72.3% had low haematocrit, only 22.4% were anaemic per haemoglobin cut-off demonstrating a poor haemoglobin-haematocrit correlation. Also, whereas 30% of participants had low TBW, 22.9% had hypernatraemia, with 97.1% reporting DWI of $< 3$ L. Bland-Altman plot showed that calculated haematocrit (HCT = Hb*3) underestimated HCT by a factor of 1.788 ($p = 0.0314$). A scatter-plot showed a trend towards higher haematocrit-haemoglobin deviations as haemoglobin increased. Furthermore, 32.6% of participants with normal haemoglobin levels had low TBW. Moreover, whereas haemoglobin and serum K+ significantly positively correlated to TBW, serum Na+ was inversely related to TBW.

Conclusion: The low DWI is suggestive that measuring plasma volume and/or haemoglobin mass may be required to correctly diagnose anaemia.

1. Introduction

Water makes up about 60% of the total body weight of healthy young adult compared to 75% body weight in infants. The total body water is distributed into intracellular and extracellular fluid compartments [1]. Research has demonstrated the importance of adequate hydration for better physical and cognitive functions [2, 3]. It has been estimated that a healthy adult loses approximately 2.5 L of water per day through what has been termed insensible (through skin and lungs) and obligatory (through kidneys and gastrointestinal tract) water loses. Requirements for daily water intake is dependent on factors such as age, level of physical activity and metabolic needs of the individual. Evidently, high ambient environmental temperature and physiological stress due to strenuous physical exertion in extreme temperature may increase not only insensible water loss but also sweating. The European Food Safety Authority recommends daily water intake of 2.0L for adults [4]. This daily water requirement needs to be adjusted depending on work load, atmospheric temperature and level of stress. For example, the WHO recommends a daily water intake of 4.5 L for both children and adults performing manual labour in warmer environments [5].

Failure to adjust daily water intake to meet an individual's daily requirement has been shown to result in negative effects on exercise capacity as well as headache, migraine and impaired cognitive function [6, 7, 8]. Additionally, volume depletion and dehydration may lead to plasma volume contraction and subsequent haemoconcentration and thus confound laboratory assay interpretation. This is particularly important in sub-Saharan Africa where anaemia is a prevailing condition and prompt diagnoses may be critical to improve patient outcomes. For example, in a study by MarcCordick, where participants red cell parameters were estimated pre- and post-intravenous saline administration, it was concluded that dehydrated individuals may have spuriously high red cell counts [9]. A recent study has also advocated for routine

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measurement of plasma volume to ensure accurate determination of anaemia to remove the confounding role of expanded plasma volume in certain categories of patients [10, 11]. In the present study, we used an anthropometric-derived equation to estimate the total body water in participants in a tropical region and assessed the association between total body water and haematocrit/haemoglobin relationship as well as participants’ daily water consumption.

2. Materials and methods

2.1. Study design/setting

This cross-sectional study employed a convenience sampling method to recruit 170 participants at the University of Cape Coast campus in the Central region of Ghana. The study population included 109 students and 61 workers; 19 commercial vehicle drivers and 42 food vendors within the university. The study was undertaken between February 2018 to May 2018; the average daily environmental temperature during the period of the study was 32 °C. Participant recruitment was restricted to 8:00 am to 11:00 am each day.

2.2. Questionnaire administration

A semi-structured questionnaire was administered to each participant to capture socio-demographic variables. Other anthropometric measurements such as height (m) and weight (kg) were also measured. Written informed consent was obtained from each participant after the test procedures and rationale for the study had been explained to participants.

2.3. Exclusion criteria

Individuals below 20 years were excluded from the study. The rationale for this exclusion criterion was based on the fact that the anthropometric equation for estimating total body water in blacks was developed using individuals ≥20 years old. Also, individuals with a known kidney pathology were excluded since kidney disorders are possible risk factors to electrolyte disturbances and abnormalities in urinalysis results. Additionally, individuals taking any form of alcoholic beverage, smoking, on anti-diuretic therapy, or with any form of diarrhoeal disease in the two months preceding the study period were excluded.

2.4. Experimental protocols

2.4.1. Measurement of height and weight of participants

The height (to the nearest 0.1 m) and weight (to the nearest 0.1 kg) were measured using a meter rule (BAHCO, UK) and scale (Seca GmbH, Germany) respectively. The values obtained for each participant were recorded and used to calculate the total body water (TBW).

Total body water (TBW) was estimated using the Chumlea et al [12] equation for Blacks:

\[
\text{TBW}_{\text{males}} \text{ (in liters)} = -18.37 - (0.09 \times \text{Age}) + (0.34 \times \text{Weight}) + (0.25 \times \text{Height}).
\]

\[
\text{TBW}_{\text{females}} \text{ (in liters)} = -16.71 - (0.05 \times \text{Age}) + (0.22 \times \text{Weight}) + (0.24 \times \text{Height}).
\]

The age, weight and height were respectively measured in years, kilogram (kg) and centimetre (cm).

2.4.2. Estimation of daily water intake

The daily total water intake was estimated per participants’ reported record of the number of sachet water consumed per day. In Ghana, drinking water is packaged and sold in 500 ml volume sachets.

2.4.3. Urine dipstick analysis

Participants were directed to collect a midstream urine into a sterile container. Urine specific gravity and urine protein analysis were performed using urine dipsticks (URIT medical Electronics Co., Ltd, China) in accordance with manufacturer’s protocol.

2.4.4. Blood sample collection

All individuals who agreed to be part of the study were provided seats to sit and rest for a minimum of 30 min before blood sampling was undertaken. During the resting period, participants answered questionnaire items; researchers were on hand to explain questionnaire items. A total of 5 ml of venous blood was obtained from the forearm of each participant using syringe and needle in accordance with standard protocols; immediately, three (3) ml of the blood was dispensed into EDTA tubes whiles 2 ml was dispensed into serum separator tubes (SST). The EDTA anticoagulated samples were used for full blood count (FBC) and haemoglobin electrophoresis. Samples in the serum separator tubes were allowed to clot and centrifuged at 2500 rpm for 5 min to obtain serum. Sera were then transferred into Eppendorf tubes and stored frozen at -25 °C until when required (for serum sodium and potassium estimation).

2.4.5. Analysis of full blood count

The haematocrit and haemoglobin concentration of participants were estimated using a three-part automated haematology analyser (Mindray BC 2800, China) in accordance with manufacturer’s specifications. The Mindray BC 2800 employs electrical impedance and cyanide-free methods for cell counting and haemoglobin estimations respectively. Based on WHO guidelines [13], anaemia was defined as haemoglobin <11.5 g/dl or 12.5 g/dl respectively for females and males in sub-Saharan Africa. Also, haematocrit was categorized using cut-off 0.45±0.05% or 0.41±0.05% for males and females respectively [14].

2.4.6. Haemoglobin variant determination

The inherited haemoglobin type was determined using cellulose acetate electrophoresis (pH 8.2–8.4) according to previously published protocols [14]. For each electrophoretic run, control sample having haemoglobin A, C, S and F was run to provide internal validation of the assay results. Whereas haemoglobin A and haemoglobin F are respectively normal adult and foetal haemoglobins, haemoglobins S (glutamic acid is replaced by valine at position 6 of globin chain) and C (glutamic acid is replaced by lysine at position 6 of globin chain) are structural variants that may result in haemoglobin disorders [14].

2.4.7. Electrolyte analysis (sodium and potassium)

Analysis of serum Sodium and Potassium concentrations was carried out using a fully automated chemistry analyzer (Convergy’s® ISE Electrolyte Analyzer, Germany). The analyzer works on Ion selective based electrolyte analysis to determine the ion concentration of Sodium and Potassium by a fully automated aspiration of 100μl of serum sample.

2.5. Ethical approval and consent to participate

The protocols for the study were approved by the Institutional Review Board, University of Cape Coast (ethical clearance ID: UCCIRB/CHAS/2016/46). Also, the rationale for the research was explained to all participants in either local dialect or English (as may be applicable) to ensure that participants thoroughly understood the research. Only those who gave informed consent were recruited for the study. No personal identifiers were recorded with the primary data to ensure that the data cannot be traced directly to any participant by any third party.

2.6. Statistical analysis

Data was entered into Microsoft Excel 2016 and analysed using GraphPad prism version 8.0.2 for Windows (GraphPad Inco, USA). Data were analysed for normality using D’Agostino-Pearson omnibus
normality testing. For correlation analyses, the Spearman correlation coefficient analyses were used to establish relationship between variables. The haemoglobin (Hb)-haematocrit (HCT) threefold conversion was employed to calculate haemoglobin as: \( H_{b, \text{calc}} = \frac{\text{HCT}}{3} \) and calculated haematocrit as \( \text{HCT}_{\text{calc}} = \frac{\text{Hb}}{3} \). This is also called the “rule of three” which literally means that multiplying a patient's haemoglobin by 3 will give haematocrit [15]. Bland-Altman plot was used to test the agreement between calculated and measured haemoglobin, as well as calculated and measured haematocrit. All statistical testing was undertaken using the two-tailed assumption and statistical significance was established using p-values < 0.05.

3. Results

The demographic and haematological characteristics of the participants are presented in Table 1. Males comprised the majority of the participants (61.8% vs 38.2% females). Whereas 73.6% of the participants were in their twenties, 64.1% were university students. Also, 27.7% of the participants had inherited haemoglobin variants. Moreover, whereas 72.3% of the participants had low haematocrit and would have qualified as anaemic, only 22.4% were anaemic per haemoglobin levels cut-off.

The serum electrolytes and anthropometrically estimated total body water are presented in Table 2. Whereas 30% of the participants had low estimated total body water, only 2.9% of the participants reported taking \( \geq 3 \) L of water per day. Also, whereas 19.4% of the participants had low serum potassium, 22.9% of participants had elevated serum sodium.

Figure 1 shows the relationship between measured haemoglobin/haematocrit and calculated haemoglobin/haematocrit based on the rule of three used in validation of complete blood count results. On average, measured haematocrit was lower than calculated haematocrit (haematocrit = \( \frac{\text{Hb}}{3} \)) by a factor of 1.788 (Figure 1A, \( p = 0.0314; t = 2.171; \) one-sample t-test). Also, measured haemoglobin was averagely greater than calculated haemoglobin (haematocrit/3) by a factor of 0.5962 (Figure 1B). When the haemoglobin values (measured and calculated) were explored through scatter-plot, there was a trend towards higher deviations between measured haemoglobin and calculated haemoglobin as haemoglobin concentration increased (Figure 1C, \( p = 0.0157; t = 2.447, \) one-sample t-test).

The total body water was explored per the demographic and haematological parameters of study participants (Table 3). Significantly higher proportion of males had low body water compared to females (40.9% vs 12.3%). However, the total body water did not significantly differ when the data was stratified per either the occupation or the inherited haemoglobin type of participants. Also, whereas 32.6% of participants with normal haemoglobin had low estimated total body water, 17.1% of participants with low haematocrit had low estimated total body water. Moreover, 33.3% of participants with low total body water had normal serum sodium levels. Participants’ reported daily water intake was categorized and used to explore variables assessed in the present study (see supplementary table 1).

The relationship between total body water and biochemical and haematological variables of participants were also assessed using Spearman's correlation coefficient (Table 4). Whereas water intake, haemoglobin levels and serum potassium were significantly positively correlated with total body water, serum sodium was significantly, but negatively correlated with total body water. Additionally, haematocrit and urine specific gravity were each negatively correlated with total body water although none of these reached statistical significance (supplementary table 2 gives the correlations between all variables assessed in the study).

4. Discussion

It has been advocated that measurement of haematocrit could be used as a surrogate means of determining anaemia in resource-limited setting.

### Table 1. Demographic and haematological characteristics of participants.

| Characteristic       | N     | Percentage (%) |
|----------------------|-------|----------------|
| Gender               |       |                |
| Female               | 65    | 38.2           |
| Male                 | 105   | 61.8           |
| Age (years)          |       |                |
| 20–24                | 104   | 61.2           |
| 25–29                | 21    | 12.4           |
| 30–34                | 11    | 6.5            |
| 35–39                | 8     | 4.7            |
| 40–44                | 6     | 3.5            |
| 45–49                | 10    | 5.9            |
| >50                  | 10    | 5.9            |
| Occupation           |       |                |
| Driver               | 19    | 11.2           |
| Student              | 109   | 64.1           |
| Trader               | 42    | 24.7           |
| Haemoglobin type     |       |                |
| AA                   | 112   | 72.3           |
| AC                   | 22    | 14.2           |
| AS                   | 20    | 12.9           |
| SS                   | 1     | 0.6            |
| HCT (%)              |       |                |
| Low (33.20 ± 5.46)   | 102   | 72.3           |
| Normal (43.78 ± 4.26)| 35    | 24.8           |
| High (53.72 ± 6.69)  | 4     | 2.8            |
| Hb (g/dL)            |       |                |
| Low (10.86 ± 0.91)   | 38    | 22.4           |
| Normal (13.57 ± 1.16)| 132   | 77.6           |

N: number of participants; HCT: haematocrit; haematocrit was categorized using 0.45±0.05% or 0.41±0.05% for males and females respectively; Hb: haemoglobin; Low Hb (anaemia) was defined as Hb < 12.5 g/dl or 11.5 g/dl for males and females respectively; for Hb and HCT categories, the actual data spread of participants are presented in parenthesis as mean ± standard deviation.
This is based on the widely applied rule of three in which measured haematocrit divided by a factor of three estimates haemoglobin. Recently, the applicability of this haematocrit-haemoglobin rule of three in malaria-endemic regions has been challenged [16, 17]. Using a cohort of adults (aged 20–68 years) we provide evidence that further argues against the adoption of this haematocrit-haemoglobin rule of three in a sub-Saharan African country. Additionally, our study found that water intake is generally inadequate in these individuals with overwhelming 97.1% taking less than 3 L of water per 24 h in spite of the warm temperature that may precipitate water loss during the day. This DWI is woefully inadequate when compared to the WHO recommended 4.5 L for individuals in tropical settings [5].

Plasma volume contraction consequent to dehydration and/or intravascular volume depletion could lead to spuriously high/normal haemoglobin values with tremendous implications on detection and subsequent diagnosis of anaemia based on complete blood count results [18]. Therefore, patients may present definite anaemic symptoms such as headache, easy fatigability, and shortness of breath and yet have persistently high red cell count and haemoglobin (Hb) levels [9]. This cross-sectional study found 30% of participants with estimated low total body water. Although this study found an overall 22.4% of participants with low haemoglobin levels and were therefore anaemic, 32.6% of the participants with normal haemoglobin levels also had low total body water. Taken together with the fact that 22.9% also had hypernatraemia, we hypothesise that both isotonic volume depletion (due to excess sweating) and hypernatraemic volume depletion (due to low daily water intake) may be at play which ultimately masked the true state of anaemia [19]. In view of the warmer environmental temperature in sub-Saharan Africa the WHO recommends an average daily water intake of 4.5 L [5].

This study did not investigate causality, we speculate that the reported low daily water consumption and its associated plasma contraction may partly account for the deviation between measured haemoglobin/haematocrit and calculated haematocrit/haemoglobin as per the rule of three. We are advancing this hypothesis based on the well-recognised physiologic response of cell shrinkage in hypertonic environment [20]. Thus, the low daily water intake and consequent tonicity of plasma might have led to shrinkage of red blood cells with potential to impact haematocrit estimation. Since manual haematocrit

| Parameter                  | N     | Percentage (%) |
|----------------------------|-------|----------------|
| Total Body Water (L)       |       |                |
| Low (40.43 ± 2.17)         | 51    | 30.0           |
| Normal (44.95 ± 4.03)      | 112   | 65.9           |
| High (58.43 ± 6.38)        | 7     | 4.1            |
| Daily water intake (L)     |       |                |
| 0–0.99                     | 3     | 1.8            |
| 1–1.99                     | 53    | 31.2           |
| 2–2.99                     | 109   | 64.1           |
| 3–3.99                     | 5     | 2.9            |
| Urine protein              |       |                |
| Positive                   | 30    | 17.6           |
| Negative                   | 140   | 82.4           |
| Serum K⁺                   |       |                |
| <3.0 (2.74 ± 0.19)         | 33    | 19.4           |
| 3.0–4.7 (4.12 ± 0.46)      | 108   | 63.5           |
| >4.7 (5.02 ± 0.42)         | 29    | 17.1           |
| Serum Na⁺                  |       |                |
| <135 (130.00 ± 3.32)       | 8     | 4.7            |
| 135–145 (140.30 ± 2.67)    | 123   | 72.4           |
| >145 (147.80 ± 1.55)       | 39    | 22.9           |

N: number of participants; L: litre; the actual data spread in each of the categories of the total body water, serum K⁺ & Na⁺ of participants are presented in parenthesis as Mean ± standard deviation.

| Parameter                  | N     | Percentage (%) |
|----------------------------|-------|----------------|
| Water intake (L)           |       |                |
| 0–0.99                     | 3     | 1.8            |
| 1–1.99                     | 53    | 31.2           |
| 2–2.99                     | 109   | 64.1           |
| 3–3.99                     | 5     | 2.9            |

Table 2. Total body water and electrolytes in participants.
Table 3. Total body water of participants stratified per demographic and haematological variables.

|                      | Total Body Water (L) | p-value |
|----------------------|----------------------|---------|
|                      | Low N (%)            | Normal N (%) | High N (%) |       |
| Age (years)          |                      |          |            |       |
| 20–24                | 35 (33.6)            | 68 (65.4) | 1 (1.0)    | 0.001*|
| 25–29                | 7 (33.3)             | 13 (61.9) | 1 (4.8)    |       |
| 30–34                | 3 (27.3)             | 7 (64.7)  | 1 (9.1)    |       |
| 35–39                | 1 (12.5)             | 5 (62.5)  | 2 (25.0)   |       |
| 40–44                | 2 (33.3)             | 4 (66.7)  | 0 (0.0)    |       |
| 45–49                | 3 (30.0)             | 6 (60.0)  | 1 (10.0)   |       |
| ≥50                  | 0 (0.0)              | 9 (90.0)  | 1 (10.0)   |       |
| Gender               |                      |          |            |       |
| Female               | 8 (12.3)             | 53 (81.5) | 4 (6.2)    | <0.001*|
| Male                 | 43 (40.9)            | 59 (56.2) | 3 (2.9)    |       |
| Occupation           |                      |          |            |       |
| Driver               | 5 (26.3)             | 13 (68.4) | 1 (5.3)    | 0.248 |
| Student              | 36 (33.0)            | 71 (65.1) | 2 (1.8)    |       |
| Trader               | 10 (23.8)            | 26 (66.7) | 4 (9.5)    |       |
| DWI (L)              | 0.0–0.99             | 2 (66.7)  | 1 (33.3)   | 0.437 |
|                      | 1.0–1.99             | 17 (32.1) | 35 (66.0)  | 1 (1.9) |
|                      | 2.0–2.99             | 29 (26.6) | 74 (67.9)  | 6 (5.5) |
|                      | 3.0–3.99             | 3 (60.0)  | 2 (40.0)   | 0 (0.0) |
| Hb type              | AA                   | 33 (29.5) | 77 (68.7)  | 2 (1.8) |
|                      | AC                   | 8 (36.4)  | 12 (54.5)  | 2 (9.1) |
|                      | AS                   | 5 (25.0)  | 12 (60.0)  | 3 (15.0)|
| Haemoglobin          | Low                  | 8 (21.1)  | 29 (76.1)  | 1 (2.6) |
|                      | Normal               | 43 (32.4) | 83 (61.9)  | 6 (4.5) |
|                      | High                 | 36 (35.3) | 62 (60.8)  | 4 (3.9) |
| HCT                  | Low                  | 6 (17.1)  | 26 (74.3)  | 3 (8.6) |
|                      | Normal               | 6 (17.1)  | 26 (74.3)  | 3 (8.6) |
|                      | High                 | 2 (50.0)  | 2 (50.0)   | 0 (0.0) |
| Serum Na+            | <135                 | 2 (25.0)  | 5 (62.5)   | 1 (12.5) |
|                      | 135–145              | 41 (33.3) | 76 (61.8)  | 6 (4.9) |
|                      | >145                 | 8 (20.5)  | 31 (79.5)  | 0 (0.0) |

DWI: Daily water intake; N: number of participants; HCT: haematocrit; haematocrit was categorized using 0.45±0.05% or 0.41±0.05% for males and females respectively; Hb: haemoglobin; Low Hb (anaemia) was defined as Hb < 12.5 g/dl or 11.5 g/dl for males and females respectively; proportions in categories were compared using the Chi-square test. *Indicates that TBW significantly differed across the group sub-categories at p < 0.05.

Table 4. Correlation between total body water and biochemical and haematological variables of participants.

| Variable              | r       | p       |
|-----------------------|---------|---------|
| Age (years)           | 0.098   | 0.202   |
| Water intake (L)      | 0.168   | 0.028   |
| Haemoglobin (g/dl)    | 0.534   | 0.001   |
| Haematocrit (%)       | -0.046  | 0.549   |
| Urine Specific gravity| -0.054  | 0.484   |
| Serum K+              | 0.160   | 0.037   |
| Serum Na+             | -0.151  | 0.049   |

Bold indicates that TBW significantly differed across the group sub-categories at p < 0.05.

One potential problematic area is in the area of blood donation where dehydration-induced plasma volume contraction may mask detection of anaemia and allow subsequent red blood cell donation by such individuals. Previous studies have documented that blood donor population in sub-Saharan Africa are overwhelmingly males. Taken together with our findings that significantly higher proportion of the males had low estimated body water, it is not far-fetching to suppose that this is a practical daily occurrence. We propose that a repeated-measure, controlled trial that evaluate the impact of increased hydration as well as estimates the osmolarity in healthy volunteers will shed light on the extent to which this low water intake confound clinical decisions made from complete blood count results of indigenes of the study area. Our proposed study is further supported by the significant correlations between total body water and participants’ variables such as haemoglobin, serum sodium and potassium reported herein. We are of the view that the impact of such a study would be far-reaching.

In spite of the conclusions drawn from the present study, our inability to measure plasma volume and/or haemoglobin mass limits the impact of the data presented herein. Recently, Otto et al made a strong argument for the inclusion of plasma volume estimation to allow accurate interpretation of haemoglobin-based anaemia classification [10]. Schmidt and colleagues have also optimized the CO-inspiration method for routine haemoglobin mass estimation to enable better determination of a patient's oxygen carrying capacity [21]. Additionally, although the anthropometric equation employed herein was optimized in Blacks in the United States, it is plausible to suppose that there might be some differences in total body water as a consequence of differences in geography and dietary intakes. In spite of these acknowledged limitations, our study brings to light an important public health challenge that needs addressing.

Declarations

Author contribution statement

Patrick Adu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Grace Ali-Baya, Emmanuel Zenile and Bridgette Obour Aikin: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Regina Elorm Amoaning, David Larbi Simpong: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article supplementary material referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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