Total body protein in healthy adolescent girls: validation of estimates derived from simpler measures with neutron activation analysis[1-3]

Verena K Haas, Jane R Allen, Michael R Kohn, Simon D Clarke, ShuHua Zhang, Julie N Briody, Margie Gruca, Sloane Madden, Manfred J Müller, and Kevin J Gaskin

ABSTRACT

Background: Little recent and accurate information about body protein content in healthy adolescent girls is available.

Objective: The objective was to assess the total body nitrogen (TBN) and total body protein (TBPr) contents of fat-free mass (P: FFM) in a group of healthy adolescent girls and to validate previously published TBN prediction equations.

Design: TBN was measured with in vivo neutron activation analysis (TBN_{NAA}). Bone mineral density and FFM were measured with dual-energy X-ray absorptiometry (FFM_{DXA}), total body water and FFM were measured with bioimpedance analysis, and FFM was assessed by measuring skinfold thicknesses in 51 girls with a mean ($\pm$SD) age of 14.7 $\pm$ 0.7 y. The validity of the TBN prediction equations was assessed with Bland-Altman analysis.

Results: TBN_{NAA} in our adolescent group was higher (1.49 kg) than values reported in earlier studies of women (1.25 and 1.31 kg), and P:FFM was slightly higher (23%) than that documented in adults (19–21%). Previously published TBN equations showed either systematic bias or wide limits of agreement.

Conclusion: A predictive equation derived from the present study population based on FFM_{DXA} improves the prediction of TBN for groups of young girls but may not be helpful for individuals in clinical settings. *Am J Clin Nutr* 2007;85:66–72.

KEY WORDS Body composition, adolescents, total body protein, total body nitrogen, fat-free mass, neutron activation analysis, dual-energy X-ray absorptiometry, anthropometric measures, bioimpedance analysis

INTRODUCTION

Adequate dietary protein intake and protein deposition are essential to achieve normal growth in children and adolescents. However, what constitutes sufficient protein intake and deposition is less than certain. To determine minimal protein requirements for growing populations, the World Health Organization (WHO) developed guidelines in 1985. These guidelines relied on short-term nitrogen balance studies that were carried out in children and adults. Protein requirements for intermediate age groups were obtained by extrapolation after an allowance for growth increment was added (1). These guidelines clearly emphasize the considerable limitations of this method and the need for an accurate indicator of protein nutritional status, the need to identify protein inadequacy before clinically detectable changes occur, and the need to gather information on long-term protein accretion associated with normal growth.

Before NAA measurement, direct assessment of total body protein (TBPr) could only be achieved with chemical carcass analysis. In children, these chemical assessments were limited to very few subjects who died of severe illness or malnutrition (2–4) and thus were not representative of normal body protein content. Current studies often assess fat-free mass (FFM), which is then used as a surrogate for protein nutritional status, assuming that protein is a relatively fixed constituent of FFM (5). However, FFM is a heterogeneous body compartment consisting of water, protein, and bone. Adolescence, in particular, is a period characterized by growth and maturation known to be associated with significant changes in the composition of FFM (6–8). Common methods to measure FFM, such as dual-energy X-ray absorptiometry (DXA), anthropometric measures, and bioimpedance analysis (BIA) are only indirect measures of FFM, based on assumptions, and therefore need to be evaluated against a direct measure in children and adolescents.

TBN measurements with neutron activation analysis (NAA) provide a direct and accurate measure of TBPr. Although most centers with NAA facilities have not examined children because of unacceptably high levels of radiation, the Children's Hospital at Westmead has developed a low-radiation-dose facility specifically for pediatric use (9). Previously developed TBN prediction equations derived from NAA measurements were developed in adult groups of mixed sex with the use of a 5-compartment model based on NAA and tritiated water analysis (10) or from a regression analysis based on height and age (11). Our previously published pediatric TBN prediction equation was based on a group of

43 girls and boys aged 4–16 y in whom NAA and anthropometric measures were used (9).

The aim of this study was to accurately assess TBN, and its relation to other body compartments, in an exclusively adolescent female population to provide further reference data on body composition in this age group. Furthermore, we aimed to investigate whether TBN could be estimated with other techniques (DXA, BIA, and anthropometric measures) in adolescent girls. We therefore evaluated previously published TBN prediction equations derived from NAA measurements (9–11) and generated a new prediction equation based on data from our adolescent study population.

SUBJECTS AND METHODS

Study population

A multiethnic group of 51 healthy adolescent girls with a mean age of 14.7 y (range: 13.4–16.0 y) was recruited from a girls school in Westmead, Sydney, Australia. All study participants had been born in Australia, but had family backgrounds from 5 different ethnic groups: white or European Australian ($n = 35$), at least one parent from the Middle East ($n = 8$), at least one parent from Asia ($n = 6$), black African ($n = 1$), and Latin American ($n = 1$). Thirty-four girls were tested in the follicular phase (ie, days 1–10) and 17 were tested in the luteal phase (ie, after day 10) of their menstrual cycle. The Ethics Committees at the Children's Hospital at Westmead and Westmead Hospital approved the study protocol. Before participating, all girls and their parents gave informed written consent.

Anthropometric measurements

All measurements were performed on the same morning after the subjects had fasted overnight. Height (± 0.1 cm) was measured with a stadiometer (Holtain Ltd, Crymych, United Kingdom) and body weight (± 0.1 kg) was measured with an electronic scale (model FW-150K; AND Tokyo, Japan) while the subjects were wearing light clothing. Body mass index (BMI) was calculated as body weight/height^2 (in kg/m^2), and SD scores for weight, height, and BMI were estimated from WHO reference data. Skinfold thicknesses were measured in duplicate on the dominant side of the body at 4 sites (biceps, triceps, subscapular, and suprailiac) with a Harpenden skinfold caliper (British Indicators Ltd, Hertfordshire, United Kingdom) by 2 trained investigators. Skinfold thickness data were used to calculate percentage body fat (%BF), fat mass (FM), and FFM (body weight minus FM) according to Slaughter et al (12). This equation, derived from a multicompartment model with hydrostatic weighing, deuterium dilution, and photon absorptiometry in a population of 132 females (age: 8–29 y), has an SEE of 3.8%.

Maturation status

Pubertal stage (breast and pubic hair development) was self-reported by the study participants on the basis of criteria developed by Tanner (13) and Duke et al (14).

Bioimpedance analysis

Resistance (R) and reactance (Xc) were measured at 50 kHz with a multifrequency bioelectrical impedance meter (model SFB3, version 1.0; SEAC, Queensland, Australia) with a tetrapolar arrangement of self-adhesive electrodes while the subject was supine on a nonconductive surface with their limbs slightly abducted. The impedance index (ZI) was calculated as height^2 (m)/reactance. For 4 study participants, BIA data could not be obtained because of technical problems. To calculate FFM, the equation of Houtkooper et al (15) was used, which is based on underwater weighing and deuterium dilution in a study group of 225 young girls and boys aged 10–19 y (CV: 5.1%). To calculate total body water from BIA, a prediction equation was developed based on deuterium oxide dilution carried out in a subgroup of 14 normal-weight girls from our study population.

Deuterium oxide dilution

Subjects had fasted for ≥ 8 h and voided before receiving an oral dose of 0.4 g D$_2$O/kg body wt that was mixed with drinkable water. It was previously shown that deuterium oxide reaches equilibrium in body fluids 3 h after administration (16); therefore, saliva samples were collected before and 3 h after administration. The deuterium oxide concentration was measured by Fourier transform infrared spectrophotometry according to Jennings et al (17), and TBW was calculated as deuterium dilution space $\times$ 0.99 (to correct for water space in saliva) $\times$ 1.04 (to correct for overestimation of TBW by the deuterium method). The precision of this method has been shown to be 0.1–0.9% (17).

Dual-energy X-ray absorptiometry

Bone mineral content (BMC) and soft tissue lean mass (STLM) were determined with a Lunar Prodigy whole-body scanner in conjunction with EnCORE software version 8.10 (Lunar Corp, Madison, WI). A whole-body scan takes <10 min, and involves a very low radiation dose of <0.02 mSv. FFM$_{DXA}$ was calculated as LTM + BMC assessed with DXA. The precision for this technique in vivo, as assessed at the hospital, was 0.82% for LTM (18).

Neutron activation analysis

TBN was measured by using a modification of the method of prompt γ NAA with a single californium source as described previously (9). In the modified technique, we now expose subjects bilaterally to neutrons from two ^{252}Cf sources placed above and below the scan table, which halves the scan time. ^{14}N is converted to ^{15}N with the emission of a 10.8-MeV γ ray, and γ ray emission is measured with 4 detectors. TBN is calculated as the integral under the nitrogen peak centered at 10.8 MeV on the γ ray spectrum. The accuracy and precision of these techniques are both 3%, as determined from the measurement of nitrogen mass in child-sized boxes and anthropomorphic phantoms (19). The scan involves a radiation dose of <0.2 mSv and takes <10 min. Nitrogen can be assumed to be a constant constituent of protein (16% by weight), so that TBPr is derived from TBN by the equation TBPr$_{NAA}$ = 6.25 $\times$ TBN$_{NAA}$ (20).

Estimates of FFM and TBN

FFM was estimated by using skinfold thicknesses (FFM$_{SF}$), BIA (FFM$_{BIA}$), DXA (FFM$_{DXA}$), and a modified 4-compartment model (FFM$_{4CBIA}$) with TBPr derived from NAA, bone mineral from DXA, and total body water from BIA. We tested 1) the Baur equations used to calculate predicted TBN (^{P}TBN$_{Baur}$), which were developed earlier in our unit in a pediatric population on a single-source TBN machine and based on height and FFM$_{SF}$ (9);

TABLE 1
General physical characteristics of the study population ($n = 51$) and body fat estimated with different methods[1]

Age (y)	$14.7 \pm 0.7 \, (13.4–16.0)$[2]
Height (cm)	$163 \pm 7 \, (142–174)$
Weight (kg)	$58.3 \pm 13.3 \, (33.9–90.4)$
BMI (kg/m^2)	$21.8 \pm 4.1 \, (15.9–33.2)$
$\Sigma 4$ SF (mm)	$64 \pm 26 \, (30–139)$
Tanner stage[3]	$4 \, (2–5)$
FM$_{SF}$ (%)	$27.8 \pm 8.4 \, (14.1–47.9)$
FM$_{BIA}$ (%)	$33.2 \pm 6.3 \, (19.4–44.7)$
FM$_{DXA}$ (%)	$32.0 \pm 9.3 \, (14.1–51.2)$
FM$_{4CBIA}$ (%)	$29.4 \pm 5.8 \, (20.0–42.9)$

[1] $\Sigma 4$ SF, sum of biceps, triceps, subscapular, and suprailiacal skinfold thicknesses; FM, fat mass; SF, skinfold thickness; BIA, bioimpedance analysis; DXA, dual-energy X-ray absorptiometry; 4C, 4-compartment model.

[2] $\bar{x} \pm$ SD; range in parentheses (all such values).

[3] Self-estimated by the girls using questionnaires; range in parantheses.

TABLE 2
Fat-free mass (FFM) and FFM composition of the study population ($n = 51$)[1]

TBN$_{NAA}$ (kg)	$1.49 \pm 0.19 \, (1.03–1.82)$
TBPr$_{NAA}$ (kg)	$9.3 \pm 1.2 \, (6.4–11.4)$
BMC$_{DXA}$ (g)	$2281 \pm 421 \, (1319–3253)$
R50$_{BIA}$ (Ω)	$732 \pm 78 \, (585–905)$
Xc50$_{BIA}$ (Ω)	$71 \pm 8 \, (58–86)$
TBW$_{BIA}$ (L)[2]	$28.8 \pm 5.0 \, (18.3–41.2)$
FFM$_{DXA}$ (kg)	$39.1 \pm 5.3 \, (26.5–49.6)$
FFM$_{BIA}$ (kg)	$38.2 \pm 6.2 \, (24.6–54.1)$
FFM$_{SF}$ (kg)	$41.7 \pm 7.6 \, (22.7–66.1)$
FFM$_{4CBIA}$ (kg)	$40.4 \pm 6.3 \, (26.1–55.5)$
TBW:FFM$_{4CBIA}$ (%)	$71.2 \pm 1.8 \, (68.2–76.5)$
TBPr:FFM$_{4CBIA}$ (%)	$23.2 \pm 1.7 \, (17.8–25.6)$
BMC:FFM$_{4CBIA}$ (%)	$5.6 \pm 0.5 \, (4.5–6.7)$

[1] All values are $\bar{x} \pm$ SD; range in parentheses. TBN, total body nitrogen; NAA, neutron activation analysis; TBPr, total body protein; BMC, bone mineral content; DXA, dual-energy X-ray absorptiometry; R50, resistance at 50 kHz; BIA, bioimpedance analysis; Xc50, reactance at 50 kHz; TBW, total body water; SF, skinfold thickness; 4C, 4-compartment model.

[2] Calculated with a prediction equation developed in this study population.

2) the Ryde equation developed with 31 men and women and based on a 5-compartment model of body composition (10); and *3)* the Ellis equation developed with a group of 61 women and based on height and age (11).

$$^{P}TBN_{Baur} \, (g) = -1821 + 19.9 \times height \, (cm) \quad (1)$$

$$^{P}TBN_{Baur} \, (g) = -295 + 46.0 \times FFM \, (kg) \quad (2)$$

$$^{P}TBN_{Ryde} \, (kg) = -0.9 + [31 \times FFM \, (kg)] \quad (3)$$

$$^{P}TBN_{Ellis} \, (g) = [1481.4 \times height \, (m)] -$$
$$(3.6 \times age) - 853.5 \quad (4)$$

Prediction equations based on weight or SF measurements were not included because these parameters are commonly used to correct for hydrogen background when calculating TBN (19).

Statistical analysis

Statistical analyses were performed by using SPSS (version 11.5.1; SPSS Inc, Cary, NC). All data are presented as means $\pm$ SDs at a significance level of $P < 0.05$. Bland-Altman analysis was used to compare agreement between methods to assess TBN (21). Pearson correlation coefficients were used to describe the relation between 2 continuous variables, and stepwise regression was carried out to assess the predictive value of FFM measures to estimate TBN. Analysis was carried out with and without overweight subjects.

RESULTS

Body composition

General physical characteristics of the study population, maturation status, and body fat estimated with different methods are shown in **Table 1**. SD scores (SDS) relative to WHO reference data were 0.3 ± 1.1 for height, 0.4 ± 1.3 for weight, and 0.1 ± 1.0 for BMI. Many girls were overweight: eleven (22% of the study sample) according to the cutoff established by Cole et al of a BMI > 23.9 for 15-y-old girls (22); 4 of these girls could be classified as obese (BMI > 29.1). The prevalence of overweight subjects in our study sample reflected the population prevalence

of overweight and obese persons reported in a large survey of young Australians (23).

Body protein, FFM determined according to the different techniques used in this study, and the composition of FFM are shown in **Table 2**. A 4-compartment model of body composition obtained by combining several measurement techniques is more robust to interindividual variability in the composition of FFM (8). Because our 4-compartment model includes BIA measurements of TBW and not a "gold standard" for TBW assessment, we compared TBW estimates from BIA with deuterium dilution in a subgroup of 14 normal-weight girls from our study group. Mean ($\pm$SD) values for TBW predicted with BIA and deuterium dilution were 28.2 ± 3.0 and 28.1 ± 3.6 L, respectively. No significant difference was observed between the means of the 2 methods ($P = 0.914$), and the r^2 value was 0.70 when TBW values measured with the 2 methods were regressed against each other. The prediction equation was as follows:

$$TBW_{BIA} = [0.271 \times body \, weight \, (kg)] +$$
$$(0.321 \times Zl_{50}) + 1.294 \quad (5)$$

where ZI_{50} is the impedance index measured at 50 kHz. No significant difference was observed between the mean FFM values measured with different techniques. TBN content itself provides only limited information about nutritional status. It is common to express TBN in relation to weight or height (**Figure 1**). A strong relation was observed between TBN and both weight and height. The best fitting model to describe TBN in relation to weight was quadratic, whereas the association between TBN and height was linear. Even though the "surplus" weight of the overweight subgroup did not appear to be stored as nitrogen, all overweight subjects had a relatively high TBN content. No significant difference was observed in TBN between the 13 girls with a Tanner stage of ≤ 3 (1.44 ± 0.22 kg N) and those with a Tanner stage of 4 to 5 (1.52 ± 0.18 kg N).

To determine whether TBN can be estimated with simpler techniques that assess FFM, we examined the relation between TBN and estimates of FFM from DXA (**Figure 2**) and BIA
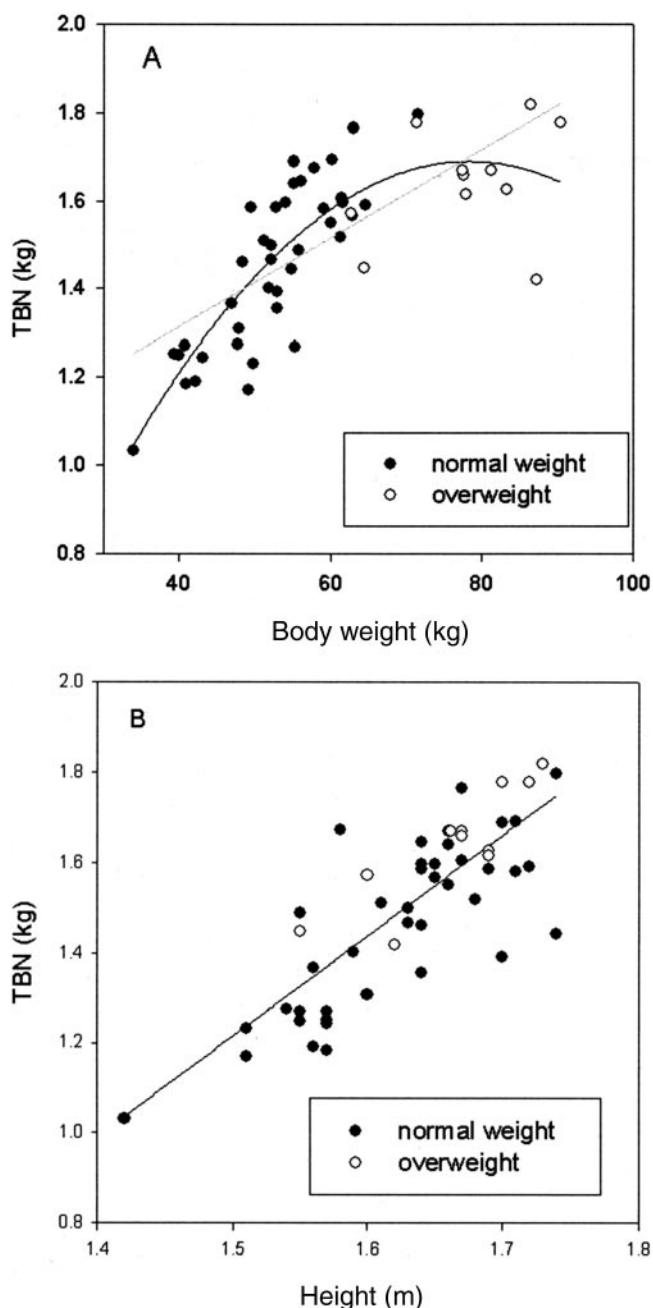
FIGURE 1. Relation between total body nitrogen (TBN) measured by neutron activation analysis and body weight and height in 51 adolescent girls by regression analysis. *Normal weight* and *overweight* were defined according to Cole et al (22). A: $y = 0.011x + 0.881$ (linear regression), $r^2 = 0.54$, $P < 0.001$; $y = -0.0003x^2 + 0.0514x - 0.3218$ (quadratic regression), $r^2 = 0.70$, $P < 0.001$. B: $y = 2.1469x - 2.002$, $r^2 = 0.65$ (linear regression), $P < 0.001$.
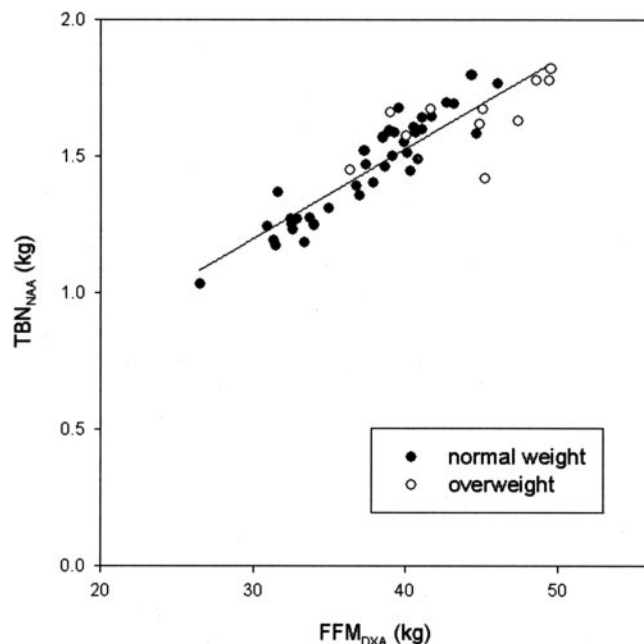
FIGURE 2. Relation between fat-free mass measured by dual-energy X-ray absorptiometry (FFM_{DXA}) and total body nitrogen measured by neutron activation analysis (TBN_{NAA}) in 51 adolescent girls by linear regression analysis. Regression equation: $y = 0.033x + 0.218$, $r^2 = 0.81$, SEE = 2.3 kg FFM_{DXA}, or 6% of the group mean FFM_{DXA}.

(**Table 3**). FFM_{SF} was not included because %fat based on SF is part of the TBN calculation to correct for hydrogen background (19), and FFM_{4CBIA} was not included because our 4-compartment model was derived with the use of TBN data. On the basis of the methods and equations used in the current study, FFM_{DXA} was more closely associated with TBN than was FFM_{BIA} ($r^2 = 0.81$ compared with $r^2 = 0.64$; Table 3) and was

therefore used subsequently to generate TBN prediction equations. Exclusion of the overweight participants improved the associations between TBN and FFM_{DXA} (Table 3).

TBN predicted from height, weight, and FFM

Agreement between TBN_{NAA} and previously developed TBN prediction equations, and the prediction equation developed with regression analysis of our current data, is shown in **Table 4**. Because techniques to assess body composition have different accuracies in normal-weight and overweight populations, the normal-weight subgroup was also analyzed separately. For both groups, close agreement was provided by the prediction equations derived from FFM_{DXA} that were developed in our unit. For these equations, prediction power improved slightly when overweight subjects were excluded. The Ellis equation performed well in both groups, although it was developed in women. With the Ryde equation, which was developed in a group of adults, including males, TBN was underestimated in both of our adolescent groups. The Bland-Altman plots shown in **Figure 3** give more detailed information about the agreement between selected methods.

As expected, TBN predicted according to the equation derived from the normal-weight group in the present study population showed the smallest limits of agreement (-134 to 136 g N; data not shown), which, however, is still equivalent to just $<10\%$ of the mean TBN_{NAA}. TBN predicted according to Baur on the basis of height showed a negative systematic bias, which suggests that the underestimation of TBN increases as TBN increases. In a regression analysis with weight as an independent variable and this bias as a dependent variable, weight was a significant predictor of the bias ($P = 0.001$). This implies that TBN in the heavier and taller girls will be underestimated when based on height, which is also evident from Figure 1. The mean bias close

TABLE 3
Associations between fat-free mass (FFM) assessed with different methods and total body nitrogen measured by neutron activation analysis (TBN$_{NAA}$)[1]

	r for TBN$_{NAA}$[2]	P:FFM	Regression equation to predict TBN (g)	r^2
		%		
All girls ($n = 51$)				
FFM$_{DXA}$	0.900[3]	24.0 ± 1.3[4]	TBN = 32.66 × FFM$_{DXA}$ + 217	0.81
FFM$_{BIA}$	0.798[3]	24.6 ± 2.1	TBN = 24.68 × FFM$_{BIA}$ + 546	0.64
Normal-weight girls ($n = 40$)				
FFM$_{DXA}$	0.937[3]	24.2 ± 1.1	TBN = 38.98 × FFM$_{DXA}$ − 11	0.88
FFM$_{BIA}$	0.751[3]	25.0 ± 2.1	TBN = 30.41 × FFM$_{BIA}$ + 346	0.57
Overweight girls ($n = 11$)				
FFM$_{DXA}$	0.63[5]	23.3 ± 1.9	TBN = 18.14 × FFM$_{DXA}$ + 838	0.40
FFM$_{BIA}$	0.330	22.3 ± 2.3	TBN = 11.44 × FFM$_{BIA}$ + 1103	0.19

[1] P:FFM, protein content of FFM; DXA, dual-energy X-ray absorptiometry; BIA, bioimpedance analysis.

[2] Pearson correlation coefficients.

[3] $P < 0.001$.

[4] $\bar{x}$ ± SD (all such values).

[5] $P < 0.05$.

to zero for TBN predicted according to Baur and FFM$_{BIA}$ for all girls could be explained by an underestimation of TBN in the normal-weight participants that was compensated for by an overestimation of TBN in the overweight participants (Figure 3B). In the normal-weight group, TBN was underestimated when based on FFM$_{BIA}$ (Table 4).

DISCUSSION

This study accurately describes total body nitrogen (TBN) content and its relation to other body compartments in a group of adolescent girls. The TBN values of the adolescent girls were higher than those reported previously for women (10, 11), and the protein content of FFM was slightly higher than that expected from early chemical studies of the protein content of children (2–4).

TABLE 4
Analysis of agreement for total body nitrogen (TBN) prediction equations[1]

| Prediction equation[2]	x ± SD	Bias[3]	95% CI
All girls ($n = 51$)		g	
Measured TBN$_{NAA}$	1493 ± 191	—	—
PTBN$_{Baur, height}$	1420 ± 144	−77 ± 113	−107 to −37
PTBN$_{Baur, FFM(DXA)}$	1503 ± 242	7 ± 111	−25 to 40
PTBN$_{Baur, FFM(BIA)}$	1487 ± 287	−3 ± 177	−56 to 50
PTBN$_{Ryde, FFM(DXA)}$	1211 ± 163	−286 ± 84	−308 to −257
PTBN$_{Ellis, height+age}$	1512 ± 107	16 ± 122	−16 to 58
PTBN$_{Gaskin, FFM(DXA)}$	1497 ± 168	1 ± 84	−22 to 29
Normal-weight girls ($n = 40$)			
Measured TBN$_{NAA}$	1455 ± 188	—	—
PTBN$_{Baur, height}$	1406 ± 144	−51 ± 115	−88 to −5
PTBN$_{Baur, FFM(DXA)}$	1439 ± 206	−18 ± 74	−38 to 11
PTBN$_{Baur, FFM(BIA)}$	1379 ± 217	−74 ± 145	−125 to −23
PTBN$_{Ryde, FFM(DXA)}$	1168 ± 139	−290 ± 75	−311 to −259
PTBN$_{Ellis, height+age}$	1502 ± 107	45 ± 121	5 to 93
PTBN$_{Gaskin, FFM(DXA)}$	1458 ± 169	1 ± 69	−17 to 29

[1] p, predicted; FFM, fat-free mass; DXA, dual-energy X-ray absorptiometry; BIA, bioimpedance analysis; NAA, neutron activation analysis.

[2] The equations of Baur et al (9), Ryde et al (10), Ellis (11), and Gaskin et al (developed in the present study population; see Table 3) were used.

[3] Calculated as means ± 1.96 SD.

TBN in relation to weight, height, and FFM in adolescent girls

In our group of healthy girls, TBN was closely associated with height (Figure 1B) and also with weight, although not linearly (Figure 1A). Describing nitrogen in relation to weight does not account for the fact that most of the "surplus" weight in overweight subjects normally consists of fat and not protein. Even though this was true for our overweight subjects, we also showed that the body nitrogen content of all overweight girls was relatively high when considering the entire study group. The adolescent group in our study had higher TBN values (1.49 kg) than did adult women in 2 other studies: 1.25 kg (10) and 1.31 kg (11). This difference can be explained by the fact that protein mass is building up during puberty (24) and decreases in adulthood with increasing age. Our data agree with those of Cohn et al (25), which showed that TBN was 1.45 kg in healthy women 20–29 y of age and steadily decreased to 1.15 kg at 70–79 y of age.

Composition of FFM

From earlier chemical studies, it is suggested that protein is a relative constant of FFM in specific age groups, rising from 3.7% at birth to ≈17% at 1 y and to between 19% and 21% in adults (2, 4). More recent studies involving NAA have confirmed these numbers in adults (10). To our knowledge, no comparable data are available for adolescents. The P:FFM ratio calculated with the help of a 4-component BIA model in the current study, 23.2%, is slightly greater than the value of 19–21% reported for adults. FFM hydration in our study population (71.2%) was also similar to adult values of between 72% and 73% (26). However, the FFM mineral content of 5.6% in our study was lower than the value of 7% reported for adults and closer to the value of 5% reported previously for children (8). Our analysis of FFM composition also showed that the mineral and protein contents varied more than did the content of water; the CVs for mineral and protein contents were 8.0% and 9.1%, respectively, but the CV for water content was only 2.8%. This finding is consistent with the study by Wells et al (8) in children. Our data confirm earlier findings that the protein chemical maturity of FFM is reached by 14.5 y in girls (27). The higher P:FFM ratio in the 4-compartment BIA model than in earlier chemical studies may be variously
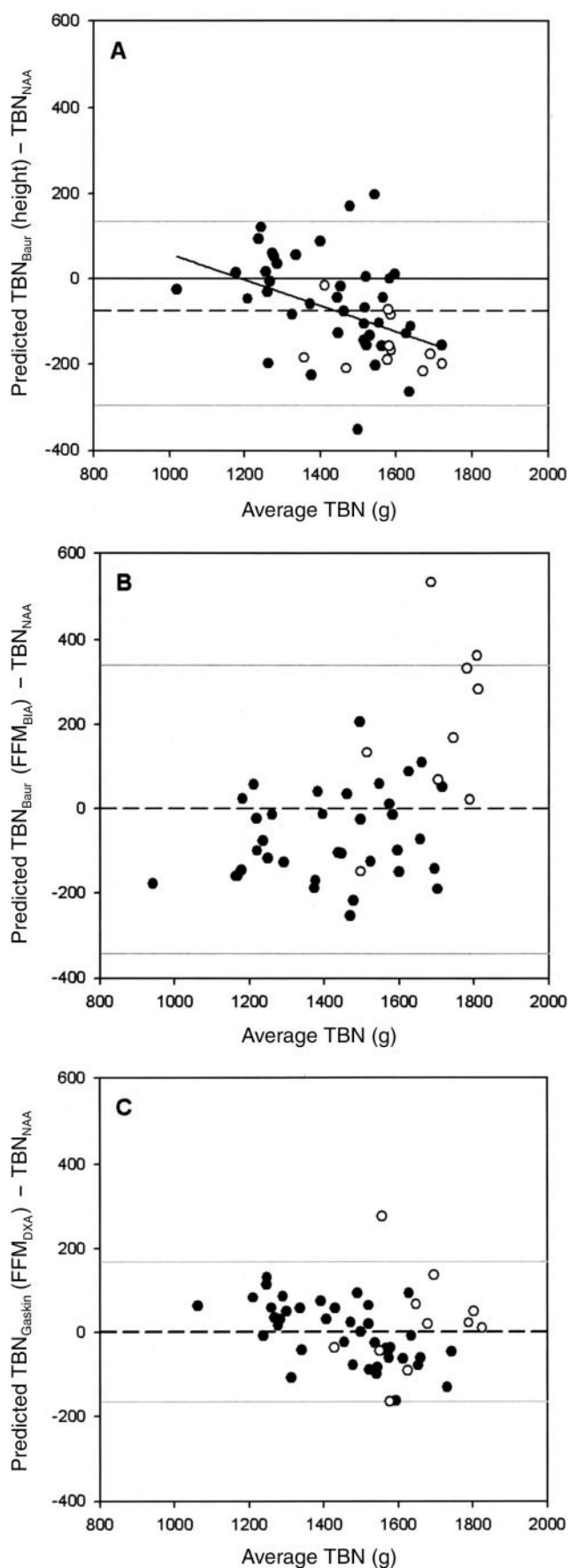
explained. FFM estimates depend on the methodology and their assumptions and could be inaccurate. Unfortunately, our study did not involve a "gold standard" test for FFM, and underestimating FFM will lead to an overestimation of the protein content of FFM. However, there might be more than just a methodologic reason for the observed discrepancy. Studies involving chemical carcass analyses were done in severely malnourished or diseased persons in whom not only absolute protein stores, but also TBPr in proportion to FFM, might have been significantly altered. Evidence exists that P:FFM adapts to nutritional status. The nitrogen content of lean body mass was found to be lower in malnourished cystic fibrosis patients than in well-nourished cystic fibrosis patients (28) and to increase in patients with anorexia nervosa during refeeding (29). Lastly, because of these early chemical studies, people have changed their lifestyle and dietary habits and are most likely better nourished than they were 40 y ago. It is tempting to speculate that the body protein content of FFM is gradually and steadily increasing, both in developing civilizations and in advancing generations.

Validation of TBN prediction equations

When cross-validating previously published TBN formulas with our study population, it became clear that formulas are less suitable when based on height or weight than when based on a DXA-derived measure of FFM and the inclusion of overweight subjects weakens their predictive power. We also conclude that both the sex and age of the study population used to derive the TBN prediction models are important factors that should be taken into account, as has been documented by other authors for different body-composition models. Additionally, we generated a prediction equation from our study population, based on FFM measured with DXA, which is a useful tool when estimating TBN in groups of adolescent girls in research settings where NAA is not available. Specifically, it could be helpful in future studies involving malnourished young girls to detect and quantify changes in body protein. However, clinical application to achieve TBPr assessment without NAA measurements cannot be achieved with our DXA model because of a residual variance of 10% in the TBN group mean. We suggest 2 explanations for this residual variance. First, it might originate from methodologic bias when FFM is measured indirectly with DXA. Studies that have assessed the body composition of animals in vivo and chemically show that DXA can provide accurate measures of FFM (30, 31). However, many authors have cautioned against the use of DXA as a reference method for body composition, other than for bone mineral content, in humans (32, 33). Second, the variance might point to the fact that the nitrogen content per unit FFM is an individual characteristic and is thus subject to biological variation.

FIGURE 3. Bland-Altman plots comparing total body nitrogen measured by neutron activation analysis (TBN_{NAA}) with TBN predicted with the Baur equation on the basis of height (A), with TBN predicted with the Baur equation on the basis of fat-free mass measured by bioimpedance analysis (FFM_{BIA}) (B), and with TBN predicted with the Gaskin equation on the basis of FFM measured by dual-energy X-ray absorptiometry (FFM_{DXA}) (C) in 51 normal-weight (●) and overweight (○) adolescent girls. The broken lines represent mean bias, and the unbroken lines represent the upper and lower limits of agreement ($\bar{x}$ bias ± 1.96 SD).

Study limitations

Because the cohort was relatively small, it did not allow for random division of the participants into 2 groups to test the regression equations developed in the current study. Therefore, and also because of the narrow age range and diverse ethnicity of our study population, caution should be used when extrapolating the data to other populations. In addition, our analysis was complicated by the fact that >20% of our study participants were overweight. Even though our group represented a typical female adolescent Australian population, our overweight group was too small to carry out a separate analysis to better elucidate the effect of overweight on body-composition techniques and the protein content of FFM.

Conclusions

In our adolescent study population, TBN values were higher than those previously described for women, and the protein content of FFM was also higher than values observed in early chemical studies. A prediction model based on DXA measurements of FFM developed in this study can accurately predict the TBN content in a group of young adolescent girls, but not in individuals. This model will be helpful in future studies of the body nitrogen content of malnourished adolescent girls.

REFERENCES

1. Energy and protein requirements: report of a joint FAO/WHO/UNU Expert Consultation. Geneva, Switzerland: World Health Organization, 1985.
2. Garrow JS, Fletcher K, Halliday D. Body composition in severe infantile malnutrition. J Clin Invest 1965;44:417–25.
3. Halliday D. Chemical composition of the whole body and individual tissues of two Jamaican children whose death resulted primarily from malnutrition. Clin Science 1967;33:365–70.
4. Widdowson EM, Dickerson JWT. Chemical composition of the body. In: Comar CL, Bronner F, ed. Mineral metabolism. An advanced treatise. New York, NY: Academic Press, 1964.
5. Borovnicar DJ, Wong KC, Kerr PG, et al. Total body protein status assessed by different estimates of fat-free mass in adult peritoneal dialysis patients. Eur J Clin Nutr 1996;50:607–16.
6. Roemmich JN, Clark PA, Weltman A, Rogol AD. Alterations in growth and body composition during puberty. I. Comparing multicompartment body composition models J Appl Physiol 1997;83:927–35.
7. Wong WW, Hergenroeder AC, Stuff JE, et al. Evaluating body fat in girls and female adolescents: advantages and disadvantages of dual-energy X-ray absorptiometry. Am J Clin Nutr 2002;76:384–9.
8. Wells JCK, Fuller NJ, Dewit O, Fewtrell MS, Elia M, Cole TJ. Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models. Br J Nutr 2001;86:45–52.
9. Baur LA, Allen JR, Humphries IR, Gaskin KJ. Measurement of total body protein in childhood. In: Jürimäe T, Hills AP. Medicine and sport science. Vol 44. Body composition assessment in children and adolescents. Basel, Switzerland: Karger Verlag, 2001.
10. Ryde SJS, Birks JL, Morgan WD, Evans CJ, Dutton J. A five-compartment model of body composition of healthy subjects assessed using in vivo neutron activation analysis. Eur J Clin Nutr 1993;47:863–74.
11. Ellis KJ. Reference man and women more fully characterized. Variations on the basis of body size, age, sex, and race. Biol Trace Elem Res 1990;26–27:385–400.
12. Slaughter MM, Lohman TG, Boileau RA, et al. Skinfold equation for estimation of body fatness in children and youth. Hum Biol 1988;60:709–23.
13. Tanner JM. Growth at adolescence with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity. Oxford, United Kingdom: Blackwell Scientific, 1962.
14. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66:918–20.
15. Houtkooper LB, Going SB, Lohman TG, Roche AF, Van Loan M. Bioelectrical impedance estimation of fat-free mass in children and youth: a cross-validation study. J Appl Physiol 1992;72:366–73.
16. Schoeller DA, Dietz W, van Santen E, Klein PD. Validation of saliva sampling for total body water determination by $H_2^{18}O$ dilution. Am J Clin Nutr 1982;35:591–4.
17. Jennings G, Bluck L, Wright A, Elia M. The use of infrared spectrophotometry for measuring body water spaces. Clin Chem 1999;45:1077–81.
18. Ogle GD, Allen JR, Humphries IR, et al. Body composition assessment by dual-energy X-ray absorptiometry in subjects aged 4–26 y. Am J Clin Nutr 1995;61:746–53.
19. Humphries IRJ, Allen BJ, Blagojevic N, Gaskin KJ. Hydrogen background in total nitrogen estimations. Phys Med Biol 1995;40:201–7.
20. Sutcliffe JF. A review of in vivo experimental methods to determine the composition of the human body. Phys Med Biol 1996;41:791–833.
21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
22. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320:1240–3.
23. Booth ML, Wake M, Armstrong T, Chey T, Hesketh K, Mathur S. The epidemiology of overweight and obesity among Australian children and adolescents, 1995–97. Aust N Z J Public Health, 2001;25:162–9.
24. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am J Clin Nutr 1982;35:1169–75.
25. Cohn SH, Vartsky D, Yasumura S, et al. Compartmental body composition based on total-body nitrogen, potassium, and calcium. Am J Physiol 1980;239:E524–30.
26. Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: review and critique of a classic body-composition constant. Am J Clin Nutr 1999;69:833–41.
27. Humphries IRJ. Multi-compartment paediatric body composition assessment—a methodological validation and clinical evaluation in healthy children and children with disease. PhD dissertation. University of Sydney, Sydney, Australia, 2004.
28. Allen BJ, Blagojevic N, McGregor BJ, et al. In vivo determination of protein in malnourished patients. In: Ellis KJ, Yasumura S, Morgan WD. In vivo body composition studies. London, United Kingdom: The Institute of Physical Sciences in Medicine, 1987.
29. Russell JD, Mira M, Allen BJ, et al. Protein repletion and treatment in anorexia nervosa. Am J Clin Nutr 1994;59:98–102.
30. Svendsen OL, Haarbo J, Hassager C, Christiansen C. Accuracy of measurements of body composition by dual-energy X-ray absorptiometry in vivo. Am J Clin Nutr 1993;57:605–8.
31. Lukaski HC, Marchello MJ, Hall CB, Schafer DM, Siders WA. Soft tissue composition of pigs measured with dual X-ray absorptiometry: comparison with chemical analyses and effects of carcass thicknesses. Nutrition 1999;15:697–703.
32. Van der Pleug G, Withers RT, Laforgia J. Percent body fat via DXA: comparison with a four-compartment model. J Appl Physiol 2003;94:499–506.
33. Roubenoff R, Kehayas J, Dawson-Hughes B, Heymsfield SB. Use of dual-energy X-ray absorptiometry in body-composition studies: not yet a "gold standard." Am J Clin Nutr 1993;58:589–91.