Title page: Associations of age and body mass index with hydration and density of fat-free mass from 4 to 22 years

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Running title: Hydration and density of the fat-free mass

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Abstract

Background: Most body composition techniques assume constant properties of Fat Free Mass (FFM) (hydration and density) regardless of nutritional status, which may lead to biased values. **Aim**: To evaluate the interactive associations of age and Body Mass Index (BMI) with hydration and density of FFM. **Methods**: Data from subjects aged between 4 and 22 years old from several studies conducted in London, UK were assessed. Hydration (H_{FFM}) and density (D_{FFM}) of FFM obtained from 4 component model in 936 and 905 individuals, respectively, were assessed. BMI was converted in z-scores, and categorised into five groups using z-score cut-offs (thin, normal weight, overweight, obese and severely obese). Linear regression models for H_{FFM} and D_{FFM} were developed using age, sex and BMI group as predictors. **Results**: Nearly 30% of the variability in H_{FFM} was explained by models including age and BMI groups, showing increasing H_{FFM} values in heavier BMI groups. On the other hand, ~40% of variability of the D_{FFM} was explained by age, sex and BMI groups, with D_{FFM} values decreasing in association with higher BMI groups. **Conclusion**: Nutritional status should be considered when assessing body composition using two-component methods, and reference data for H_{FFM} and D_{FFM} is needed to higher BMI groups to avoid bias. Further research is needed to explain intra-individual variability of FFM properties.

Introduction

Body composition is useful to assess as it is related to diverse health and disease conditions, either as cause or consequence (1). For instance, lean mass is associated with bone deposition and, in turn, is the main tissue consuming glucose and determining energy expenditure (2,3). On the other hand, an increased fat mass (FM) early in life is associated to insulin resistance, adulthood obesity and cardiovascular risk (4–6) and a reduced lean mass deposition in childhood could predict osteoporosis in the adult age but also morbidity and mortality.

Although Body Mass Index (BMI) is considered as the accepted clinical standard to assess weight in relation to height, and is widely used to diagnose both under-nutrition and overweight or obesity, BMI does not have a constant association with body composition across age, gender and ethnicity (7), and therefore can be misleading. Assessing body composition in nutrition-related diseases is useful for monitoring clinical progress and response to treatment, and to inform more specific individual management of the disease (1).

Given the fact we cannot use the gold standard technique, which is cadaver dissection (8), several techniques for assessing body composition *in vivo* have been developed and improved over the years to measure different components of the human body.

Body composition in children is usually assessed using 2-component (2C) methods, which partition body weight into its major components FM and fat-free mass (FFM, used here synonymously with lean mass). For example, hydrometry measures total body water (TBW) and converts this to FFM by taking into account hydration of FFM (H_{FFM}), while densitometry measures total body density and calculates FFM and FM using Archimedes principle, in combination with values for the density of fat and the density of FFM (D_{FFM}). However, these techniques lose accuracy in many human conditions, such as disease, or hormone cycle in women, due to the effect on variability in H_{FFM} under these situations. Second, nutritional status may also influence FFM properties. Such variability may therefore challenge techniques for measuring TBW like isotopic dilution or bioelectrical impedance, or densitometric techniques such as air-displacement plethysmography.

Many studies have shown differences in FFM properties between children and adults, due to chemical maturation of the FFM. Differences between adults and children in FFM properties are due to the fact that children have higher levels of water and lower levels of mineral and proteins (9,10). In addition, other factors can be involved in FFM properties such as nutritional status, but more data is needed to understand this issue (11,12).

We previously analysed associations of BMI SDS with hydration in small samples of children aged 7-14 years (12,13) (n=50 and n=107 respectively). The aim of this study is to evaluate associations of age and BMI with both H_{FFM} and D_{FFM} over a wider age-range (4-22 years), drawing on a substantially larger sample size. Understanding how FFM properties differ not only by age but also by BMI may help to assess body composition in those with higher levels of BMI, in whom body composition assessment is clinically important.

Methods

Subjects

Body composition data from a total of 1014 healthy subjects aged 4 to 22 years old were available from different data bases from the Childhood Nutritional Research Centre (UCL Institute of Child Health) (10,14–18). The main samples were a reference dataset of healthy children and adolescents aged 5-22 years (18), some of whom were followed at 2 year intervals for up to 10 years, and obese children participating in weight-loss trials (14,16), however other smaller studies were also incorporated (10,17). Ethical approval was provided by UCL Institute of Child Health, Cambridge Health Authority and the MRC Dunn Nutrition Unit. Written informed consent was obtained from those aged 18+ years and from parents of minors, and verbal assent from all participants.

The total sample is effectively a mixed-longitudinal dataset, with 533 contributing 1 measure, 31 contributing 2 measures, 53 contributing 3 measures, 50 contributing 4 measures and 12 contributing 5 measures. The average time between successive measurements was 2 years. However, all data-points were treated as independent in the analyses. Inclusion criteria for the original studies were either (a) to be healthy with no condition known to affect normal growth and development (high BMI was not excluded), or (b) children and adolescents recruited from obesity weight loss clinics (17 % of sample). Pooling these data provided a representation of the general population including substantial numbers of overweight and obese individuals. Distribution of the sample is represented in Supplementary figure 1.

Anthropometry

Height (HT) and weight (WT) measures were obtained in duplicate using standard operating procedures, and the average value was used in all analyses. Weight was measured wearing minimum clothing and to the nearest 0.01 kg. Height was assessed using a wall-mounted stadiometer to the nearest 0.1 cm. Body Mass Index (BMI kg/m^2) was calculated as weight (kg) divided by height squared (m^2). These values were converted into standard deviation score (SDS) using current UK 1990 reference data (19) to assess representativity of the sample compared to the UK population. Categories of BMI were defined as follows: 1= Thinness (<-1 BMI SDS), 2 = Normal (-0.999 to 1 BMI SDS), 3 = Overweight (1.001 to 2 BMI SDS), 4 = Obese (2.001 to 3 BMI SDS), 5 = Severe Obese (> 3 BMI SDS).

Body Volume

Underwater weighing

Body volume of 30 children was measured by weighing the subject underwater. Lung volume was simultaneously measured by helium dilution. Measurements were obtained in duplicate in 24 children and the mean value was used when appropriate in our analyses (10).

Air-displacement plethysmography

For all other participants, body volume was measured by BODPOD instrumentation (Cosmed Inc., Concord, CA, USA) according to manufacturer's instructions and recommendations and as described previously (20). Subjects wore a tight-fitting swimsuit and a swimming cap. The test consisted in two measures of body volume. If these measures differed by >150mL, a third measure was undertaken. Then, the mean of the measures, or the mean of the two closest measures when three performances were needed, were used in subsequent analysis. Lung volume was predicted as previously described (17).

Bone Mineral Content

Bone mineral content (BMC) was determined by dual-energy X-ray absorptiometry. A subsample of 30 children were assessed by using a Hologic QDR 1000W whole body scanner (Hologic Inc, Waltham, MA) and CHILDREN'S WHOLE BODY software (version 5.61; Vertec Scientific Ltd, Reading, United Kingdom) (10). BMC for all other participants was determined by a Lunar Prodigy scanner (GE Medical Systems, Madison, WI, USA) with Encore 2002 software (15). Both protocols have been previously described.

Total Body Water

Deuterium Dilution (D2O)

TBW was determined by isotopic dilution using deuterium-labelled water. Dosing was equivalent to 0.05 g/Kg of body weight (99.99% D2O). Doses were given as water, or made up as fruit squash or juice. Saliva samples were taken before dosing and either 4 (for normal body fatness) to 6 hours (for obese subjects) post-dose by using a cotton wool swab. Subjects were instructed to not eat or drink during the 30 minutes period before taking a saliva sample. Isotopic enrichment of saliva samples was analysed by two different protocols. Most samples were analysed by Iso-Analytical Ltd (Sandbach, UK) using an equilibration method (14). Deuterium dilution space was assumed to overestimate TBW by a factor of 1.044 and correction was made for fluid intake during the equilibrium period to derive actual body water (15).

Four-component model

The 4-component (4C) model is based on the fact that the body is mainly composed of fat, water, mineral and protein. Assuming constant densities for all 4 components, FM and FFM can be calculated by the following equation:

$$FM\ [kg] = (2.747\ x\ BV) - (0.710\ x\ TBW) + (1.460\ x\ BMC) - (2.050\ x\ WT) \quad (21)$$

where BV= body volume in litres (from ADP), TBW= total body water volume in litres (from deuterium dilution), BMC = bone mineral content in kg from DXA and WT = body weight in kg.

FFM is obtained by difference of FM from WT. This model has been considered the most accurate *in vivo* approach for assessing fat and fat-free masses.

Hydration and density of FFM

As previously described (10), H_{FFM} (%) was calculated as:

$$H_{FFM}[\%] = \frac{TBW}{FFM}\ x\ 100$$

Protein mass (PM) was calculated in kg as follows:

$$Protein\ mass\ [kg] = WT - (TBWm + FM + TMM)$$

D_{FFM} was then calculated as follows:

$$D_{FFM}[kg/L] = \frac{TBWm+PM+TMM}{TBWv+PV+TMV}\ x\ 100\ (21)$$

Where TBWm = Total body water mass in kg, and TBWv = Total body water volume in L, calculated by dividing TBWm by the density of water at body temperature; Protein volume (PV) was then calculated by dividing PM by the density of protein; TMM = total mineral mass in kg and was calculated by multiplying BMC by a constant of 1.2741 (22), and TMV = total mineral volume calculated by dividing TMM by the density of mineral.

Statistics

All data were analysed by using IBM SPSS version 24 for Windows. A t-test for independent samples was applied to assess anthropometry and body composition differences between males and females. A 1-sample Kolmogorov-Smirnov test was used to assess normality of H_{FFM} and D_{FFM}. Equality of variance between groups was assessed using Levene's test.

A one-way ANOVA with post-hoc Bonferroni correction (alpha 0.05) was performed to assess any differences for hydration and density among the nutritional status groups.

A univariate general linear model with post-hoc Bonferroni correction (alpha 0.05) was conducted to assess the interactive associations of BMI SDS groups and age with H_{FFM} and D_{FFM}.

Linear regression analyses were performed to investigate the associations of age, sex and BMI with H_{FFM} and D_{FFM}. The regression model was constructed using the independent variables age, sex (1 = male, 2 = females) and BMI SDS groups, included both as a continue variable and as dummy variables for each nutritional status. The normal BMI group was chosen as the reference group. Identified outliers (n=1) for H_{FFM} (<68%) and (n= 4) D_{FFM} (<1.068 kg/L) values were considered implausible and were removed from the analyses. We additionally fitted age-BMI group interaction terms, to test whether the association of age with H_{FFM} and D_{FFM} varied by BMI-group.

RESULTS

After screening for implausible values for H_{FFM} and D_{FFM}, and accounting for missing data which prevented full calculation of the 4C model for H_{FFM} and D_{FFM} (n=77 and n=105 respectively), a total of 936 data points for H_{FFM} and 905 for D_{FFM} were analysed. Both these outcomes were normally distributed.

Table 1 shows a description of the characteristics of the sample stratified by gender and age. Females presented greater FM (Δ = 5.91 kg, 95%CI 4.48, 7.34; $p < 0.001$) and lower FFM than males (Δ = -2.57 kg, 95%CI -4.20, -0.94; $p = 0.002$ respectively).

The BMI SDS distribution of the sample by age and gender is shown in Figure 1, showing wide variability at all ages. Supplementary Table1 provides mean and SD of age, and the ratio of males to females, for each BMI category.

Hydration of FFM values are illustrated in Figure 2, which shows how hydration of FFM varies in association with nutritional status and age. Heavier groups (obese and severely obese) showed clearly higher hydration levels of FFM at all ages. Furthermore, hydration decreases with age in all BMI groups, but with different patterns. While the decrease is marked in lower BMI groups, heavier groups showed a weaker decrease, trending to a plateau. Beyond these patterns, wide variability range of hydration values can be found within each BMI group. Variance in H_{FFM} did not differ between the groups.

Density of FFM shows patterns with age and BMI that are broadly inverse to those for hydration of FFM (Figure 3), though with a stronger overall age-association (the higher the hydration level, the lower the density). Lower BMI groups presented higher levels of density for FFM while higher BMI-groups showed lower levels of D_{FFM}. Moreover, density of FFM increases with age for all nutritional status groups but this increase is more obvious in lower BMI groups. In addition, differences in density among lighter and heavier BMI groups seem to be more striking with increasing age. Variance in D_{FFM} did not differ between the groups.

All BMI groups showed differences ($p<0.001$) in hydration of FFM except the two highest ones, with differences not statistically significant between obese and severely obese ($p=0.121$). On the other hand, no significant differences were found for density among thin, normal and overweight nutritional groups ($P>0.05$) but highly significant differences appeared between these three groups and the two heaviest ones ($p<0.001$). In addition, a highly significant statistical difference was observed between obese and severely obese groups ($p<0.001$). Also, BMI group showed a significant interaction with age for both H_{FFM} and D_{FFM} ($p=0.007$ and $p=0.014$ respectively), confirming the fact that not only age but also nutritional status is influencing H_{FFM} and D_{FFM} levels and their trends.

Prediction of hydration and density of FFM in growing ages by nutritional status is given in Table 2. While age and BMI SDS explain between 30% and 40% of the variability in both hydration and density, sex was only significant in models for density. These models also showed “dose-response” associations of hydration and density with age and BMI SDS group and their interaction, taking the “Normal” group as the reference.

Discussion

This work reports evidence on variability in FFM properties in association to BMI shown by the gold standard method to assess body composition in vivo, the 4-component model. The relevance of this study is that 2-component model-based techniques rely on constant properties of the FFM. Our study has shown that hydration and density of FFM vary not only with age, as previously reported (23), but also with nutritional status. The study benefits from a large sample size, and wide ranges of age and BMI.

Previous work has reported poor accuracy of predictive techniques such as bioelectrical impedance for measuring body composition in obese patients. Among the underlying reasons for such bias may be differences in body proportions or anatomical distribution of tissue masses, or differences in FFM properties, none of which may be addressed by the manufacturers’ equations (16,23,24).

In 1999, Wang *et al.* (25) suggested that adiposity might influence hydration of FFM in adult mammals but few studies have addressed this question since then and the issue remains poorly understood.

A previous study lead by Battistini (26) proposed that increasing hydration in obese can be related to an expanded extracellular water space. Other studies supported this hypothesis also in adults (27,28). However, the fact that after weight-loss treatments, both nutritional and surgical options, over-hydration persisted comparing to never-obese people, suggests there might be other mechanisms involved in over-hydration in obese people (29).

Haroun *et al.* showed significant differences in the composition of FFM between non-obese and obese in a sample of 50 children. They found out that water and mineral content were higher in obese children and, thus, the proportion of protein was reduced. Consequently, obese children had lower values for density of FFM and higher hydration (12).

Our study goes further, by revealing interactions of BMI status with age, i.e. values change with age differently depending on BMI. For H_{FFM} we showed that the combination of age and BMI group explained ~30% of variability. Thus, H_{FFM} models showed as expected decreasing values with age, but also interactions between BMI and age, with BMI increments associated with obesity greater at older ages. Also, age-BMI interactions were stronger for overweight and obese subjects. On the other hand, D_{FFM} models showed differences not only by age and BMI group, demonstrating a strong association of age and BMI in higher BMI groups, but also by gender, where females showed increased values of D_{FFM}.

These regression models proposed can be used to predict individual H_{FFM} and D_{FFM} values, either from their individual BMI SDS value, or from their BMI SDS category, as well as their age and gender. Despite this, more than half of the inter-individual variability in H_{FFM} and D_{FFM} cannot be explained by our predictors. Methodological error and other unknown biological properties are likely to contribute.

Our research therefore supports previous reports about changes in FFM properties due to age but also by BMI. The current study showed that variability associated with age is amplified by BMI, due in part to the fact that in higher BMI groups, changes with age are weaker.

The most important application of these findings is that body composition analyses in obese children could be in the future performed by an individual prediction of hydration or density combined with a 2-component model technique such as Body density (i.e. BodPod ®) or bioimpedance. Further research should validate the applicability of the predictive equations of hydration and density combined with these 2-component based techniques.

Strengths and limitations

A strength of this study is the large sample size with a wide range of BMI and age. A limitation is that we treated mixed longitudinal data as independent data-points, thus ignoring how some individuals contribute correlated values of FFM properties and BMI. However, since the average time between measurements was 2 years, this correlation is unlikely to introduce spurious results, and also allows us to describe age effects with greater confidence. A small proportion of the sample (30 out of 1014) had mineral content assessed with a different device (Hologic) than the majority of the study sample (Lunar) which may cause a small bias in FFM properties (30). Likewise, differences between underwater weighing and air-displacement measures can exist, although body density by underwater weighing and air-displacement plethysmography is known to be highly correlated (31).

Conclusions

Nutritional status should be considered when assessing body composition in children, adolescents and young adults by two-component techniques in order to improve accuracy. This issue is relevant not only for research studies, but also for the follow-up assessments of disease and treatment.

Our study demonstrates that two-component techniques such as bio-electric impedance or air-displacement plethysmography that use constant values for FFM properties might introduce bias especially in obese subjects. Our results demonstrate that reference data for FFM properties is needed to improve accuracy of body composition measurements in obese children, adolescents and young adults.

Conflict of interests

The authors declare no conflicts of interest.

Author contributions

DGM performed analyses and drafted the article; JCKW and VL designed the study; JCKW, VL, MF, JW and NF supported the analyses and critically review the manuscript. All authors approved the final version of the manuscript.

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Supplementary information is available at EJCN's website.

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Figure legends

Figure 1. BMI SD (z-score) distribution of the sample by age and gender.

Figure 2. Dispersion (A) and distribution (B) of hydration of the fat-free mass (FFM) values stratified by nutritional status grouped by BMI SD score.

Figure 3. Dispersion (A) and distribution (B) of density of the fat-free mass (FFM) values stratified by nutritional status grouped by BMI SD score.

Table 1. Description of the sample.

	Whole sample			Age group 1			Age group 2			Age group 3			Age group 4			Age group 5			Age group 6		
	n	mean	± SD	n	mean	± SD	n	mean	± SD	n	mean	± SD	n	mean	± SD	n	mean	± SD	n	mean	± SD
MALES																					
Age (years)	416	12.9	4.1	30	4.2	0.9	72	7	0.8	128	10	0.9	94	13	0.9	59	16.1	1.4	34	20	0.4
Weight (kg)	416	49.6	20.8	30	15.2	3.6	72	17,8*	13.1	128	25,7‡	16.9	94	31,2‡	16.3	59	46,8‡	14.4	34	50,1†	10.8
Height (m)	416	153.2	20.4	30	102.5	7.6	72	113.6	8.3	128	126†	9.2	94	145,2†	9.9	59	164,2‡	7.1	34	158,1‡	6.4
BMI (kg /m2)	416	20.2	5.2	30	14.1	1.5	72	13*	5.3	128	13,8‡	5.8	94	13,9‡	4.9	59	15.8	4.5	34	17.9	4.1
BMI SDS	416	0.45	1.42	30	-1.21	0.97	72	-2,42*	1.61	128	-2,43†	1.58	94	-3,09†	1.31	59	-2.66	1.32	34	-2.22	1.26
Fat mass (4C - kg)	404	12.1	10.1	21	1*	1.8	69	2,1†	8.9	128	2,4‡	11.4	94	3,1‡	11.1	59	2,9†	10.2	34	2,9‡	7.8
Fat-free mass (4C - kg)	404	38.3	14.4	21	12.8	2.9	69	15.1	5	128	20,7‡	7	94	25,6†	9.2	59	41,4‡	6.7	34	45,6‡	5.8
Body volume (L)	245	52.1	22.8	30	14.5	3.5	34	18.5	17.7	66	24†	21	39	29†	22	43	43,7†	16	34	46,4*	11.3
Total body water (L)	261	29.4	11.6	30	9.4	2.1	45	11.3	4.5	71	16.9	6	39	18.5	8.1	43	30,1‡	5.8	34	32,5‡	4.3
Protein mass (kg)	376	7.3	3	21	2.1	2	58	2.4	0.7	123	2,9†	1.6	93	4,6†	1.9	58	4,7‡	1.7	24	9‡	1.4
Mineral mass (kg)	376	2.4	1	21	0.6	0.6	58	0.7	0.4	123	1,1‡	0.4	93	1,3‡	0.6	58	1,6‡	0.6	24	3,1‡	0.5
Density of the FFM (kg/L)	404	1.092	0.01	21	1.072	0.006	69	1,013*	0.011	128	1.015	0.01	94	1.047	0.008	59	1,081*	0.006	34	1,087†	0.006
Hydration of the FFM (%)	416	75	2.2	30	72.9	2.1	72	71.4	2	128	65.1	2.2	94	70	1.9	59	69*	1.7	34	70,3‡	1.4
FEMALES																					
Age (years)	520	13.4	4.4	33	4.4	0.8	97	7	0.9	134	10	0.8	121	13	0.9	73	16	1.4	62	20	0.4
Weight (kg)	520	52.8	19.9	33	16.1	8.9	97	17*	15.2	134	25,5‡	20.2	121	29,6‡	18	73	38,4‡	12.9	62	35,7†	12.3
Height (m)	520	151.8	15.6	33	103.9	8	97	112.2	9	134	130†	8.6	121	145,2†	6.3	73	146,9‡	6.6	62	146,9‡	6.9
BMI (kg /m2)	520	22.2	6.2	33	14.2	4.5	97	12,8*	5.8	134	12,5‡	7.1	121	13,4‡	6.7	73	16	4.2	62	15.8	4.7
BMI SDS	520	0.79	1.52	33	-0.96	1.5	97	-2,31*	1.57	134	-3,32‡	1.57	121	-3,33†	1.53	73	-2.84	1.24	62	-3.32	1.36
Fat mass (4C - kg)	504	18	11.8	21	2,6*	6.6	93	2,3†	9.7	134	3,3‡	13.6	121	5,6‡	12.8	73	7,7†	8.7	62	9‡	9
Fat-free mass (4C - kg)	504	35.7	9.4	21	12.1	4.3	93	13.5	6	134	22,3‡	7.9	121	23,6†	6.7	73	30,7‡	5.2	62	26,5‡	4.8
Body volume (L)	352	54.3	22.6	31	15.3	9.5	66	16.2	18.2	75	25,9†	24	64	27,9†	23.5	54	36,4†	14.5	62	34*	13
Total body water (L)	366	26.5	8.1	31	9.8	3	74	8.1	5.4	81	17.1	7	64	16.9	6.5	54	22,4‡	4.4	62	19,1‡	3.8
Protein mass (kg)	471	6.5	1.8	21	1.7	0.8	85	2.5	1.1	128	2,8†	1.5	121	5,2†	1.2	73	5,3‡	1	43	4,4‡	1.2
Mineral mass (kg)	471	2.4	0.8	21	0.6	0.3	85	0.8	0.4	128	1,2‡	0.6	121	1,6‡	0.6	73	2,2‡	0.5	43	1,9‡	0.4
Density of the FFM (kg/L)	504	1.095	0.008	21	1.071	0.007	93	1,072*	0.006	134	1.077	0.006	121	1.081	0.007	73	1,087*	0.006	62	1,084†	0.006
Hydration of the FFM (%)	520	75.1	1.9	33	72	1.6	97	70.8	2.1	134	71.8	1.8	121	69.1	1.8	73	70.4	1.6	62	71,1‡	1.8

Abbreviations: BMI = body mass index; SDS = standard deviation scores; FFM = fat-free mass. Age groups: 1 = 4 to 6.99 years; 2 = 7-9.99 years; 3 = 10 to 12.9
years; 4 = 13 to 15.99 years; 5 = 16 to 19.99 years; 6 = 20 to 22.99 years. Significances * = P < 0.05; † = P < 0.01; ‡ = P < 0.001.

Males
Females
BMI [z-score]
6
4
2
0
-2
-4
-6
4
6
8
10
12
14
16
18
20
22
24
Age (years)

Figure 1

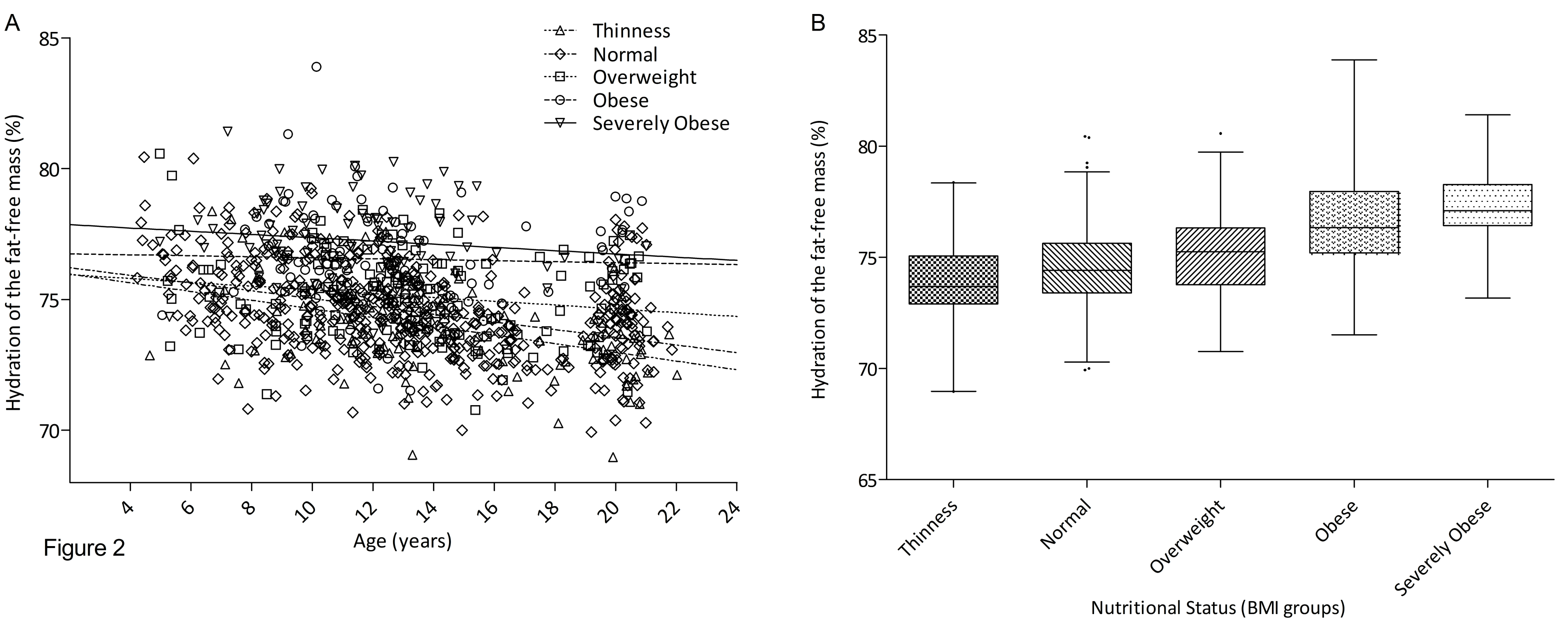

A
Thinness
Normal
Overweight
Obese
Severely Obese
Hydration of the fat-free mass (%)
Age (years)
B
Hydration of the fat-free mass (%)
Thinness
Normal
Overweight
Obese
Severely Obese
Nutritional Status (BMI groups)

Figure 2

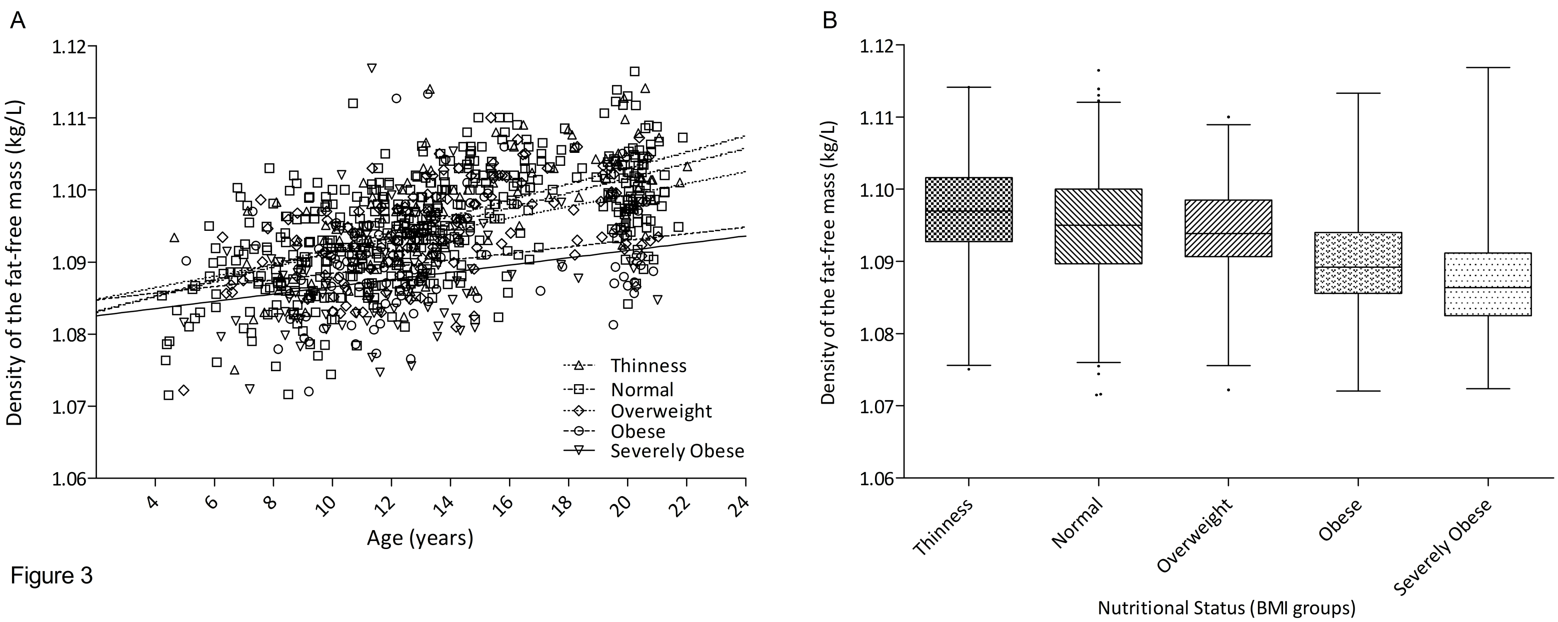

A
B
Density of the fat-free mass (kg/L)
1.12
1.11
1.10
1.09
1.08
1.07
1.06
4
6
8
10
12
14
16
18
20
22
24
Age (years)
Thinness
Normal
Overweight
Obese
Severely Obese
Nutritional Status (BMI groups)

Figure 3

Table 2. Prediction of hydration (A) and density (B) of FFM from age and BMI SD scores

		HYDRATION					
A.		B	SE	t	p value	r2	s.e.e
Model 1.	Constant	74,611	0.231	412,472	**<0.001**		
	age (years)	-0.124	0.013	-9,355	**<0.001**	0.292	1,692
	BMI SDS (continuous)	0.596	0.037	15,908	**<0.001**		
Model 2.	Constant	76,212	0.186	409,696	**<0.001**		
	age (years)	-0.124	0.013	-9,608	**<0.001**		
	Thinness	-0.545	0.179	-3,055	**0.002**	0.303	1,677
	Overweight	0.565	0.158	3,567	**<0.001**		
	Obese	1,976	0.189	10,438	**<0.001**		
	Severely Obese	2,495	0.197	12,690	**<0.001**		
Model 3.	Constant	76,514	0.229	334,369	**<0.001**		
	age (years)	-0.147	0.016	-8,961	**<0.001**		
	Thinness	-0.238	0.613	-0.388	0.698		
	Overweight	-0.451	0.534	-0.845	0.398		
	Obese	0.296	0.658	0.450	0.653	0.309	1,670
	Severely Obese	1,478	0.720	2,051	**0.041**		
	Interaction age-thinness	-0.019	0.041	-0.470	0.639		
	Interaction age-overweight	0.076	0.038	1,997	**0.046**		
	Interaction age-obese	0.130	0.049	2,660	**0.008**		
	Interaction age- severely obese	0.084	0.059	1,433	0.152		

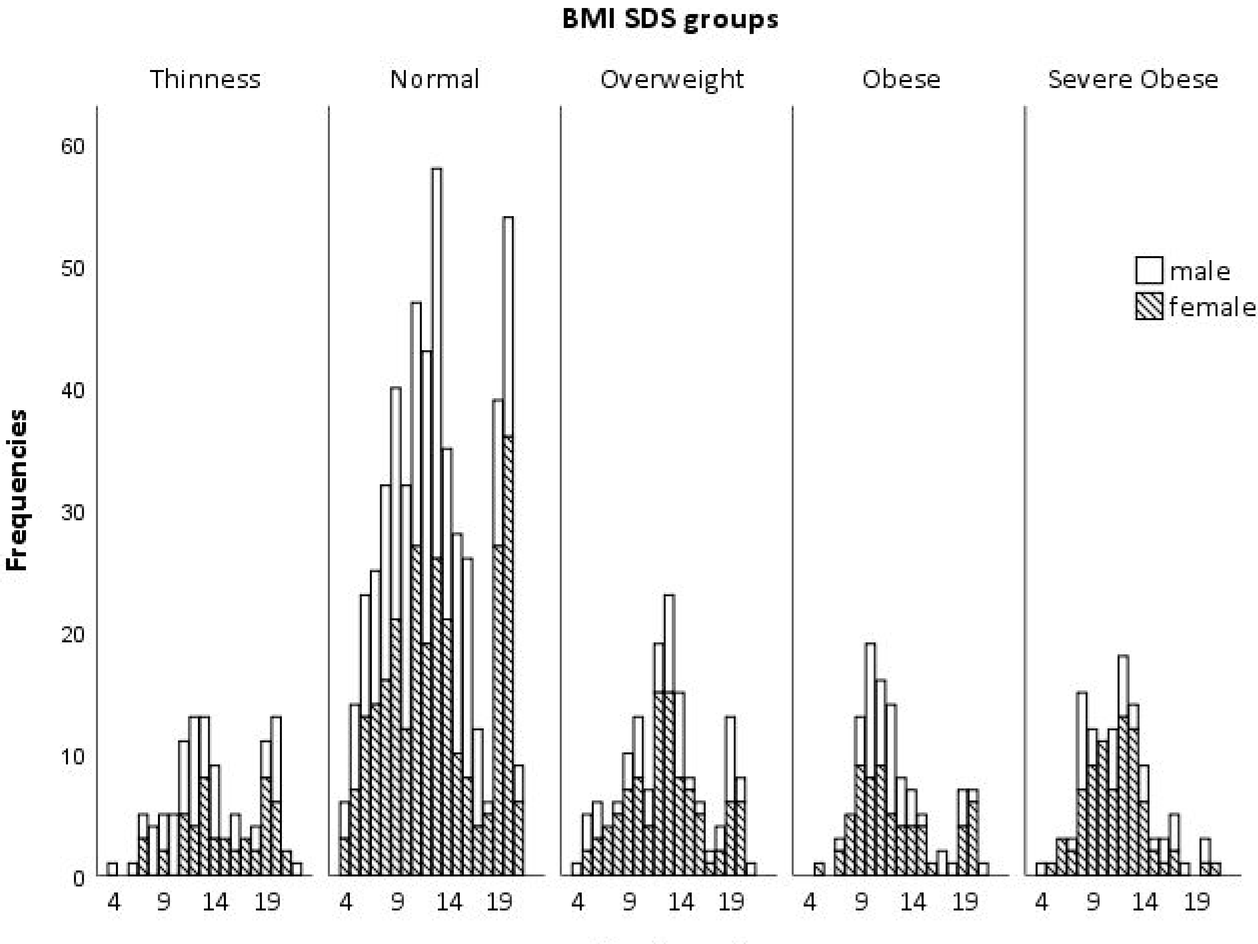
		DENSITY					
B.		B	SE	t	p value	r2	s.e.e
Model 1.	Constant	10,791	0.001	1,162,028	**<0.001**		
	age (years)	0.0009	0.0000	18,233	**<0.001**	0.375	0.006
	sex	0.0021	0.0004	5,192	**<0.001**		
	BMI SDS (continuous)	-0.0014	0.0001	-9,925	**<0.001**		
Model 2.	Constant	10,793	0.0009	1,161,661	**<0.001**		
	age (years)	0.0009	0.0000	18,350	**<0.001**		
	sex	0.0022	0.0004	5,227	**<0.001**		
	Thinness	0.0012	0.0007	1,830	0.066	0.378	0.006
	Overweight	-0.0012	0.0006	-1,972	0.050		
	Obese	-0.0048	0.0007	-6,773	**<0.001**		
	Severely Obese	-0.0063	0.0007	-8,595	**<0.001**		
Model 3.	Constant	10,782	0.0001	1,014,878	**<0.001**		
	age (years)	0.0010	0.0001	15,911	**<0.001**		
	sex	0.0021	0.0004	5,072	**<0.001**		
	Thinness	0.0004	0.0023	0.189	0.850		
	Overweight	0.0015	0.0022	0.680	0.497		
	Obese	0.0024	0.0025	0.954	0.340	0.385	0.006
	Severely Obese	-0.0001	0.0027	-0.046	0.964		
	Interaction age-thinness	-0.0001	0.0002	0.302	0.763		
	Interaction age-overweight	0.0002	0.0002	-1,279	0.201		
	Interaction age-obese	-0.0005	0.0002	-2,999	**0.003**		
	Interaction age-severely obese	-0.0005	0.0002	-2,304	**0.021**		

The nutritional group "Normal" has been chosen as the reference group for regressions.
Significance at $p<0.05$.

Supplementary table 1. Comparison of age and sex between BMI groups.

	BMI SDS group					
	Thinness (n = 108)	Normal (n = 505)	Overweight (n = 144)	Obese (n = 93)	Severe Obese (n = 86)	p-value
Age	14.4 (± 4.3)	13.2 (± 4.5)	13.4 (±4.04)	12.8 (±3.8)	11.7 (±3.2)	<0.001
Sex (M/F)	58/50	241/264	51/93	41/52	25/61	<0.001

Abbreviations: BMI SDS = Body Mass Index in standard deviation score (z-score);
M= Male and F= Female. Significance at p<0.05.



Supplementary figure 1