BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN

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INTERNATIONAL DOCTORAL THESIS

Directed by Dra. Verònica Luque Moreno

Medicine and Surgery Department
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FAIG CONSTAR que aquest treball, titulat "*Valoració de la composició corporal en el pacient pediàtric. Validació de nous mètodes de mesura de la composició corporal en nens obesos*", que presenta Desirée Gutiérrez Marín per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament de Medicina i Cirurgia d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "*Valoración de la composición corporal en el paciente pediátrico. Validación de nuevos métodos de medida de la composición corporal en niños obesos*", que presenta Desirée Gutiérrez Marín para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Medicina y Cirugía de esta universidad.

I STATE that the present study, entitled "*Body composition assessment in pediatric patients. Validation of new methods of body composition measurements in obese children*", presented by Desirée Gutiérrez Marín for the award of the degree of Doctor, has been carried out under my supervision at the Department of Medicine and Surgery of this university.

Reus, 26 d'abril de 2019

La directora de la tesi doctoral
La directora de la tesis doctoral
Doctoral Thesis Supervisor/s

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"To me, there has never been a higher source of earthly honour or distinction than that connected with advances in science."

Isaac Newton

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A mis padres,
por su apoyo infinito e incondicional.
A Albert,
por ser el mejor compañero de vida que jamás pude soñar.
Os quiero.

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LIST OF ABBREVIATIONS

%	Percentage
%$BF_{D\&BV}$	Body fat percentage calculated from density of the FFM and BV
µA	microamperes
Ω	Ohms
2C	Two-component model
3C	Three-component model
4C	Four-component model
AC	Alternating electric current
ADHD	Attention deficit hyperactivity disorder
ADP	Air-displacement plethysmography
BC	Body Composition
BF	Body fat
BIA	Bioelectrical impedance analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BV	Body Volume
C1	Constant 1
C2	Constant 2
CEIC	*Comitès d'ètica d'investigació clínica*
CI	Confidence interval
cm	Centimetres
CT	Computerized tomography
D&BV	Calculations derived from density of the fat-free mass (new equation) and body volume
D_b	Body Density
DD	Deuterium dilution
D_{FFM}	Density of the fat-free mass
D_{FM}	Density of the fat mass
DPA	Double-photon absorptiometry
D_W	Water density
DXA	Dual energy X-ray absorptiometry
e.g.	"*exempli gratia*"; For example

List of Abbreviations

ECW	Extracellular water
et al.	"and collaborators"
FaC	Forearm circumference
FFM	Fat-free mass
FFM_{4C}	Fat-free mass from four-component model calculations
FFM_{BODPOD}	Fat-free mass from BOD POD output
$FFM_{D\&BV}$	Fat-free mass calculated from density of the FFM and BV
FFM_{TANITA}	Fat-free mass from TANITA output
FFM_{Z}	Fat-free from the new equation with impedance
FM	Fat mass
FM_{4C}	Fat mass from four-component model calculations
FM_{BODPOD}	Fat mass from BOD POD output
$FM_{D\&BV}$	Fat mass calculated from density of the FFM and BV
FM_{TANITA}	Fat mass from TANITA output
FM_{Z}	Fat mass from the new equation with impedance
FTIR	Fourier transform infrared spectrometry
g	gram
HT	Height
HT^2/Z	Impedance index
Hz	Hertz
i.e.	"*id est*"; "that is"
ICC	Intraclass correlation coefficient
ICW	Intracellular water
IRMS	Isotope-ratio mass spectrometry
kg	Kilogram
kHz	Kilohertz
L	Litre
LM	Lean mass
m	Metres
MM	Mineral mass
MV	Mineral Volume
n	Sample size
NMR	Nuclear magnetic resonance

P	Pressure
P97	97th percentile
PM	Protein mass
PV	Protein volume
R	Resistance
RV	Remaining volume of the air in the lungs
SD	Standard deviation
SDS	Standard deviation scores
SKT	Skinfold thickness
SPA	Single-photon absorptiometry
SS	Subscapular skinfold
TBW	Total Body Water
TOBEC	Total body electrical conductance
TP	Triceps skinfold
USS	Ultrasonography
UW	Underwater weighing
V	Volume
WC	Waist circumference
WHO	World Health Organization
WT	Body Weight
Xc	Reactance
y	Years
Z	Impedance

SUMMARY

Title: Body composition assessment in paediatric patients. Validation of new methods of body composition measurements in obese children.

Background: Childhood obesity is one of the most important health problems at global level and it has reached epidemic levels at both developed and developing countries around the world. Obesity is defined as an excess of fat in the body but it is usually diagnosed by methods which cannot actually measure or estimate the adipose tissue of the body, i.e. body mass index (BMI). There are many existing techniques which can differentiate body compartments *in vivo* and then, fat can be estimated with a relative high level of accuracy, i.e. dual energy X-ray absorptiometry (DXA), air-displacement plethysmography (ADP), isotopic dilutions, multi-compartment models, among others. The gold standard method to assess body composition in vivo is the four-compartment model. However, techniques have some limitations, and mainly, all of them are expensive and implausible for clinical practice. Bioelectrical impedance analysis (BIA) has been proposed as a suitable technique to assess body composition in a wide range of populations, including obese children. However, there are research evidences that showed a poor accuracy and precision of BIA body composition assessments in this population.

Aim: To improve the accuracy and precision of the body composition assessment techniques in obese children and adolescents to make them suitable to clinical practice.

Methods: Three studies have been done to perform this thesis:

- Study 1: This was a cross-sectional observational study to describe the fat-free mass (FFM) properties from childhood to young adulthood considering the total

body mass. A British cohort of 1014 measurements from participants aged 4 to 22 years old with no BMI restriction was analysed. The whole sample was classified by five BMI z-scores groups (thinness, normal weight, overweight, obese and severely obese) according to WHO classification. Body composition (fat mass (FM) and FFM) was assessed by the four-component model and then, the fat-free mass (FFM) properties, hydration and density of the FFM, were calculated. The hydration and the density of the FFM were then compared between the five BMI groups. A linear regression analysis was then performed to develop different predictive models for hydration and density of the FFM calculations.

- Study 2: This was a cross-sectional validation study, secondary to a randomized clustered clinical trial (OBEMAT2.0). The baseline body composition data of 66 participants enrolled in the OBEMAT2.0 clinical trial was used. We obtained ADP body composition measurements (FM, FFM and body volume) with a BOD POD® device. The density of the FFM was calculated using the equation developed in study 1. These calculations where then used together with body volume obtained from ADP to assess FM and FFM. The four-component model was calculated with FM Fuller's equation. DXA, ADP and deuterium dilution where performed to obtain bone mineral content, body volume and total body water respectively to perform the four-component calculation. The agreement of the FM and FFM calculated measurements from density of the FFM and ADP outcomes with the four-component model was analysed by Bland & Altman analyses.
- Study 3: This was a cross-sectional validation study, secondary to a randomized clustered clinical trial (OBEMAT2.0). The baseline body composition data of 315 participants enrolled in the OBEMAT2.0 clinical trial was used. Body composition measurements where obtained from the four-component model likewise in study 2. BIA was also performed with a TANITA 418-BC device. A

bootstrap linear regression analysis was undertaken in 249 participants (train sample) to develop a new FFM predictive equation derived from impedance raw data. This equation was then tested in the test sample (n =66) and FM and FFM was obtained. The agreement between the new equation and TANITA outputs with the four-component model was analysed with a Bland & Altman analysis.

Results: The results obtained from study 1 showed that FFM properties (hydration and density) are different between obese and non-obese people and the assumption of constant values for these properties lead to obtain biases in body composition assessment in obese children.

The study 2 results demonstrate that using calculated density of the FFM values unlike assumed constant values of the density in two-component based techniques as ADP, improves the accuracy of the body composition assessment in obese children by this technique.

The study 3 showed that the new equation derived from body impedance improves the accuracy and the precision of the BIA body composition assessment in obese children.

Conclusions: The FFM properties (hydration and density) are different when compared between obese and non-obese people. These differences might lead to assume biases when assessing body composition by current techniques based on two-component model, which usually assume constant values of this properties. The use of density of the FFM calculated measurements instead constant values improves the accuracy and precision of the body composition assessment in obese children by ADP, which is a two-component based technique.

However, ADP stills being an unaffordable technique for body composition assessment in clinical practice. The new predictive equation to assess body

composition derived from body impedance improves the accuracy and the precision of BIA's body composition assessment and it is suitable to clinical and research use.

Finally, further studies are needed to assess the accuracy and the precision of this findings in longitudinal studies and clinics follow-up to evaluate body composition changes.

11.1.

1. INTRODUCTION

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1.1. Childhood Obesity: effect on health during childhood and beyond

1.1.1. Definition

According to the World Health Organization (WHO), obesity can be defined as an abnormal or an excessive accumulation of fat that can be harmful to health (1).

Obesity is a chronic preventable multisystem disease caused with a multifactorial origin including genetics, environmental and behavioural causes which can begin during childhood and adolescence (2,3). Also, ethnicity can be considered as an obesogenic factor in developmental countries (4). Obesity in the second decade of childhood is a great predictor of adulthood's obesity (5).

Nevertheless, defining obesity during childhood and adolescence may be difficult because of the variability in growth rates and the natural, gender-specific variations in body composition that occur at different maturational stages. Nowadays, weight-for-height indices are the most widely used for assessing childhood obesity, as body mass index (BMI).

In adults, BMI cut offs to define obesity are a fixed term. However, in children, the physiologic relation between weight and height changes according to maturation, age and gender. Therefore, overweight and obesity are diagnosed by standardization of BMI as z-scores (or standard deviations (SD)) from mean BMI in the normal population of the same age and gender (4).

1.1.2. Prevalence

According to WHO, childhood obesity is one of the most serious public health problems of the 21st century. The prevalence of childhood obesity in Spain has been increasing since 1985. Nowadays, prevalence of childhood obesity has reached

about 15% on infancy and adolescence according to several national studies as AVENA, enKid and ALADINO (5–7). Moreover, children aged 2 to 9 have higher values of obesity than other age ranges and it is more prevalent among people with lowest socio-economic and educational levels. In Catalonia, the prevalence of obesity was 15.4% in 2010-2012 period, which remained stable from 2006 (8).

With all this, world prevalence of childhood and adolescent obesity seems to be increasing and a recent article published in *The Lancet* by Bentham *et al.* (3) showed a worldwide increase of childhood obesity of tenfold in the last four decades. Based on data from 128.9 million participants aged 5 to 19 years old from 200 different countries, Bentham et al. found that if this trend continues, by 2022 the number of obese infants and adolescents will be higher than those classified as moderate-to-severe underweight.

Childhood and adolescence obesity rates in the world increased from less than 1% (equivalent to five million girls and six million boys) in 1975 to nearly 6% in girls (50 million) and nearly 8% in boys (74 million) in 2016. Combined, the number of obese 5 to 19 year olds is more than tenfold globally, from 11 million in 1975 to 124 million in 2016. An additional 213 million were overweight in 2016 but fell below the threshold for obesity (3).

Nevertheless, childhood obesity prevalence seems to be plateaued in high-income countries whereas it is still increasing in low-income and middle-income countries.

1.1.3. Aetiology

Body weight is regulated by numerous physiological mechanisms that maintain the balance between energy intake and energy expenditure. Thus, any factor that raises energy intake or decreases energy expenditure will cause obesity in the long-term.

The aetiology of childhood obesity is extremely complex, being clearly influenced by genetic, environmental and behavioural factors.

1.1.4. Genetic factors

There is strong evidence of the heritability of body weight but only a small fraction of the BMI variability can be explained by genetic factors.

A total of 97 BMI-associated loci have been identified to be involved in obesogenic processes. Many of these loci were in or near genes, which play a role in different biological processes, e.g. the neuronal development, hypothalamic expression and regulatory function, limb development, lipid biosynthesis and metabolism, cell proliferation and survival, and immune system. These 97 loci account for 2.7% of BMI variation and it was suggested that their common variants influence nearly 21% cases of obesity (9).

Many genetic diseases, as endocrine disorders, may cause weight gain, e.g. hypothyroidism, GH deficiency and pseudo-hypoparathyroidism type 1a (10). Also, some syndromic diseases involve obesity as Cushing, Prader-Willi, Bardet- Biedl, Cohen, and Alstrom syndromes, but the molecular causes of these obesity syndromes have not yet been identified (2).

Common forms of obesity, which are present in most cases, are caused by a complex interaction of environmental factors with many gene variants of minor effect. However, single gene mutations account for a small fraction of human early-onset severe obesity (before the age of 5 years and BMI > 3SD). These genes encode enzymes and receptors that, mainly, have a physiologic role in the development of the hypothalamus and the leptin-melanocortin system (9). This system is an

important pathway to energy balance, involving food-intake regulation and body weight management.

Nevertheless, most of the obesity genes remain to be discovered, or their interaction is unknown.

1.1.5. Environmental factors

Hence, childhood and adolescent obesity is a complex combination of different factors that lead to an obesogenic status.

Perinatal factors and early life conditions seem to be related to childhood overweight and obesity as birth size, catch-up growth or first year feeding habits as formula feeding (10,11) and early introduction of solids foods at weaning (< 4 months of age) (12).

Psychosocial and emotional distress, adverse childhood events, stress and depression can lead to overweight and obesity by overfeeding to suppress negative emotions.

Also, parental characteristics as maternal pre-pregnancy obesity, maternal smoking habits at pregnancy, overweight parents, low parental education and low socio-economic status have been identified as important risk factors for children's overweight and obesity (13).

Other proposed environmental factors are microbiota composition, environmental chemicals exposure and some medications as antibiotic use, glucocorticoids, antipsychotic and antiepileptic drugs (10).

1.1.6. Behavioural factors

There are behavioural and psychological factors that raise energy intake or decrease energy expenditure and that will cause obesity in the long-therm. The increased caloric consumption is mostly due to excess of fat and sugar intake from sweetened beverages, sweet snacks, ultra-processed and fast foods, but also larger portions sizes. This, accompanied by a deficient caloric expenditure due to common sedentary activities involving television, computers, tablets, mobile phones and electronic games are important contributors to childhood obesity (10).

Sleeping habits have been proposed to be a factor of obesity, diabetes mellitus type II and metabolic syndrome in adolescents. Koren *et al* found a strong relationship between insufficient, excessive or poor quality sleep, and hyperglycaemia and decreased insulin secretion and sensitivity (14).

1.1.7. Complications of childhood obesity

Preventing and reversing excess weight in children and adolescents is important for many reasons. First, childhood obesity leads to a lifelong overweight and obesity and weight loss and maintenance after weight loss are hard to achieve. Second, childhood obesity is associated with greater risk and earlier onset of chronic disorders that may affect any body system. Third, obesity in childhood and youth is related to psychosocial consequences and lower educational attainment (3).

Most of the current comorbidities of childhood obesity used to be considered as adult diseases and the severity of the consequences increases with the severity of the obesity.

These comorbidities are associated to almost every system in the body including, but not only, cardiovascular, endocrine, pulmonary, gastrointestinal, musculo-skeletal, dermatologic, neurologic and psychosocial systems. Some of the diseases involved in childhood obesity are hyperinsulinemia, insulin resistance, prediabetes and further diabetes type 2, early onset of sexual maturation in girls, higher risk of developing hyperandrogenism and polycystic ovary syndrome in girls too, metabolic syndrome, obstructive sleep apnoea, alveolar hypoventilation, asthma, non-alcoholic fatty liver disease, steatohepatitis, impairment in mobility, risk increased of bone fractures, lower extremity joint pain and malalignment, bilateral slipped capital femoral epiphysis, tibia vara, genu valgum, acanthosis nigricans, intertrigo, hidradenitis suppurativa, furunculosis, stretch marks, idiopathic hypertension intracranial, poor self-esteem, anxiety, depression, and social difficulties, among many other dysfunctions (2).

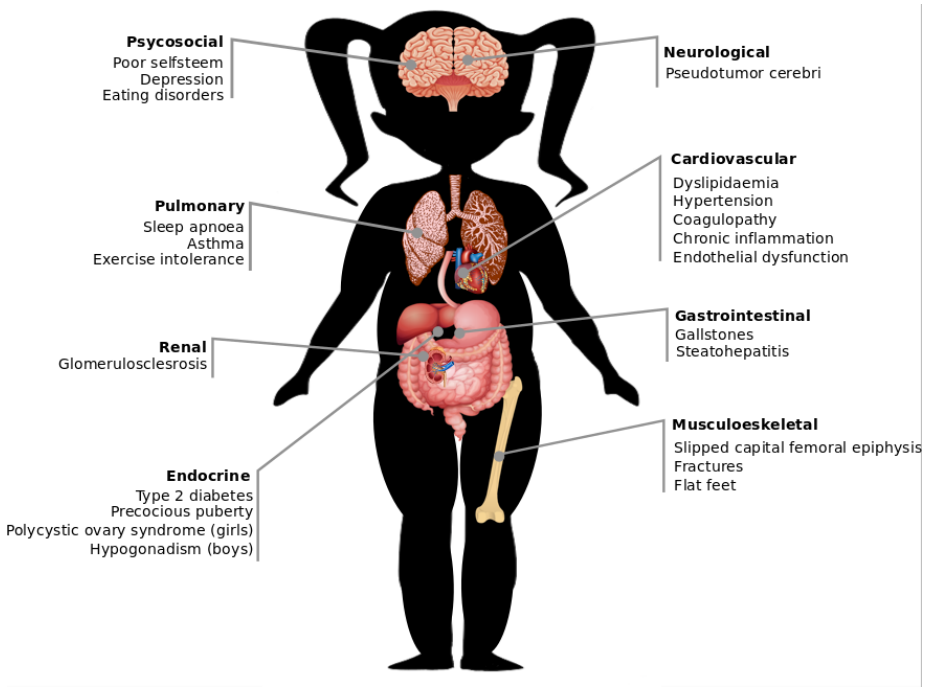

Figure 1. Complications of childhood obesity by biological systems. *Adapted from Ebbeling* et al. *2002* (2).

1.1.8. Diagnosis

A complete history and physical examination are usually sufficient in determining the cause of childhood obesity.

Physical examination usually consists of anthropometrical measures as weight and height measures, but also waist circumference measures. Childhood obesity is usually diagnosed by body mass index (BMI), which consists of dividing weight by the square of the height expressed in kg/m^2. This proportion is then compared to percentiles for children of the same age and sex (15). BMI is widely used to determine the presence of overweight or obesity. Nevertheless, BMI as only tool for childhood obesity diagnosis might be misleading.

Dietary history should consist of details of eating habits including frequency, content, and location of meals and snacks as well as intake of calorie-dense foods such as fruit juice and soda. Physical activity assessment should include details of time spent in unstructured play, organized sports, school recess, and physical education as well as screen time (television, video games, mobile phones, and tablets).

Medical history should include details about medications that may cause weight gain such as glucocorticoids or drugs indicated for neurologic diseases. A complete review of systems is helpful in determining an underlying aetiology for the weight gain. The review of symptoms is also helpful in screening for obesity-related comorbidities (10).

There is a lack of standardization and consensus on when to screen and the types of laboratory screening tests to perform in children with obesity. Most experts recommend that children afflicted with overweight, who are free from etiologic risk factors, should have measurement of a fasting lipid profile, fasting blood glucose or

haemoglobin A1c, aspartate aminotransferase, alanine aminotransferase levels if they are 10 years and older and have 1 or more of the cardiometabolic risk factors as elevated blood pressure and or family history of obesity-related diseases. In addition, all these lab tests are recommended to all children with BMI up to 95th percentile even in the absence of risk factors. Children with signs and symptoms suggestive of a genetic or endocrine cause for the weight gain may need specific testing.

These exploring measures contribute to determine which are the causes of obesity and whether there is any consequence associated. However, BMI is the tool widely used to diagnose the presence of overweight or obesity, both in children and adults. Notwithstanding, BMI shows some limitations when diagnosing obesity. Obesity is the excess of body fat, as we have mentioned before. BMI is a ratio of body weight to height squared, but cannot distinguish between body components. Thus, changes in BMI are usually associated to changes in body fat and high values of BMI are associated to obesity but it is known that main changes in children's body composition are due to maturation and mineralisation of the fat-free mass (FFM), especially in non-obese children (16). This assumptions can lead to misclassify overweight or obesity and may not accurately assess significant changes in body composition over time (17).

Hence, assessing body composition sheds some light on the diagnosis and monitoring of childhood obesity.

1.2. Body composition

1.2.1. What is body composition and why should be important to study it?

It is not easy to find a good and agreed definition of body composition (BC) but, in general biological terms, we can describe it as all about the body is made of.

It is known that almost all diseases exert different effects, to a lesser or greater extent, on body composition in adults but also in children. In some disease states, alterations in body composition are one of the primary symptoms, e.g. eating disorders involving severe weight gain or loss, or many kidney or gastrointestinal diseases may be responsible on lean mass proportions (18).

Therefore, in some cases, such effects are transient and might be of little clinical importance, but many diseases impose stronger and persistent effects.

Many studies have been done in adults but not so many in children. However, knowledge about body composition can benefit when monitoring disease progress and treatment efficacy, predict risks and outcomes or tailor nutrition and treatment according to the moment of the illness (19).

Assessing body composition and monitoring its changes are quite difficult to perform and no agreements have been reached among researchers. These might be some of the reasons why body composition analyses are not widely used in paediatric clinical practice.

1.2.2. Body components

Body is composed of many substances forming our human body. There are different approaches to body composition analysis, including molecular, histological and

anatomical analysis (**Figure 2**). Thus, we can describe body components from different points of view according to the body level of interest to study (20).

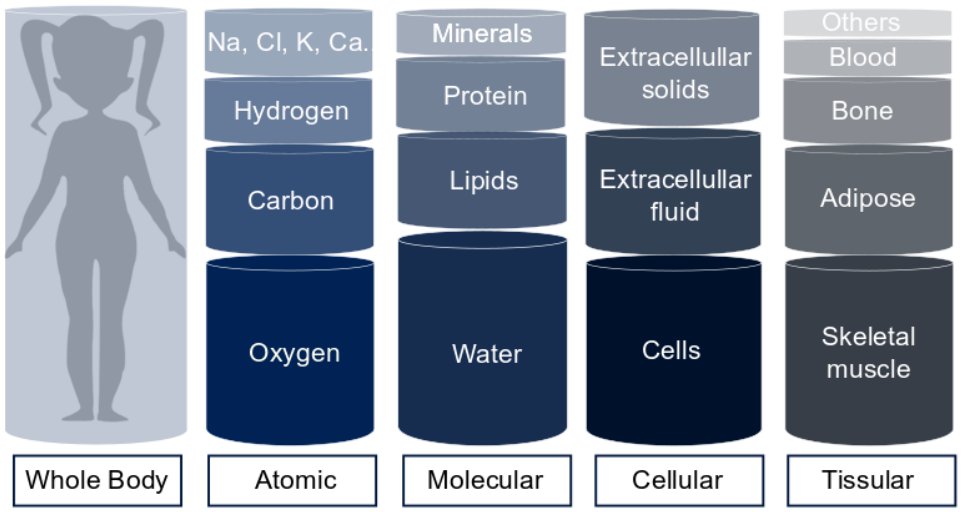

Figure 2. Scheme of the four different levels (atomic, molecular, cellular and tissular) to study body composition and its components. Abbreviations: Na = sodium; Cl = chlorine; K = potassium; Ca = calcium. *Adapted from Wang* et al*. 1992* (21).

To the interest of the present thesis, this work focused on the tissular level; essentially, fat mass and fat-free mass.

- **Fat mass**

Fat mass (FM) or adipose tissue is a body component which functions are to store energy as fat for metabolic demands,to protect the internal organs from hits and to isolate the body from heat and cold, and also behaves as an endocrine organ by releasing hormones (e.g. leptin and steroid hormones), citokines (TNF-α and IL-6) and other proteins (e.g. adipsin, ASP, angiotensinogen, etc.) (22,23). Fat mass is almost totally mollecularly composed of triglycerides and represent ~99% of total body lipids.

The density of the FM is assumed to be constant at 0.9007kg/L, which is the density of triglycerides. Although there are other lipids with a higher density (i.e. cholesterol: 1.067 kg/L and phospholipids: 1.035 kg/L, they represent only ~1% of total body lipids and this proportion is maintained between individuals (24). Thus, to assume constant density for FM is reasonabily accurate.

- **Fat-free mass**

Fat-free mass (FFM) can be described as "all of our body components except fat". It includes water, bone, internal organs, connective tissue (different of adipose tissue) and muscle. It is frequent to find in the literature lean mass (LM) instead of FFM but there are subtle differences: since FFM excludes all kind of fats, LM includes a small percentage of essential fat found in bone marrow and internal organs. Although that, they are both used interchangeabily. However, when it comes to body composition assessment, FFM refers mainly to muscle and bone.

FFM properties, hydration and density, are usually asumed constant but recent studies have demonstrated that both properties can change even between healthy subjects just due to age or gender. First, between birth and adulthood the body suffers many changes due to chemical maturation (25,26). Other biological conditions, both phisiological and pathological, as pregnancy or disease states may be involved in muscle wasting, mineral loss, oedema and all lead to an over- or underhydration of the FFM, affecting both hydration and density properties of the FFM. This situations may compromise the accuracy of the 2-component methods (2C) (27).

Hydration of the FFM is assumed constant in healthy adults at 73.2% and the density at 1.1 kg/L. For children and adolescents, it is well known that total body water decreases and mineral content of FFM increases along growing ages so, hydration

and density of the FFM are different. Lohman (28) and more recently Wells (26) have published age and sex specific constant tables to assess body composition in healthy children and adolescents (**Table 1** and **Table 2**).

Table 1. Reference data of the hydration of the fat-free mass by age and gender published by Lohman. *Adapted from Lohman 1989* (25).

	MALES		FEMALES	
Age *(years)*	**Density** *(kg/L)*	**Hydration** *(%)*	**Density** *(kg/L)*	**Hydration** *(%)*
1	1.068	79.0	1.069	78.8
1-2	1.071	78.6	1.071	78.5
3-5	1.075	77.8	1.073	78.3
5-6	1.079	77.0	1.075	78.0
7-8	1.081	76.8	1.079	77.6
9-10	1.084	76.2	1.082	77.0
11-12	1.087	75.4	1.086	76.6
13-14	1.094	74.7	1.092	75.5
15-16	1.096	74.2	1.094	75.0
17-20	-	73.8	-	74.5

Table 2. Reference data of the hydration and density of the fat-free mass by age and gender published by Wells. *Adapted from Wells 2010* (29).

	MALES (n = 261)		**FEMALES** (n = 272)	
Age *(years)*	**Density** *(kg/L)*	**Hydration** *(%)*	**Density** *(kg/L)*	**Hydration** *(%)*
4–5.99	1.0826	76.6	1.0821	77.3
6–7.99	1.0865	76.1	1.0899	75.3
8–9.99	1.0887	75.8	1.0905	75.2
10–11.99	1.0917	75.2	1.0926	75.0
12–13.99	1.0914	75.2	1.0951	74.8
14–15.99	1.0923	75.0	1.0996	74.1
16–17.99	1.0992	73.1	1.1021	73.7
18–19.99	1.0995	73.4	1.1034	73.5
20–22.99	1.1013	73.6	1.1037	73.7

There are some evidence that these FFM properties could be different for healthy and obese children. Thus, assuming constant values of hydration and density of the FFM to assess body composition in children could be misleading.

As said before, FFM is composed of water, protein and mineral. These three component also have their particular properties:

- Body water density at 36ºC is 0.9937 kg/L and at 37ºC is 0.9934 kg/L. These density values are usually used to convert volume of water into mass of water and *vice versa*. Body water is used both in 3- and 4-component model.
- Protein can be defined as the muscle in essence but specificly density of the proteins can vary between 1.27 kg/L and 1.36 kg/L. This is due to the fact that most proteins ara binded to water in human body and can be difficult to isolate them but also specific proteins differ in density.
- Mineral of the FFM is mainly considered bone mineral content (BMC). The density of the bone mineral is considered 2.982 kg/L but can vary to 3.01 kg/L. This data was obtained from a mixture of different mammal bones and bone minerals are considered similar in composition across mammals.

1.2.3. Techniques for body composition assessment

The gold standard technique for measuring body composition is cadaver dissection (30–32). Due to obvious limitations of cadaver dissection and the need of assessing body composition in vivo has led to develop and improve many different techniques over the years.

The assessment of body composition can be carried out starting from different premises. Depending on the level of fractionation of the total body mass we are interested in, two to five components can be studied. This versatility allows developing different types of studies depending on the component that is going to be analysed but each model has advantages and limitations that must be considered.

Methods to assess body composition can be classified according the way we obtain the measure (**Figure 3**). Techniques that measure body fat are often unavailable and very expensive. Therefore, body mass index (BMI) has emerged as the accepted clinical standard measure of overweight and obesity for children 2 years old and older (10). However, many techniques have been developed and adapted over the last decades to perform body composition analyses.

Figure 3. Scheme of different methods to study body composition and its components. Abbreviations: TC = computed tomography; DXA = dual energy X-ray absorptiometry; NMR = nuclear magnetic resonance; US = ultrasonography; TOBEC = total body electrical conductivity; BIA = bioelectrical impedance analysis.

ANTHROPOMETRY

Anthropometry comprises a wide range of different measurements: **weight**, **height**, different **body circumferences**, **skinfold thicknesses** and **BMI**. Anthropometry techniques are, essentially, double-indirect methods for the body composition assessment because they are used in predictive equations to get body composition results.

Background

Anthropometry has been used to assess nutritional status, physical proportions and body composition since the beginning of the 20th century. Motiegcka was one of the firsts authors to publish his "somatotechnic" methods in 1921 (33).

Due to the different possibilities that the different anthropometric measurements offers, many specific studies have been published during the second part of the 20th century. The most widely studied measures have been the skinfold thickness due to their relationship with fatness and body density (34,35) and BMI (36).

Measurements and instrumentation

- Body weight

Body weight (WT) is usually badly used as a reference measure of body composition, especially among people who are trying to gain muscle or lose fat. WT includes masses of every single body component and it is implausible to distinguish between FM, FFM or body water from the direct WT measure. In paediatrics, WT is also used together with height to evaluate the children's growth through specific age and sex tables and charts (37). Body weight taken without other measures of body size is

misleading because a person's weight is highly related to height and other body conditions. However, WT is needed to assess body composition with any other technique.

WT is measured with a scale that can be analogue or digital. However, digital scales are easier to read than scales with moving dials. There are thousands of different types of scale commercialized and they are accessible to everyone.

- Length/height

Length or height (HT) can be described as the distance from the bottom to the top of someone. The difference of these two words is the position of the subject to be measured. Length usually refers to the decubitus measure (i.e. babies) and HT, to the standing measure. HT is used together with weight to evaluate the children's growth through specific age and sex tables and charts (37).

HT is measured with a stadiometer and there are many different types of this instrument widely available for everyone. For individuals who are unable to stand, recumbent length (also known as crown-to-heel length) can be measured.

Further, WT and HT are used to calculate BMI.

- Body mass index

Quetelet described for the first time the relation between WT and HT of a person in 1869 (Quetelet Index) (38) but it was not until 1972 that Keys recovered Quetelet's concept and established BMI as the WT expressed in kg divided by the squared HT expressed in meters (39).

BMI is used in clinical settings, field surveys, and large scale population studies as a screening tool to indicate the nutritional status of a person or a population

(underweight, overweight, obese or normal or healthy weight for their height) (see **table 3**). BMI is widely used as screening tool for overweight and obesity for both adults and children and it is often associated with health conditions. It is supported by the World Health Organization (WHO) due to its easy and fast calculation (40).

Table 3. World Health Organization Body Mass Index cut-offs for defining nutritional status in adults.

Nutritional status classification	Body mass index (kg/m^2)
Obese class III	≥ 40
Obese class II	35.0 – 39.9
Obese class I	30.0 – 34.9
Overweight	25.0 - 29.9
Normal	18.5 - 24.9
Mild underweight	17.0 - 18.4
Moderate underweight	16.0 - 16.9
Severe underweight	< 16.0

In children, BMI is calculated and then compared to percentiles. However, BMI in children is often converted into SDS adjusted by age and sex and then compared to z-scores as showed in **table 4**.

Table 4. WHO BMI z-scores cut-offs for defining nutritional status in children (41).

Nutritional status classification	BMI z-score
Obesity	> +2SD
Overweight	> +1SD
Normality	< +1SD > -2SD
Thinness	< -2SD

Although there are studies that support the use of BMI as a fatness predictor in children and adolescents (42) it is well known that variability in FFM is an important

source of variability in BMI, not only in children (43,44) but also in adults, especially in athletes (45,46).

- Body circumferences and bone breadths

Body circumferences might give an insight of how fat is distributed around the body and they might be a useful tool when monitoring body changes. **Figure 4** shows some of the body locations where circumference measures are standardized.

The most used measures are mid-upper arm, waist, hip and mid-thigh. Mid-upper arm circumference indices have been used to estimate muscle mass (47) and it is frequently used when assessing malnutrition in developing countries (48).

Measures of circumference can also be combined to calculate ratios and are often used to assess some pathological conditions. Waist to hip ratio it is indicative of the masculine (upper body) or feminine (lower body) distribution of adiposity. People with an increasing waist-to-hip ratio are riskier of metabolic disorders such as obesity and diabetes than those who have a low waist-to-hip ratio. Waist-to-calf ratio or waist-to-thigh ratio can be used to predict sarcopenic obesity, typically found in middle age and older individuals as lean mass gradually decreases with age, even if body fat remains stable or slightly increases.

In the last decades, many studies have related waist circumference with obesity-related risks, mainly cardiometabolic risk (49–51). Although waist circumference has been demonstrated to be correlated to %FM (52), stills being difficult to differentiate body components from body circumferences measures. Despite all, some colleges have published predictive equations to predict body density and, then, body components (53) (**Table 5**).

Body circumferences are measured by a non-stretch measuring tape and the measure is often taken in cm.

Bone breadths (also called widths) were usually used to assess growth in children (54). Due to the relatively expensive specialized callipers needed to perform these measures and the lack of evidence of their reliability and accuracy, bone breadths are not so popular, nowadays. Few studies have used these measures to predict body composition instead of circumferences, but there is a lack of evidence of their utility in body composition assessments.

- Skinfold thickness

Skinfold measurements are performed with a calliper, usually precise to the nearest 0.1mm and, preferably with a constant pressure of 10g/cm^3 between its jaws.

The most commonly used skinfolds are subscapular and triceps but there are many other anatomical sites that can be assessed (see **Figure 4**).

Skinfold thickness values are often used and they act as reliable indicators of regional fatness and they can be converted into standard deviation scores (SDS) and then, they can be compared to reference data, provided by WHO (55).

To convert raw skinfold thickness values into a %FM, predictive-equations can be used. These equations are usually derived from empirical relationships between skinfold thickness and body density. Many equations firstly calculate BD and require an additional calculation to estimate %FM. The Brožek et al (1963)(56) and the Siri (1961)(57) equations are often used for this last calculation.

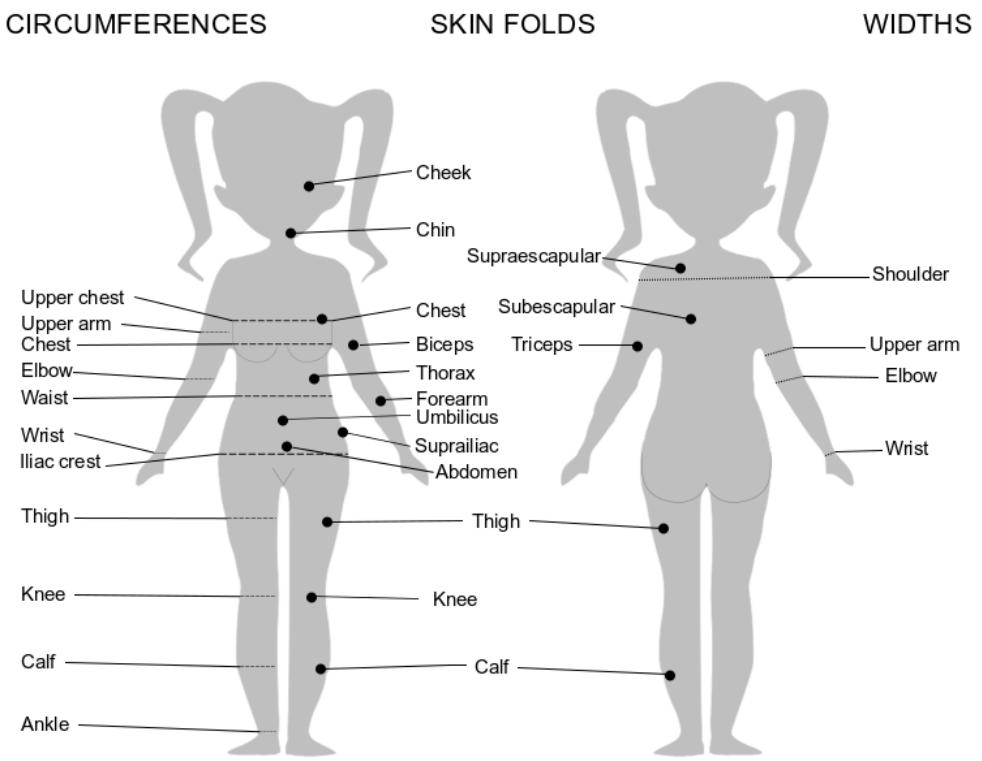

Figure 4. Anatomic sites for anthropometric measurements of circumferences, skin fold thicknesses and widths from left to right. *Adapted from Wang et al. 2000* (54).

The first to published body fat predictive equations from skinfold thickness was Matiegka in 1921 (33). Early as 1951, Brožek and Keys saw the relationship between the skinfold thickness and the body density for assessing FM (34). After this, several predictive equations have been published. However, the most often used in literature are those predicted by Durnin and Womersley (58) in 1974 and Jackson and Pollock in 1978 (59), but others as Slaughter (60) and Goran (61) have been popular, too (**Table 5**).

No equations are available for estimating body fat from a single-site skinfold measurement.

Table 5. Predictive equations from skinfold thickness and body circumferences.

Authors (Year)	**Population**	**Predictive equations**
Jackson&Pollock (1978) (54)	General	BD = 1.101 − 0.00041150 ($\sum_1$) + 0.00000069 ($\sum_2$)^2 − 0.000226310 (age) − 0.0059239 (WC) + 0.0190632 (FaC)
	General	BD = 1.17615 − 0.02394 (log $\sum_1$) − 0.00022 (age) − 0.0070 (WC) + 0.02120 (FaC)
Jackson&Pollock (1985) (62)	Men	% FM = 0.29288 ($\sum_3$) – 0.0005 ($\sum_3^2$) + 0.15845(age) – 5.76377
	Women	% FM = 0.29669 ($\sum_3$) – 0.00043 ($\sum_3^2$) + 0.02963 (age) + 1.4072
Durnin&Rahaman (1967) (35)	Boys 13–15.9 y	BD = 1.1533 − 0.0643 (log $\sum_4$)
	Girls 13–15.9 y	BD = 1.1369 − 0.0598 (log $\sum_4$)
Durnin and Womersley (1974) (58)	Boys 16–19.9 y	BD = 1.162 − 0.063 (log $\sum_4$)
	Girls 16–19.9 y	BD= 1.1549 − 0.0678 (log $\sum_4$)
Slaughter (1988) (55)	Girls 9-16 y	%FM = 1.33 ($\sum_5$) − 0.013 ($\sum_5$)^2 − 2.5
	Boys 9.8 ± 1.3 y	%FM =1.21 ($\sum_5$) − 0.008 ($\sum_5$)^2 − 1.7
	Boys 12.2 ± 1.4 y	%FM = 1.21 ($\sum_5$) − 0.008 ($\sum_5$)^2 − 3.4
	Boys 15.8 ± 1.6 y	%FM = 1.21 ($\sum_5$)− 0.008 ($\sum_5$)^2 − 5.5
	Boys (TP+SS) > 35 mm	%FM = 0.783 x ($\sum_5$)+ 1.7
	Girls (TP+SS) > 35 mm	%FM = 0.546 x ($\sum_5$) + 9.7
Lean (1996)(63)	Women 18–64.3 y	%FM = 0.730 BMI + 0.548 TP + 0.270 Age − 5.9
	Men 18–64.3 y	%FM = 0.742 BMI + 0.95 TP + 0.335 Age − 20
Goran (1998) (61)	4-10y	FM = 0.23 x SS + 0.18 x WT + 0.13 x TP - 3.0 kg

Abbreviations: i.e. = examples; BD = body density in kg/L; %FM = percentage of fat mass; $\sum_1$ sum of chest, axilla, triceps, subscapular, abdomen, suprailiac and front thigh skinfolds in mm; $\sum_2$ sum of chest, abdomen and thigh skinfolds; WC = waist circumference in cm; FaC = forearm circumference in cm; WT= body weight; SS = subscapular skinfold; TP = triceps skinfold; BMI = body mass index in kg/m^2.

Strengths and limitations

Anthropometry has the advantage of not needing complex and expensive equipment, it is useful in all ages and it is very quick and non-invasive. The instrumentation is portable and requires little space of storage. It is simple to obtain in most age groups. Skinfolds are useful in monitoring changes in fatness in non-obese children because of their small body size, and the majority of fat is subcutaneous. These aspects make the method usable in epidemiological studies.

On the other hand, the disadvantages are that it is less accurate and operator-dependent in the sense that the accuracy of the measurements depends on the training of the evaluator. Soft tissues undergo compression and change quickly, it takes a significant training to get the measurement in just few seconds, after which the measurable location may vary. Compression may be due to displacement of extracellular water or adipose tissue to places of lower pressure. This compressibility varies according to the body region (64,65). In addition, many predictive equations that are applied have not been developed for specific populations and/or are not validated, making the method less reliable and accurate. Measurements should be made under standardized conditions to ensure reproducibility and reliability, but still being operator-dependent (54,66).

In addition, accuracy and precision are poorer in obese individuals as it is difficult to hold a large skinfold while reading the calliper dial and most callipers have an upper measurement limit of 45 to 55 mm, which limits their use to obese and very thin subjects (67,68).

Many body conditions may influence on skinfolds size. For instance, hydration status can influence the measurements: dehydration reduces the skinfold size; oedema and dermatitis increase the skinfold size. Finally, available published predictive equations may not always be applicable to a specific-study population.

DENSITOMETRY

Densitometry techniques are based on the 2C model; that is, they estimate FM and FFM. These methods use body density and body volume to calculate body fat percentage (%BF).

Two densitometry techniques are **underwater weighing** (UW) and **air-displacement plethysmography** (ADP). Both techniques are known as very accurate methods for measuring body volume, and have been considered as the "gold standard" strategies for body composition assessment (69,70).

- ### UNDERWATER WEIGHING (UW)

UW, also referred to as hydrostatic weighing or hydrodensitometry, is a 2C model-based method of determining body composition from body density measurements.

Background

UW was first explored by Behnke, Feen, and Welham in 1942 to estimate the relative proportion of fat and lean tissues (71). In the 1950's, Brožek and Keys used this approach and state the need to measure the remaining air in the lungs at the time of weighing (72) and confirmed the scientific use of the UW (73). Since then, many old and new studies have used UW as the criterion method for assessing body composition *in vivo* and it has been the gold standard technique for many years (74–77).

Physical Principles of underwater weighing

UW is based on Archimedes' principle (78), which states that when an object (or a person) is immersed in a fluid (i.e. water), the object displaces the fluid and it takes

its place. The volume of fluid displaced can be measured and, thus, the volume of the immersed object can be deduced.

UW is based on 2C model of body composition so assumes constant densities of the FFM and the FM. The density of the whole body (D_b), therefore, depends on the relative content of these two components: as bone and muscle are denser than water, a person with a larger %FFM will weigh more in the water, and have a lower %FM. Conversely, fat is less dense than water. Therefore, a large amount of FM will make the body lighter in the water and have a higher %FM.

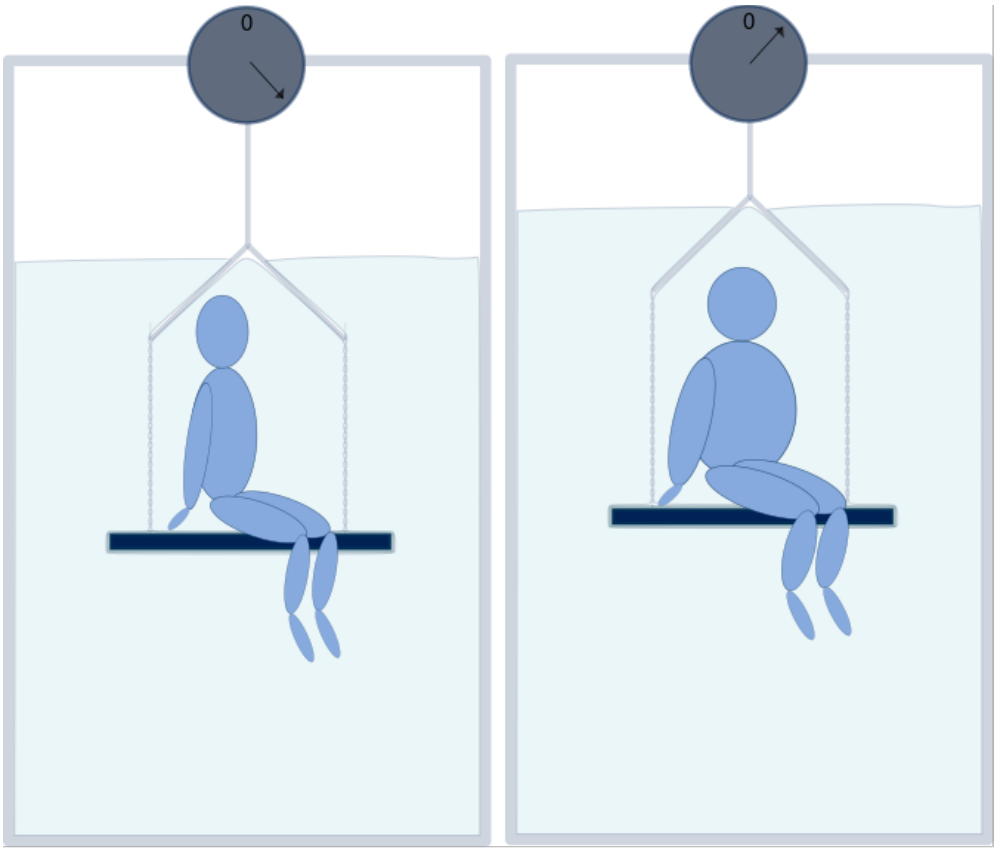

Figure 5. Underwater weighing technique illustration for leaner (left) and fatter (right) subjects. Fatter subjects weight less than leaner ones in an aqueous environment due to the lower density of the fat. *Adapted from Ningthoujam et al. 2016* (79).

D_b is calculated relating mass and volume, where mass is the weight of the body in air, and volume is the difference between the weight in air and the weight of the body submersed during underwater weighing (**Figure 6**).

Figure 6. Calculation of body density.

$$D_b = \frac{WT}{[(WT - WT_{UW})/(D_W - RV)]} \quad (80)$$

D_b is body density in kg/L; WT is body weight measured outside water in kg; WT_{UW} is body weight measured entirely submerged in the water in kg; D_W is density of the water where the participant is submerged in kg/L; RV is the remaining volume of air in the lungs in L.

Then, the equation has to be corrected for the remaining volume of air in the lungs, which is measured using closed circuit dilution (i.e. helium dilution or oxygen dilution) or nitrogen washout (81). If these methods cannot be performed, the air remaining in the lungs can be estimated by published predictive equations based on age and height.

Once body density is calculated, it is possible to convert this into %FM using Siri's or Brožek's equations as previously described.

Strengths and limitations

Many studies have shown that UW is a reliable and accurate technique for body composition assessment, it has been the gold standard technique for the measurement of body composition for many years and it is often used to validate other methods.

However, there are many limitations to restrict its use: it is time consuming, requires a high level of participant cooperation and a specific installation (**Figure 5**), it is uncomfortable for the subjects and not recommended for all age-ranges (i.e. elderly or disabled) and well-trained technicians are needed. In addition, the technique is unable to assess fat distribution and, as all 2C model-based methods, UW assumes constant properties (density of the FM and FFM) and distribution of the body components, which may induce some error. Finally, another source of error can be miscalculation of the air remaining in the lungs.

Nevertheless, new more sophisticated methods may make underwater weighing obsolete in the near future.

- **AIR-DISPLACEMENT PLETHYSMOGRAPHY (ADP)**

ADP is and indirect densitometry technique based on the 2C model that assesses body volume (BV). From BV together with body mass, body density (D_b) can be calculated, and further, FM and FFM can estimated.

Background

ADP has been used to measure human body composition since the early 1900's, when was mostly applied to the measurement of the body volume and composition of infants. However, because of technical difficulties as the maintenance of constant room conditions, applications in common use for human were limited. Although the first commercially available air-displacement plethysmograph for adults was developed in the 1960s (82) it was not developed into a viable system for routine use until the mid-1990s when the technique was well-established for adults.

The device was developed by the manufacturer of the BOD POD® Body Composition System (Life Measurements Inc., Concord, CA, USA) (83). The same manufacturer developed de PEA POD® Infant Body Composition System in the early 2000's, thus consolidating the technique for infants (84). Nowadays, the BOD POD manufacturer is COSMED Inc (Concord, CA, USA).

Physical Principles of air-displacement plethysmography

The physical principles of ADP are based on the application of Boyle's Law and Poisson's Law, which are relevant gas laws.

Boyle's law says that in a room with constant temperature (isothermal conditions), volume and pressure vary inversely with one another. It means, when pressure (P) increases, volume (V) vary inversely decreasing and *vice versa*, following the equation: P1/P2 = V1/V2.

On the other hand, Poisson's law explains the relationship between volume and pressure when there are changes in temperature, i.e. under adiabatic conditions. This follows the equation: $P1/P2 = (V1/V2)^{\gamma}$, where γ is the ratio of the specific heat of the gas at constant pressure to that constant volume (γ = 1.4 for air). Thus, BOD POD uses these two laws to assess body volume (77).

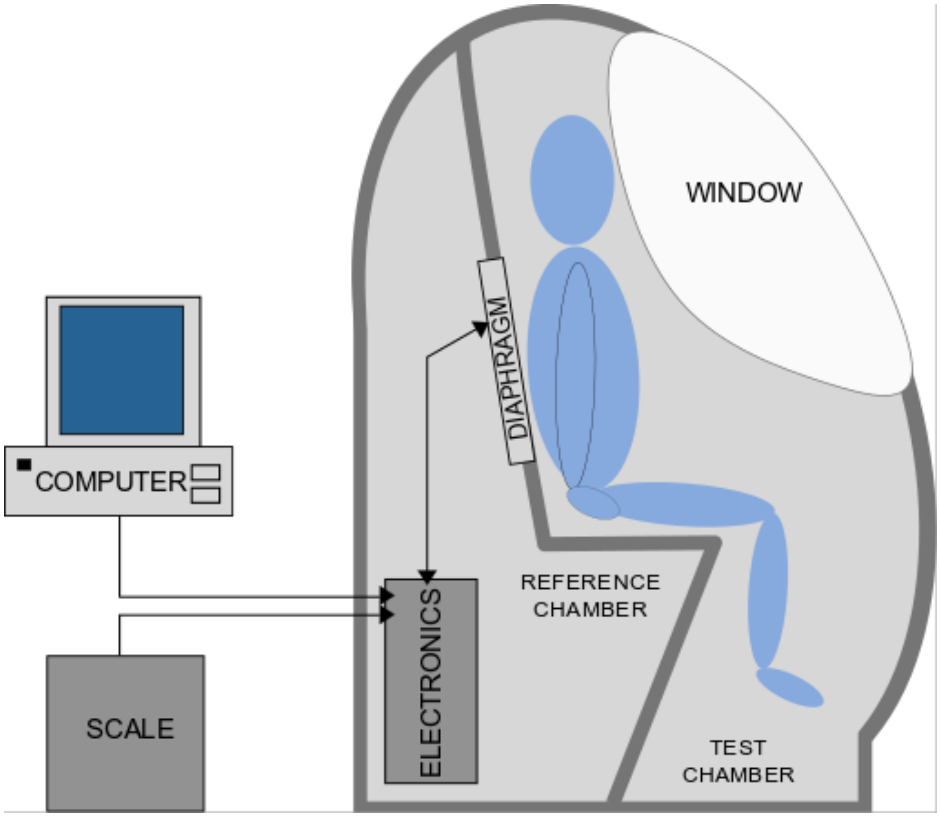

Figure 7. Diagram of a BOD POD device and its system components. *Adapted from Dempster et al. 1995* (85).

The BOD POD device consists of two chambers (**Figure 7**): the test chamber and the reference chamber. Both of them have an air volume content known and they are separated by the seat (made of molded fiberglass) where the subject stays during the test. Based on Boyle and Poisson's laws, when the volume increases in one of the chambers, it decreases by the same amount in the other one. Thus, body volume is equal to the reduction of volume in the chamber with the introduction of the subject, while maintaining a constant temperature (under isothermal conditions). However, it is impossible to maintain a constant temperature throughout the test. Therefore, Poisson's law shows the relationship between pressure and volume under changing temperature (adiabatic) conditions. These volume changes result in a very small pressure variation that is monitored by transducers (83).

Inside our bodies, a small amount of air exists and that should be considered when testing body volume. The thoracic gas volume remaining in lungs can be measured by the device but also it can be predicted by the average lung volume during normal tidal breathing. This last type of measure is easier when working with children (82).

The test consists in two measures of body volume, which last 50 seconds each one, opening the BOP POD's door at the end of the first measure and then closing it again. If the volume measures are consistent, it means, the volume differences between two measures must be less than 150 mL, the software calculates the mean of the two measures. If the differences are bigger than 150 mL, a third measure must be done (86).

The software calculates fat free mass (FFM) and fat mass (FM) from subject volume measures applying the principle of densitometry, where body density can be calculated by following the relation: D_b = body mass/body volume. Density of FM is known by the software used (the density of fat is assumed 0.9007 kg/L and FFM 1.1

kg/L) and with volume data measured, FM can be predicted, and then FFM as follows.

When D_b is obtained, percentages of FM and FFM can be determined. The software uses different equations according to specific populations to calculate percentage of FM (**Table 6**).

Table 6. Equations used by Bod Pod software to assess percentage of fat mass (FM%) according to specific populations.

NAME (ref)	**EQUATION**	**POPULATION**
Siri (57)	FM% = [(4.95/D_b) – 4.50] x 100	General population
Schutte (87)	FM% = [(4.374/ D_b) - 3.928] x 100	African-American black males
Ortiz (88)	FM% = [(4.83/ D_b)-4.37] x 100	African-American black females
Brožek (56)	FM% = [(4.57/ D_b)-4.142] x 100	Lean and obese individuals
Lohman (25)	FM% = [(C1/ D_b) - C2] x 100*	Children ≤ 17 years

Adapted from BOD POD Gold Standard Body Composition Tracking System Operator's Manual (89,90). *C1 and C2 are constants based on age and gender (25).

Then, %FFM can be subtracted from WT and %FM predicted (83).

Strengths and Limitations

The BOD POD device is not difficult to operate; only a little training is needed and does not require expert technicians to perform the test. The time of testing is really short (less than 2.5 min) and minimum participant cooperation is needed. It offers

a high level of accuracy. It is as accurate as hydrostatic weighing (another method based on densitometry to assess body composition), but quicker and easier to perform and the subject does not need to get wet so it is more comfortable for the participant. ADP is suitable for a wide range of populations including infants (with the Pea Pod), toddlers and young children, adolescents, adults, elderly, different ethnic groups and different nutritional status groups from thinness to obesity.

The main limitation of BOD POD is that many variables can affect the results. For instance, environment conditions (summer warm and winter cold weather), facial or body hair, body temperature, moisture, and the tightness of the spandex or swimsuit can all alter the results.

Among the assumptions inherent to this 2C model are that the densities of these two components (FM and FFM) are assumed constant at 0.9007 and 1.1 kg/L, respectively, but several studies have demonstrated that age, ethnicity and BMI can vary density and hydration of FFM (29,82,91–94) thus, some bias have to be considered.

In addition, the device is quite expensive, needs to be located in a room with specific conditions of size and environment and it is only available in a few research facilities.

Although ADP is useful in a wide age ranges, it holds some operation difficulties with children. On one hand, the PEA POD device can be used in infants weighted up to 8kg and the BOD POD is validated from 12kg onwards. Therefore, there is a weight range in which children cannot be assessed yet. On the other hand, the person being tested needs to be quiet, relaxed and still. For example, an accurate measurement of a 2 years old children, who is crying and moving inside the chamber is not feasible.

IMAGING

First evidences of imaging methods used to assess body composition were published by Stuart and colleagues in 1940 to report the first use of standard x-ray films to capture fat and muscle "shadows" in children (95).

Imaging methods provide information about the spatial distribution of adipose tissue by exploiting differences in the imaging properties of different tissues, lean and adipose tissue to be distinguished.

Current imaging techniques **are dual x-ray absorptiometry (DXA)**, **computerized tomography (CT)** and **Magnetic resonance imaging (MRI)**, for whole body assessments, and **ultrasonography (USS)** for regional assessments. The primary function of these methods is to assess tissue structures in clinical diagnoses, rather than estimating body composition. They are typically used in research to estimate fat compartments but also to assess some pathological conditions when other methods are not feasible.

A big limitation to perform these methods when assessing body composition is the lack of standardization for body composition measurements (96) when using CT, MRI or USS. In addition, a trained operator is needed to perform the test and are high costly techniques.

However, DXA is widely used in research to assess body composition.

- **DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA)**

DXA is an *in vivo* indirect-image technique for the study of the human body composition defined by the 2C model, mainly.

DXA is widely used to diagnose osteoporosis by determining bone mineral content and bone mineral density at lumbar spine and proximal femur levels, but DXA can be used to estimate body composition (bone, FM and FFM), too.

Background

First absorptiometry techniques for bone mineral density (BMD) assessments *in vivo* were originally based on single-photon absorptiometry (SPA), where a radioactive isotope (iodine-125 or americium-241) was used as a source (97,98). Then, SPA was replaced by double-photon absorptiometry (DPA), using the photon radiation of americium-241 and cesium-137 which emit two different energies. Then, the method was improved by using a gamma-radiation generated from gadolinium-153, which can emit radiation at two different energies. This makes possible to distinguish the attenuation between soft tissues and bone. These techniques were widely used in the 80's (53). The main inconvenience of both SPA and DPA was the short average life of the radioactive sources, which is less than 1 year. The precision of the method decreases by that time so the source must be replaced by another one and it is very expensive. Other disadvantages were the time of exploration, which is lengthy (approx. 1 hour).

To solve the radioactive source problems, the first-generation of modern DXA scanners replaced the radio-nucleotide generator for a current X-ray tube, which allows a higher photon flux so it increased the resolution of the images, its precision and shortened the exploration time.

Physical Principles of DXA

The DXA system uses a source that generates X-rays at two energies, a detector, and an interface with a computer system for imaging. The differential attenuation of the two energies is due to the energy emitted and the density of the subject. Then, this is used to determine body composition.

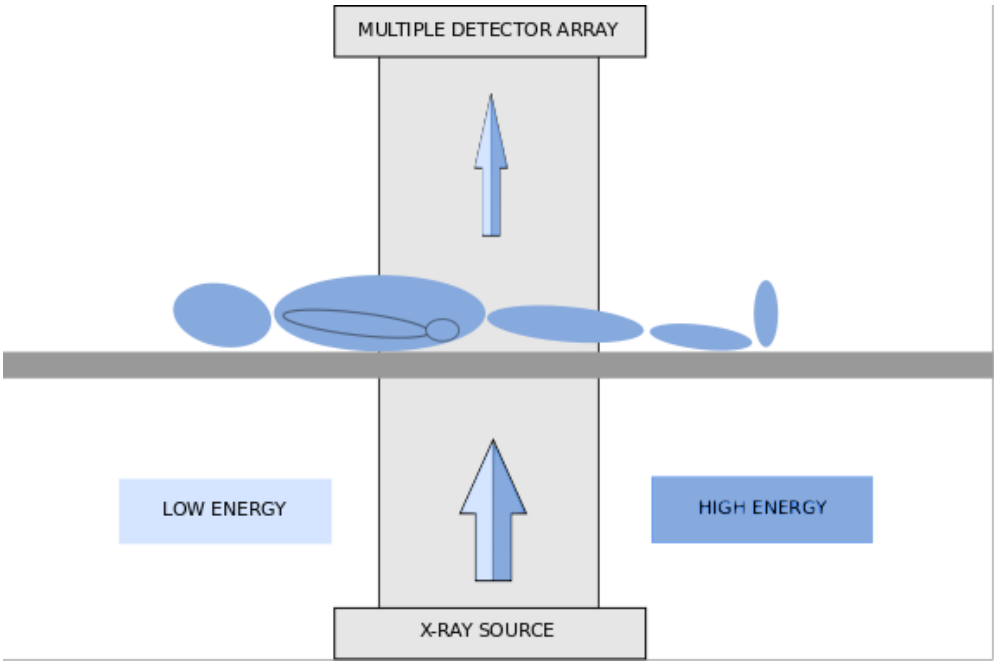

Figure 8. Diagram of the principle of dual-energy X-ray absorptiometry. *Adapted from Toombs* et al*. 2012* (99).

Based on that, there are three different commercial manufacturers of DXAs with rather different approaches: Hologic, Lunar and Norland.

The Hologic system generates two beams of X-ray alternating high and low energy pulses and the attenuated X-rays that have passed through the subject are measured sequentially with a detector located above the patient.

Other systems, as Lunar and Noland, have a constant-potential X-ray generator, which produces one beam of X-ray. Then, the X-ray beam is separated into high and low energy by a k-edge filter to achieve a beam of stable dual-energy radiation. The attenuated X-rays that have passed through the subject are measured with an energy-discriminating detector.

In all three systems, the difference of attenuation is used to estimate BMC and soft tissue composition; each tissue has its particular attenuation difference between the two energy peaks. With this, we must consider the body as a two-compartment system, bone and homogeneous soft tissue, so the soft tissue can only be measured where there is no bone (100,101).

Depending on the type of beam that DXA devices use, there are different technologies (102). The two most commonly applied densitometry technologies are (**Figure 9**):

- Pencil beam

These type of densitometers were the first generation of DXA. They are the gold standard for accuracy to measure BMC (103). The main inconvenient is that it takes 30 min to complete a whole body scan.

- Fan beam

To speed-up the process, DXA systems developed the fan beam. Narrow-angle fan beam devices can get a whole body scanning in 5-7 minutes, whereas a wide-angle fan beam system can take up to complete a whole body assessment in 3 minutes. The consequences of getting a faster scanning process are the needing of apply higher radiation than that used with the pencil beam devices, and the distortion/magnification of the images (104).

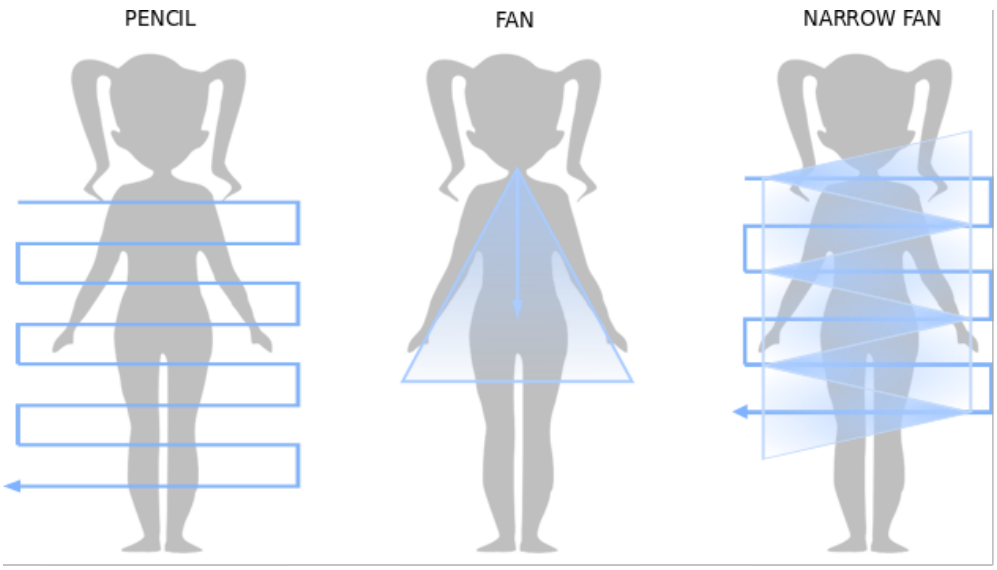

Figure 9. Characterization of the X-ray beams (blue arrow) scanning between the different dual-energy X-ray absorptiometry systems. From left to right: pencil beam, fan beam and narrow fan beam. *Adapted from Crabtree et al.* (105) *and Toombs* et al*. 2012* (99).

Pixels of attenuation of bone and soft tissue can be measured using a software, which knows the properties of attenuation of fat and fat-free mass, assuming constant attenuation, and use specific algorithms to correct the effect of body thickness.

One of the issues that may cause some concern is the dose of radiation received. The radiations dose can vary among instruments and scan modes (i.e. total body scanning or located area scanning) but it is very low for all cases, even for fan beam devices. To compare something with, we receive more accumulated radiation in only one day without doing special activities (106).

Strengths and limitations

DXA measurements requires little cooperation of the patient, it is precise, accurate and reliable. DXA can distinguish regional as well as whole body composition but also estimates overall body fat and body fat patterning and distribution through the body. The radiation dose received is very low and time of exposure is relative quick. Thus, DXA is suitable to be used in epidemiological studies.

The main limitation of DXA is the difficulty of assess FM and LM just above and/or under the bone as well as the "trunk thickness", increasing the error as the thickness increases.

Another important limitation is related to the corporal size of the subject. When the subject is very obese or exceeds the size of the worktable, some corporal zones cannot be analysed. Related to corporal size, obese people need longer radiation exposure or a large dose of radiation.

Also, the design of the equipment (pencil or fan beam), algorithms of the software or calibration systems make that parameters as quality, accuracy and precision may be different among all devices. Therefore, measured values may vary from one device to another from different manufacturers.

Finally, the equipment is expensive, needs relative big room space to keep the device and a trained radiology operator is need to perform the test (107).

HYDROMETRY

- ### ISOTOPIC DILUTION

Hydrometry is an indirect, in vivo method to assess body composition at the molecular level through assessing TBW by isotopic dilution.

Background

The first report about stable isotopes relevant to biologic studies was published in the late 1920s by Aston and hydrogen isotopes where discovered in the early 1930s (108). In 1934, Hofer and von Hevesy were the first to propose to measure TBW by using deuterium (109) based on water-soluble tracers dilution method in experiments using goldfish. Moore applied it in 1946 (110) after the firsts studies of deuterium's toxicity in the late 1930s (54,55). Since then, many studies had used deuterium oxide (^2H_2O) to assess TBW and body composition accuracy and safety.

Biochemical principles of isotopic dilution

Deuterium dilution is the gold standard technique for TBW assessment *in vivo*. TBW can be measured using labelled water with tritium ($^3H^2O$) or with stable isotopes as deuterium (^2H), oxygen-18 (^{18}O) or double-labelled water ($^2H_2{}^{18}O$). Stable isotopes are preferred because there are innocuous for humans' health so minimizes risks. Both ^2H_2O and ^{18}O are naturally found. The abundance of ^2H in the oceans of Earth is approximately one atom in 6500 hydrogen atoms (± 0.015%) and the natural abundance of ^{18}O is 0.2%. Although ^{18}O is an excellent tracer, the use of ^2H-labelling is widespread because ^2H_2O is more inexpensive than $H_2{}^{18}O$ or $^2H_2{}^{18}O$. Then, we are focusing on ^2H-labelled water technique (112).

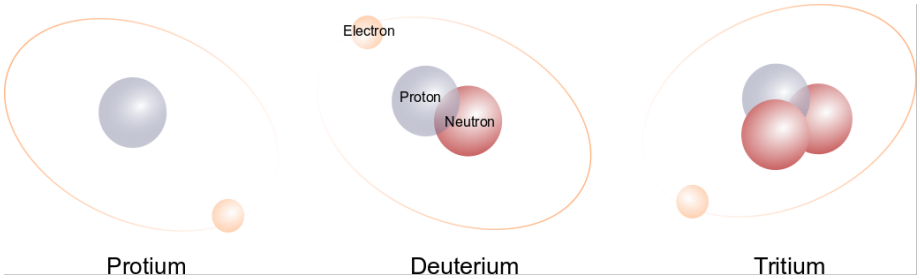

Figure 10. Diagram of the three isotopes of the hydrogen: protium, deuterium and tritium. *Adapted from* http://sdavis0709.tripod.com/id6.html

Deuterium is a stable non-radioactive isotope. After its oral administration as deuterium oxide (^2H_2O), deuterium molecules immediate exchange proportional atoms with body protium (^1H) from body fluids, but also organic molecules. After a short time, deuterium equilibrates, approximately 3 hours, and behaves as body water. This technique is based on the principle that water is distributed in all parts of the body except body fat (hydrophobic tissue). Then deuterium is eliminated by urine, saliva, sweat and breastmilk.

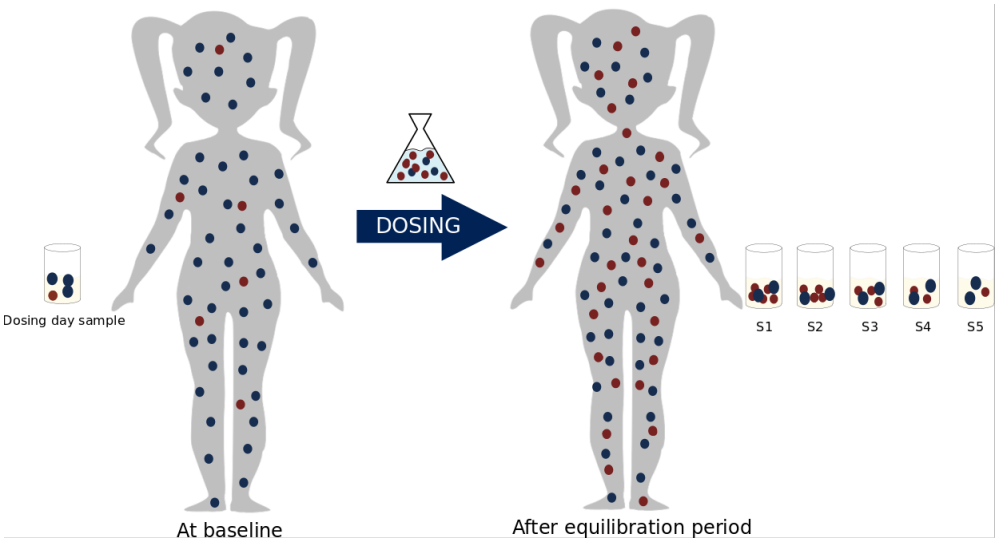

Figure 11. Concentration in body water of deuterium isotope at baseline and after dosing. Deuterium natural abundance is less than 0.1% while protium accounts for more than 99.98%, thus, small amounts of deuterium can be found in biological fluids before dosing with enriched water with deuterium. Symbols and abbreviations: Blue dots = protium; Red dots = deuterium; S1 – S5 = samples 1 to 5.

There are two basic approaches to determine deuterium in biologic samples: the intercept method and the equilibration method.

The **intercept** or **back-extrapolation method** is often used when assessing water turnover, i.e. for measuring total energy expenditure (TEE) and maternal TBW to know breastmilk intake by their babies. It is usually applied when doubly labelled water is used and it takes 7 to 14 days (3-4 water cycles of turnover) to perform.

On the other hand, the **equilibration** or **plateau method** is based on $^{2}H_2O$ – body water equilibrium, which takes approximately 3 hours to come to equilibrium with intracellular and extracellular fluid and 4-8 hours with urine. The volume of all the consumed fluids during the equilibration period should be recorded.

After sampling saliva, urine, plasma or milk, deuterium enrichment can be assessed by Isotope-ratio mass spectrometry (IRMS) or Fourier transform infrared spectrometry (FTIR). FTIR is less sensitive than IRMS, requires higher dosage of labelled water and it is not an appropriate technique to analyse urine nor breastmilk. However, FTIR requires an instrumental easier to use and maintain and cheaper than the IRMS and the analysis is less laborious (113).

Hence, the dilution space of deuterium is an output from spectrometry (in moles). Then, TBW is calculated by specific equations, and FFM can be estimated using TBW and a hydration coefficient (**Figure 12=**, which is the fraction of FFM comprised of water.

$$FFM = \frac{TBW}{hydration\ coeff.}$$

Figure 12. Calculation of the fat-free mass from the water fraction.
FFM = fat-free mass; TBW = total body water; hydration coeff. = hydration coefficient (usually assumed constant).

It is established that, in adults (21 to 70 years), hydration of the FFM is 73.2% (hydration coefficient = 0.732). Nevertheless, the hydration coefficient varies according to age and other human conditions as pregnancy and breastfeeding (58). Once the FFM is estimated, FM and percentage of total body fat (%BF) is calculated with the following equations (**Figure 13** and **Figure 14)**:

$$FM\ (kg) = WT(kg) - FFM\ (kg)$$

Figure 13. Calculation of the fat mass (FM - kg) subtracted from total body weight (WT – kg) and fat-free mass (FFM - kg)

$$\%BF = \frac{100xFM}{WT}$$

Figure 14. Calculation of the body fat percentage. FM = fat mass (kg); WT = total body weight (kg).

Strengths and limitations

One advantage of this technique is that it can be used to assess longitudinal changes in body composition because error rates are low (1-5% for TBW and 0.5% for FFM) (115).

Another advantage is that, as a stable isotope, it is safe to be used in children and in pregnancy and it is suitable for field use.

On the other hand, the preparation of the dosage might be tedious because an accurately measured dose is needed.

The cost of the equipment, analysis and isotope are pricey, which might be a limitation.

In addition, the technique makes some assumptions; ^{2}H is homogeneously distributed in all fluid compartments and only in these compartments, and no ^{2}H nor body water is lost during the equilibration time (113,116) and assumes constant hydration of the FFM.

BIOELECTRICAL TECHNIQUES

Although bioelectrical techniques are not strictly considered hydrometric methods, these techniques use the electrical properties of electrolytes in the body to estimate TBW and then, FFM. Two examples of bioelectrical methods are **total body electrical conductivity (TOBEC)** and **bioelectrical impedance analyses (BIA).**

TOBEC analysis is a double-indirect bioelectrical technique that uses measures of the body electrical conductivity to estimate LM. Accordingly, TOBEC was initially developed to determinate the composition of meat in meat-packaging industry (117). There are studies that compared TOBEC against UW and/or DD and showed an accuracy comparable to other available methods (i.e. DD) (118). In addition, it is easy to perform and no participant preparation is needed.

On the other hand, results can be affected by hydration status, the equipment is expensive, as well as other methods, it is based on regression equations to estimate body composition and it is not widely available.

BIA is an *in vivo*, double-indirect technique to assess body composition based on the 2C model and has been deeply studied in the last decades because its multiple advantages over other existing methods.

- **BIOELECTRIC IMPEDANCE ANALYSIS (BIA)**

Background

Electrical properties of tissues have been described since the last part of the 19th century (Hermann, 1871). Thomasset, in 1962 conducted an original study using electrical impedance measurements as an index of total body water (TBW), using measures of impedance at more than a single frequency with two subcutaneous

steel needles (119). Hoffer *et al*. (1969) (120) and Nyboer (1959) (121) first used single frequency measures of impedance and introduced the four-surface electrode BIA technique. By the 1970s, the foundations of BIA were established, confirming the relationship between impedance and body water content. A variety of single frequency BIA analysers then became commercially available, and by the 1990s, the market included several multi-frequency analysers (122,123).

Physical principles of BIA

To understand the basis of BIA:

- **Impedance (Z)**: Z is the opposition that an **electrical circuit** or **system** (e.g. human body) offers to the pass of an alternating electric current (AC).
- **Resistance (R)**: R is the additional opposition by the **conductors** (e.g. body fluid) to the pass of alternating electric current. It is related to the amount of water present in tissues.
- **Reactance (Xc)**: is the additional **opposition** (of the cell membrane, e.g.) to the pass of an alternating electric current.
- **Capacitance**: is the property of a **capacitor** (e.g. cell membrane, tissues interface, ECL and ICL, non-ionic tissues) to **store** an electric charge according to a potential difference.

BIA is based on the relationship between TBW and the electric body impedance.

In the human body, biological tissues can behave as electric conductors or electric insulator depending on each tissue composition. Tissues with a high electrolytic fluid composition are great conductors whereas fat and bone are not.

When an alternating electric current is applied to a human body from point A to point B, an electrical tension (difference between the current that enters from the

point A and the one that arrives to the point B) is generated. That tension is given by the opposition of body components to the pass of the current, known as resistance (R). Z can be determined by the vector relation between R and Xc following this equation (**Figure 15**):

$$Z^2 = R^2 + Xc^2$$

Figure 15. Relation between impedance (Z), resistance (R), and reactance (Xc).

R and Xc values depend on the electric current frequency: low frequencies allow AC passing through extracellular fluids (ECF) whereas high frequencies allow AC get into all watery compartments. However, when it is applied an AC at low frequency, the Xc is 0 so the Z is equal to the R. As the frequency of the electrical current increases, reactance occurs if there are multiple current ways within the conductor and some of them delay the current more than others. The value of the R increases with frequency but reaches a maximum at a specific frequency that depends on the composition of the conductor (tissue). Then, the R decreases as the frequency continues increasing, so at a certain high frequency, the Z is equal to R, again. Z vector forms an angle with R vector as the frequency changes low to high (**Figure 16.**). That is the phase angle, which can be related to physiologic, nutritional and life expectancy variables (124,125).

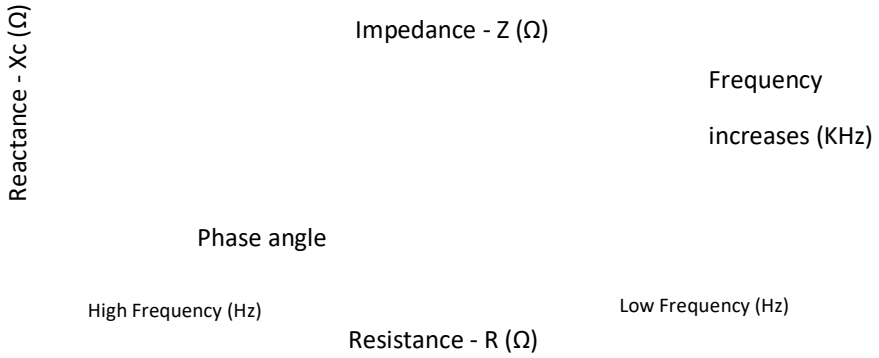

Figure 16. Diagram of the derivation of the phase angle and its relationship with reactance, resistance and impedance. *Adapted from Kyle et al. 2004* (123)

Instruments to measure BIA

To measure BIA, there are different types of analysers. They are classified according to the range of frequencies (single or multiple frequencies) and the number of electrodes (bipolar, tetrapolar, octopolar) (126).

According to **frequencies** used, the devices are single frequency analysers when these use a single frequency electrical range (frequency of 50 kHz). This type of measurers cannot determine intracellular water. Thus, multi-frequency analysers (frequencies up to 800 kHz) can measure the water both inside and outside cells (**Figure 17**).

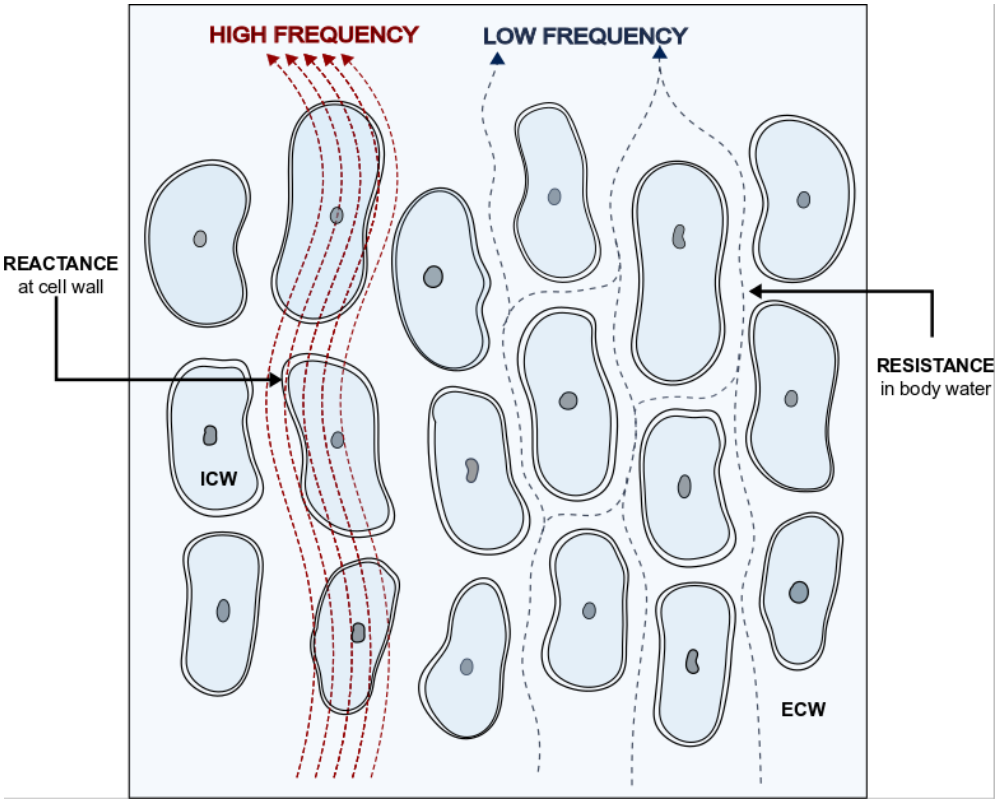

Figure 17. Current flows in tissue at different frequencies (high and low). High frequencies (red arrows) can determine both intra and extracellular fluids; low frequencies only determine extracellular water Abbreviations: ICW, intracellular water; ECW, extracellular water.

According to the **number of electrodes**, the bipolar analysers also called regional, produce results that depend on fat distribution in the individual using two electrodes placed on feet or on wrists. This means that results vary depending on fat location in the upper or lower segment of the body.

In contrast, a tetrapolar analyser offers values, which are independent of the localization of fat deposits. Two of the four electrodes are located on feet and the other two, on wrists, leading the electrical current pass across the body from the bottom to the top of the body.

Octopolar analysers use eight electrodes, two of which are placed on each foot and the other two are placed on each hand. This technique is also called segmental because determines the body composition of each segment of the body: trunk, right arm, right leg, left arm and left leg, also known as the five-cylinders' model (**Figure 18**).

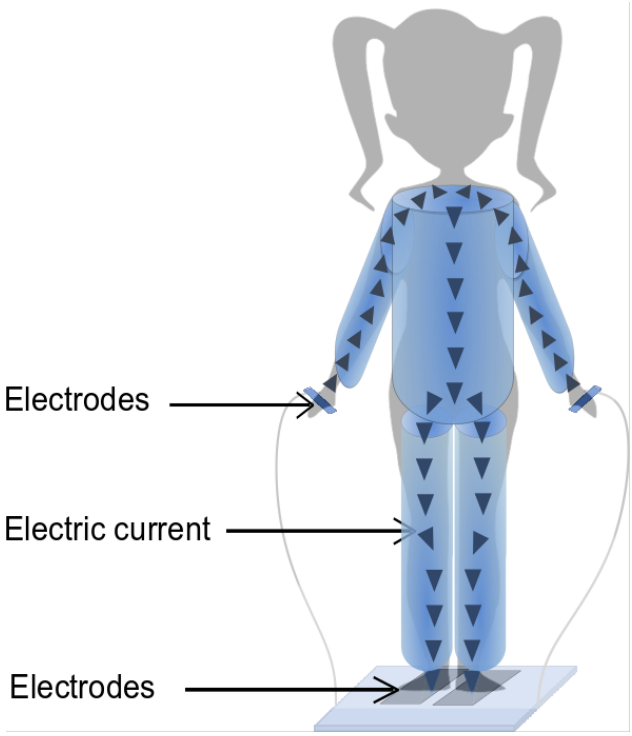

Figure 18. Bioelectrical current flow and the five cylinders' model. This diagram schematizes a tetrapolar analyser; the current flow comes out from the handgrip electrodes, goes through the body and ends at the electrodes located on the feet soles. BIA analysers considers the body as a group of five cylinders and determines body composition of each segment: right arm, right leg, trunk, left arm and left leg.

Strengths and limitations

Nowadays, the use of BIA has increased because the equipment is relatively inexpensive, non-invasive and safe. The analyser is easy to use, can be portable,

does not need a large space to be storage and requires minimal participant cooperation. The procedure and the results are reproducible and rapidly obtained (123). Furthermore, with little training, BIA is not affected by the operator.

On the other hand, BIA has been demonstrated to be influenced by body size, gender, age, ethnicity (127) and time of the day (128). BIA is a technique that uses regression equations to determine TBW and then body composition. Equations show statistical relations observed in a particular population so, each equation can be useful to populations with similar characteristics to the reference population.

Another point to consider is the intra-individual variability in the hydration state. This depends on room temperature, fasting, disease conditions, medication, menstrual cycle, proportionality of body shape, vigorous exercise, caffeine and alcohol intake, and others. Related to this, BIA trends to overestimate %FM in leaner patients and underestimates it in the heavier ones. This is because obese individuals have higher level of body mass and body water at the trunk. The trunk contributes 10% to whole body impedance whereas represents almost 50% of body mass (123). Thus, BIA is poorly accurate when assessing severely obese patients. Consequently, it is not useful detecting short term body composition changes after dietary or physical activity interventions.

Table 7 provides a summary of all body composition assessment techniques.

Table 7. Summary of the body composition assessment techniques.

TECHNIQUE	PRIMARY MEASURE	ADVANTATGES	DISADVANTADGES
Body Mass Index	Relative weight	Simple Quick Inexpensive Non invasive	Measures nutritional status not body composition. Poor accuracy. Assumes var of weight = var of fat.
Anthropometry (skinfold thickness)	% Body fat prediction	Inexpensive All age groups Quick Non invasive	Poor accuracy and precision in obesity. Operator dependent. Assumes SKF ∝ whole BF
Hydrodensitometry: Underwater weighing	Body volum Body density	Reliable and accurate	Uncomfortable for subjects Not suitable for all populations. Assumes ctt FM and FFM densities.
Air-displacement plethysmography	Body volume Body density	Relative high accuracy Fast Simple	Expensive device (BOD POD). Limited availability. Assumes constant FM and FFM densities.
Dual-X ray absortiometry	Bone mineral density	Precise and accurate Whole body and regional BC.	Biased by body size and fatness. Low radiation. Specialized technician. Assumes ctt attenuations.
Hydrometry by deuterium dilution	Total body water	Wide population groups Safe	Expensive. Specialized analysis. Assumes ctt FFM hydration.
Total Body Electrical Conductivity	Total body water	Easy Safe Non invasive	Expensive. Limited availability. Assumes conductivity ∝ TBW.
Bioelectrical impedance analysis	Total body water	Inexpensive Safe Non invasive Fast Portable	Poor accuracy. Population specific. Assumes conductivity ∝ TBW and ctt FFM hydration

Var = variability; SKT = skinfold thickness; ∝ = proportional; BF = body fat; BC = body composition; FM = fat mass; FFM = fat-free mass; ctt = constant; TBW = total body water.

MULTICOMPONENT MODELS

The need to improve the accuracy and the precision of the body composition assessment lead to develop component models of body composition assessment, which combines some of the techniques described above to solve some of the limitations of the individual techniques.

The oldest model is the bicompartmental or 2-component model (2C); but nowadays, due to technological and design advances, body composition can be assessed dividing the human body into three or more compartments (multi-components models) (**Figure 19**). This versatility allows to develop different types of studies depending on the component that is going to analyse, having each model advantages and limitations that must be considered (129).

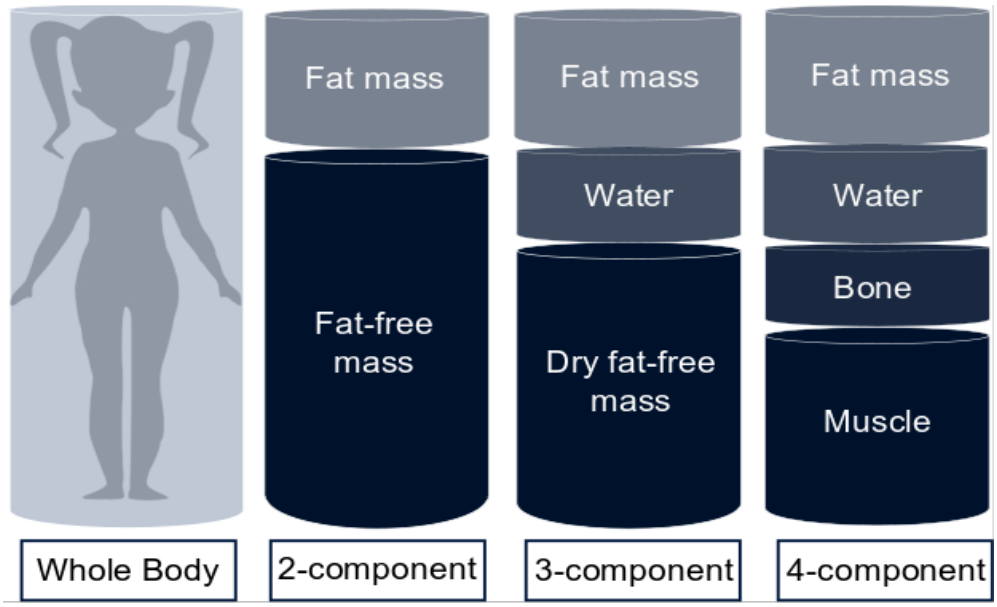

Figure 19. Scheme of the two, three and four component models to study body composition and its body components. *Adapted from Ellis, 2000* (130).

- **TWO-COMPONENT MODEL (2C)**

The 2C model divides the human body in fat mass (FM) and fat-free mass (FFM), usually from the estimation of the total body density (D_B), and traditionally using the equations of Siri (1961)(57) or Brožek *et al* (1963)(56) (**Figure 20**).

$$Siri: \%\ BF = \left(\frac{4.95}{D_B} - 4.50\right)\ x\ 100$$

$$Brožek: \%\ BF = \left(\frac{4.57}{D_B} - 4.50\right)\ x\ 100$$

Figure 20. Calculations of body fat percentage (%BF) by Siri (above) and Brožek (below). D_b = body density in L.

This model is based on the assumption of constant FM density and FFM properties, which are described later. These assumptions may lead to accept some result biases due to inter-individual variation in the composition of the FFM and unsual subject circumstances as disease or pregnancy.

Many techniques are based on the 2C model as densitometry or hydrometry, and even DXA.

- **THREE-COMPONENT MODEL (3C)**

The 3C model differentiates body water from total fat-free mass, dividing the body composition into fat, water and remaining dry-fat-free mass (proteins and minerals). This model reduces the assumption that hydration of the fat-free mass is constant, but the protein/mineral ratio of the FFM stills being assumed constant. The 3C model is based on measurements obtained from densitometry, hydrometry and body weight.

No instrumentation exists to measure these three components simultaneously but a step-calculation is needed. The method includes the previous assessment of body volume (BV), total body water (TBW) and body weight (WT) allow FM calculation using published equations. The most widely used equations are sumarized in **Table 8.**

Table 8. Equations for fat mass assessment from the three-component model.

AUTHOR (year) (ref)	EQUATIONS
Siri (1961) (131)	FM = 2.057 x BV-0.786 x TBW-1.286 x WT
Lohman (1992) (131)	FM = 6.386 x BV+3.961 x MM-6.09 x WT
Fuller (1992) (132)	FM = 2.22 x BV-0.764 x TBW-1.465 x WT

BV is body volume in L from densitometry techniques; TBW is total body water in L from hydrometry techniques; MM is mineral mass in kg estimated from hydrometry measures and WT is body weight in kg.

- **FOUR-COMPONENT MODEL (4C)**

The 3C model can be extended to four components at a molecular level, analyzing in the fat-free mass, in addition to water, the mineral content of the bone and the protein component. Thus, the 4C differentiates body fat, bone mass, muscle mass and water (**Figure 19**). Estimating bone mineral content and protein mass of the FFM avoids the assumption that the protein/mineral ratio is constant. However, 4C assumes that ratio bone mineral/non-osseous mineral content is constant.

Therefore, the 4C is currently considered the gold standard method to assess body composition *in vivo*.

The 4C calculates the fat mass of the body and then fat-free mass can be sustracted from total body weight. To perform these estimations, many equations have been published (**Table 9**) (133).

Table 9. Examples of published equations for assessing fat mass by the four-component model.

AUTHOR (year) (ref)	EQUATIONS
Lohman (1992) (131)	FM= 2.747 x BV-0.714 x TBW+1.146 x BMC-2.053 x WT
Fuller (1992) (132)	FM=2.747 x BV-0.710 x TBW+1.460 x BMC-2.05 x WT
Wang (2002) (134)	FM=2.748 x BV-0.699 x TBW+1.129 x BMC-2.051 x WT

FM is fat mass in kg; BV is body volume in L; TBW is total body water in L; BMC is bone mineral content in kg; and WT is body weight in kg.

Thus, the 4C requires individually measurements from densitometry, hydrometry, DXA and body weight. These needs make the 4C unaffordable for many research groups and unsuitable for clinical practice.

Table 10. Sumary of the multicompartment models features.

MODEL	COMPONENTS	MEASUREMENTS REQUIRED	ASSUMPTIONS
Two-component model	• Fat mass • Fat-free mass	• Densitometry *or* • Hydrometry *or* • BIA	Constant: • FFM density • FFM hydration • Bone/mineral ratio
Three-component model	• Fat mass • Water • Fat-free dry mass	• Densitometry *and* • Hydrometry	Constant Bone/mineral ratio
Four-component model	• Fat mass • Water • Protein • Bone	• Densitometry *and* • Hydrometry *and* • DXA	Constant bone mineral/non-osseous mineral ratio

BIA is bioelectrical impedance; FFM is fat-free mass; DXA is dual x-ray absortiometry.

2. RATIONALE

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

2. RATIONALE

The gold standard method to assess body composition *in vivo* is the 4C model but its cost makes it unattainable for many research groups and unsuitable for clinical practice. Therefore, the solution lies in choosing less expensive methods while maintaining a high level of precision and accuracy. However, it is also important to choose one or another method of analysis considering not only its accuracy in relation to the reference procedure but also aspects related to the transportability, whether the procedure is invasive or not, the degree of training of the personnel to conduct the measurements, the cost of the appliance and its maintenance, the type of population to which it is intended, or the purpose of the assessment of body composition (research or clinics).

Some techniques as DXA and ADP (with BOD POD) have a high level of precision in healthy populations and are accessible for individual body composition assessments, thus, they are often used in research. However, their costs are unaffordable for clinical practice and some physiological (pregnancy, exercise, age, gender…) and pathological (obesity, oedema…) circumstances need to be considered when assessing body composition with these methods; DXA's precision decreases for some populations (e.g. obese patients) whilst ADP assumes constant values of the fat-free mass properties.

BIA have been proposed to be the most suitable method to implement it in clinics due to its advantages: low-cost of the device and its maintenance, non-invasive, safe, fast, portable, minimal participant cooperation required, little training of the operators, suitable for a wide range of populations, the results are reproducible and it is highly correlated to some reference method when assessing healthy, normal-weight subjects. However, BIA is based on the assumptions of the 2C model, mainly

FFM hydration, and makes the method inaccurate when assessing participants with abnormal human conditions (i.e. pregnancy, oedema, obesity, disease, long-term pharmacological treatments, etc.). Therefore, much of the effort of body composition assessment research is to validate BIA in any clinical situation.

One of the most important applications of the body composition assessment is to evaluate obesity at different levels: diagnosing, monitoring its progress and tailoring its treatment. Hence, there is a strong need to optimize the body composition assessment methods to take them to daily clinical practice. Nowadays, the current methods available have a great degree of error in obesity and this error trends to increase with obesity level (135) (the higher the obesity level, the higher the error) and these biases may be due to assumptions in hydration and density of the FFM.

With this doctoral thesis, an analysis of the properties of the fat-free mass in the obese children will be done. This will help to understand whether different body mass index statuses may play a key role in body composition assessment. Further, gained knowledge will be used to improve the precision of 2-component based techniques in obese children.

3. HYPOTHESIS

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
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HYPOTHESES

1. Whole body mass may influence the properties of the fat-free mass, hydration and density, in children, adolescents and young adults.

2. The precision of air-displacement plethysmography (BOD POD®) body composition measurements in obese children may be improved by adjusting the density of the fat-free mass to the nutritional status.

3. The accuracy and precision of the body composition assessment, fat mass and fat-free mass, based on bioelectrical impedance in obese children, could be improved considering the specific properties of the fat-free mass specific to obesity.

4. AIM & OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

OBJECTIVES

Aim of the thesis

The main objective of the present thesis is to improve the accuracy and precision of the body composition assessment techniques in obese children and adolescents to make them suitable to epidemiology and clinical practice.

To achieve this purpose, we addressed the following objectives.

Objectives

1. To evaluate associations of age and body mass index (BMI) with fat-free mass properties, hydration and density, in children, adolescents and young adults.

2. To validate the use of the density of the fat-free mass calculations in body composition assessments by air-displacement plethysmography to improve the body composition predictions in obese children.

3. To validate the use of bioelectrical impedance analysis for body composition assessment in obese children aged 8 to 14.

4. To validate a new predictive equation to estimate the fat-free mass based on impedance index against the four-component model.

5. METHODS

UNIVERSITAT ROVIRA I VIRGILI
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Desirée Gutiérrez Marín

METHODS

The three hypotheses of this doctoral thesis have been addressed through three parts or studies which respond to the different objectives:

- Study 1 addresses the first hypothesis and the objective of the present thesis.
- Study 2 addresses the second hypothesis and the second objective of the present thesis.
- Study 3 addresses the third hypothesis and the third and fourth objectives of the present thesis

5.1. STUDY 1 METHODS

5.1.1. Study design

This was a cross-sectional observational study to describe the fat-free mass properties from childhood to young adulthood considering the total body mass.

A cohort was built from different datasets from the Childhood Nutritional Research Centre (UCL Institute of Child Health, London, UK) (10,14–18). The main samples were a reference dataset of healthy children and adolescents aged 4-22 years (26), some of whom were followed at 2 year intervals for up to 10 years after recruitment, and obese children taking part in weight-loss trials (136,137), however other smaller studies were also incorporated (92,138). 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.

Participants were classified into five nutritional status groups according to their BMI SDS, based on WHO references:

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)

Then, associations of age and whole body mass with hydration and density of the FFM were evaluated.

5.1.2. Study sample

A cohort of 1014 body composition measurements was analysed. The 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 the whole sample) without known comorbidities. 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 **Figure 21**.

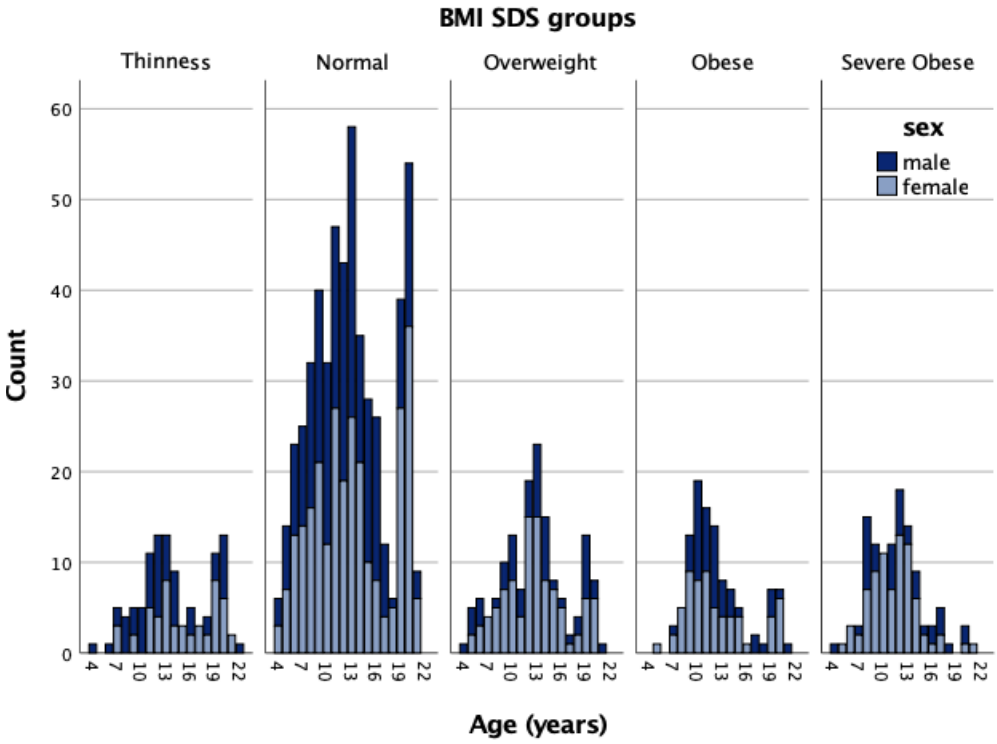

Figure 21. Distribution of the sample stratified by body mass index with standard deviation scores (BMI SDS) groups, age and gender. Groups sizes: n thinness group = 109; n normal group = 529; n overweight group = 151; n obese group = 110; n severely obese group = 115.

5.1.3. Body composition measurements and outcome measures

Body composition outcomes were:

- anthropometric measurements (weight, high and BMI)
- body volume
- bone mineral content
- total body water
- FM and FFM
- Hydration and density of the FFM

These measurements were obtained by anthropometry measurements, air-displacement plethysmography (ADP), underwater weighing, Dual X-Ray Absorptiometry (DXA), and deuterium dilution (DD) analysis. Further, body composition by 4C was calculated.

ANTHROPOMETRY

Weight (WT) and height (HT) measures were obtained in duplicate using standard operating procedures, and the average value was used in all analyses.

Body weight was measured as part of the air-displacement plethysmography protocol using the precision scale incorporated in the ADP device (see below), with minimum clothing and to the nearest 0.1 kg.

Height was obtained by using a wall-mounted stadiometer (Holtain, Dyfed, United Kingdom) without wearing footwear or socks.

BMI was then (BMI kg/m2) was calculated as weight (kg) divided by height squared (m2). These values were converted into standard deviation score (SDS) using current

UK 1990 reference data (139) to assess representativity of the sample compared to the UK population.

BODY VOLUME

Air-displacement plethysmography

BV was measured by ADP using a BOD POD® device (Life Measurements, Inc, Concord, CA). Measurements were taken following the manufacturer's instructions and recommendations, wearing tight fitting swimsuits or underclothes and swimming caps. Each test performed two body volume measures. If these duplicate measures of BV differed more than 150 mL, a third measurement were required. The average of the two measurements (or the closest two when a third measure was needed) was then used in subsequent calculations. The thoracic gas volume remaining in lungs was predicted by children's equations (82), and subtracted from total body volume in subsequent calculations.

Based on the principles of densitometry, D_b was calculated by following the general equation of densitometry (**Figure 22**):

$$Body\ Density\ (\mathrm{kg}/L) = \frac{Body\ Weight\ (kg)}{Body\ Volum\ (L)}$$

Figure 22. Equation of body density (kg/L).

Then, this D_b was used to predict FM (%) from Lohman's equation (25) with the BOD POD device software.

Underwater weighing

The BV of a little subsample (n = 30) was measured by underwater weighing. Lung volume was simultaneously measured by helium dilution. Measurements were obtained in duplicate and the mean value was used when appropriate in the analyses (92).

BONE MINERAL CONTENT

Bone mineral content (BMC) was determined by dual-energy X-ray absorptiometry (DXA). These measurements were determined by a Lunar Prodigy scanner (GE Medical Systems, Madison, WI, USA) with Encore 2002 software (140). A whole-body scan was performed while the subjects were wearing light indoor clothing and no metal objects. The typical scan duration was 5–10 min, depending on subjects' height and the radiation exposure per whole body scan was estimated to be 2.2 µSv. All scans were performed and analysed by one operator. Previously reported precision values for DXA are < 1% for whole body lean mass and < 2% for FM in adults (141).

A subsample (n = 30) was 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). Scans were performed while the subjects were wearing light indoor clothing (typically T-shirts and shorts) and no metal objects. The typical scan duration was 10–12 min, depending on the height of the subject and the radiation exposure per scan was estimated to be 5 mSv. The software package was used by only one member of the investigative team (92).

TOTAL BODY WATER

TBW was determined by isotopic dilution using deuterium-labelled water. A dose equivalent to 0.05 g/Kg of body weight (99.99% $^{2}H_2O$) were dispensed. 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 (136). 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 (140).

FOUR-COMPONENT MODEL

The 4-component model divides the human body into fat, water, mineral and protein. From the primary outcomes obtained by ADP, DXA and hydrometry, FM was calculated using the Fuller's equation (1992) (132):

$$\mathrm{FM}_{4C} = (2.747 \text{ x BV}) - (0.710 \text{ x TBW}) + (1.460 \text{ x BMC}) - (2.050 \text{ X WT})$$

Figure 23. Fat mass calculation from the Fuller's four-component model equation. FM = fat mass in kg; BV= body volume (L) from ADP; TBW= total body water volume (L) from deuterium dilution; BMC = bone mineral content (kg) from DXA and WT = body weight (kg). FFM_{4C} was then calculated as the difference of FM_{4C} from body weight, in kg.

CALCULATIONS OF THE FAT-FREE MASS PROPERTIES AND COMPONENTS

Hydration of the FFM (H_{FFM}), protein mass (PM), total mineral mass (TMM) and density of the FFM (D_{FFM}), were calculated as follows:

- Hydration of the FFM (%): $\mathrm{H_{FFM}} = \frac{\mathrm{TBW}}{\mathrm{FFM}} \mathrm{x}\ 100$
- Protein Mass (kg): $\mathrm{PM} = \mathrm{WT} - (\mathrm{TBWm} + \mathrm{FM} + \mathrm{TMM})$
- Total mineral mass (kg) (56):$\mathrm{TMM} = \mathrm{BMC\ x}\ 1.2741$
- Density of the FFM (kg/L) (132): $\mathrm{D_{FFM}} = \frac{TBWm+PM+TMM}{TBWv+PV+TMV} x\ 100$

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 TMV = total mineral volume calculated by dividing TMM by the density of mineral.

5.1.4. Statistical analysis

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 one-way ANOVA with post-hoc Bonferroni correction (alpha 0.05) was performed to assess any differences for hydration and density of the FFM 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 hydration and density of the FFM.

Linear regression analyses were performed to investigate the associations of age, sex and BMI with hydration and density of the FFM. The regression model was constructed using the independent variables age, sex (1 = male, 2 = female) 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 hydration (<68%) and (n= 4) density (<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 hydration and density of the FFM varied by BMI-group.

5.1.5. Ethics

Ethical approval was granted for the original works by the Ethical Committee of the University College London Institute of Child Health, Great Ormond Street Hospital for Children NHS Trust and and Chichester Local Research Ethics Committee. Both informed parental consent and child assent were obtained before proceeding with the studies.

5.2. STUDY 2 METHODS

5.2.1. Study design

Study 2 was a cross-sectional validation study, secondary to a randomized clustered clinical trial on a motivational intervention to treat obese children. To perform the present validation study, we used the baseline body composition data of the participants enrolled in the OBEMAT2.0 clinical trial (142) (see *addendum* num. 1).

The method to be validated consisted of applying specific calculations of the density of the FFM to improve the body composition predictions from the BOD POD, thus reducing the bias produced in obese children body composition assessments. That is, rather than assuming a constant density of the FFM as it is usually done, we considered a specific density for the obese population. The reference method for comparison was the gold standard: the 4-component model.

5.2.2. Study population

Data from 66 obese children (35 males; 31 females) aged 8 to 14 were obtained from the clinical trial OBEMAT2.0 at baseline. Children were recruited from June 2016 to March 2018 from primary health care centres belonging to the "Camp de Tarragona" healthcare area.

Patients aged 8 to 14 at recruitment time point diagnosed as obese children according to BMI values equal to or higher than percentile 97 (P97) of Hernandez (1988) (37) references were included. Patients with no informed consent signed, eating disorders, participating in another randomized clinical trial, corticoid or ADHD treatment, or presenting endocrinopathies were excluded.

5.2.3. Body composition measurements and outcome measures

Body composition outcomes were:

- anthropometric measurements (weight, high and BMI)
- body volume
- bone mineral content
- total body water
- Density of the FFM
- FM and FFM

These measurements were obtained by anthropometry measurements, air-displacement plethysmography (ADP), Dual X-Ray Absorptiometry (DXA), and deuterium dilution (DD) analysis. Further, body composition by 4C was calculated.

All examinations were taken between 8:00 a.m. and 10:00 a.m. after an overnight fast.

ANTHROPOMETRY

Body weight was measured using a digital scale (SECA 703) to the nearest 0.05 kg in underwear or minimum clothing.

Height was measured by a wall-mounted stadiometer (SECA 216) with 0.1 cm of precision.

Body mass index (BMI) was calculated as WT over height squared (HT^2) in kg/m^2. BMI was then converted into standard deviation scores (SDS) using current WHO 2007 reference data (4).

BODY VOLUME – AIR-DISPLACEMENT PLETHYSMOGRAPHY

BV was measured by ADP using a BOD POD device (Life Measurements, Inc, Concord, CA). Measurements were taken following the manufacturer's instructions and recommendations, wearing tight fitting swimsuits or underclothes and swimming caps. Each test performed two body volume measures. If these duplicate measures of BV differed more than 150 mL, a third measurement were required. The average of the two measurements (or the closest two when a third measure was needed) was then used in subsequent calculations. The thoracic gas volume remaining in lungs was predicted by children's equations (82), and subtracted from total body volume in subsequent calculations.

Based on the principles of densitometry, Db was calculated by following the general equation of densitometry (**Figure 22**):

$$Body\ Density\ (\mathrm{kg}/L) = \frac{Body\ Weight\ (kg)}{Body\ Volum\ (L)}$$

Figure 22. Equation of body density (kg/L).

Then, this D_b was used to predict FM (%) from Lohman's equation (25) with the BOD POD device software.

BONE MINERAL CONTENT – DUAL ENERGY X-RAY ABSORPTIOMETRY

To assess bone mineral content (BMC), a whole body DXA scan was performed by a specialist trained technician using a General Electric Lunar Prodigy Advance (Madison, Wi, USA) instrumentation and the GE, Axial Lunar Prodigy Full Advance (encore 2014 version 15.20.002) software. Individuals wore underclothes during the

test, laying in the supine position with arms at their side. The test duration was less than 10 minutes depending on patients' high and the estimated radiation of the scanning was 0.4 μGy.

DXA also provides FM and lean mass (LM) analyses. FFM was assumed as the sum of LM plus BMC.

TOTAL BODY WATER – DEUTERIUM OXIDE DILUTION

All participants had an oral dose equivalent to 1g/kg body weight of deuterium (^{2}H) oxide dilution. Participants collected six urine samples: one sample before dosing at study site and then, five more urine samples each 24 hours during the following 5 days, at the same time, avoiding the first urine in the morning and keeping the samples in the fridge. Families brought back the urine samples to the study site 6-10 days after dosing and samples were stored at -20ºC. Samples were shipped to the Medical Research Council Elsie Widdowson Laboratory (MRC EWL, Cambridge, UK) for their analysis.

For ^{2}H enrichment, samples of 0.4 ml were placed in 3.7 ml glass vials and flush-filled with hydrogen gas, and then equilibrated for 6 hours in the presence of a platinum catalyst. The headspace of the samples was then analysed using a continuous flow IRMS (Sercon ABCA-Hydra 20-22, Sercon Ltd, Crewe, UK). All measurements were made relative to V-SMOW (Vienna Standard Mean Ocean Water) using calibrated laboratory standards. Analytical precisions (SD) are better than ± 1.3 ppm for 2H.

Main outcome measure was TBW (kg), which was calculated using the zero-time intercept of ^{2}H turnover and corrected for non-aqueous exchange within the body. From these analysis, lean mass (LM) and FM can also be calculated using Lohman's hydration factors (7) to derive lean mass (LM) and FM can be calculated as the difference between WT and LM.

FOUR-COMPONENT MODEL

The 4-component model divides the human body into fat, water, mineral and protein. From the primary outcomes obtained by ADP, DXA and hydrometry, FM was calculated using the Fuller's equation (1992) (132):

$$\mathrm{FM}_{4C} = (2.747 \text{ x BV}) - (0.710 \text{ x TBW}) + (1.460 \text{ x BMC}) - (2.050 \text{ X WT})$$

Figure 23. Fat mass calculation from the Fuller's four-component model equation. FM = fat mass in kg; BV= body volume (L) from ADP; TBW= total body water volume (L) from deuterium dilution; BMC = bone mineral content (kg) from DXA and WT = body weight (kg).

FFM_{4C} was then calculated as the difference of FM_{4C} from body weight, in kg.

DENSITY OF THE FAT-FREE MASS

The density of the fat-free mass (D_{FFM}) was calculated from the study 1 derived predictive equation (**Figure 24**):

$$D_{FFM} = 1.0791 + (0.009 \text{ x age}) + (0.0021 \text{ x gender}) - (0.0014 \text{ x BMISDS})$$

Figure 24. Density of the fat-free mass from study 1 predictive model. Age is given in years; gender 1 = male and 2 = female; BMISDS = body mass index in z-score.

BODY FAT PERCENTAGE

Body fat percentage (%BF) was calculated with the generic Siri's equation (57) (**Figure 25**):

$$\%\mathrm{BF} = \left(\frac{\mathrm{C1}}{\mathrm{BD}} - \mathrm{C2}\right) \mathrm{x}\ 100$$

Figure 25. Siri's generic body fat percentage equation. %BF = body fat percentage; BD = body density (kg/L).

To perform this %FM, previous calculations were needed: Body Density, Body Volume, C1 and C2.

- **Body density** was calculated as: $\mathrm{BD} = \frac{\mathrm{WT}}{\mathrm{BV}}$

Where BV = body volume in L and WT = body weight.

- **Body volume** was obtained from ADP device output.
- **C1** and **C2** from generic Siri's equation are age specific constants which are calculated as (114):

$$\mathrm{C1} = \frac{(\mathrm{D_{FFM}\ x\ D_{FM}})}{(\mathrm{D_{FFM}} - \mathrm{D_{FM}})}$$

$$C2 = \frac{(\mathrm{D_{FM}})}{(\mathrm{D_{FFM}} - \mathrm{D_{FM}})}$$

Figure 26. Equations to calculate specific constants of the density of the fat-free mass. D_{FM} = 0.9007 kg/L (assumed constant) and D_{FFM}, previously calculated in section 5.2.3.

Then, %FM can be calculated (**Figure 25**).

In summary, it is worth to say that %FM was obtained mainly from calculated density (D) fat-free mass and body volume (BV) outcome ($\%BF_{D\&BV}$).

FAT MASS AND FAT-FREE MASS

From %BF, $FM_{D\&BV}$ and $FMM_{D\&BV}$ can be obtained as follows (**Figure 27** and **Figure 28**):

- **Fat mass (kg)**

$$FM_{D\&BV} = \frac{\%\text{BF x WT}}{100}$$

Figure 27. Fat mass (FM – kg) calculation from density of the fat-free mass calculations (D) and body volume (BV). %BF = body fat percentage; WT = total body weight (kg).

- **Fat-free mass (kg)**

$$FFM_{D\&BV} = WT - FM_{D\&BV}$$

Figure 28. Fat-free mass subtracted from total body weight (WT) and fat mass ($FM_{D\&BV}$).

5.2.4. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA). Descriptive characteristics for the overall sample are showed as means ± the standard deviation (SD). Kolmogorov-

Smirnov test for normality was applied and T-test were performed to assess differences between sample groups (train sample against test sample).

We assessed the association between FFM and FM measurements from BOD POD output and calculations derived from predicted density of the FFM, with the reference method (4C) by Pearson correlation coefficients after applying Kolmogorov-Smirnov test for normality. Reliability was obtained from Cronbach's α analysis. Concordance was given as intraclass correlation coefficient (ICC) with a confidence interval (CI) of 95%.

Bland and Altman plots were performed to assess agreement between methods, and the limits of agreement for FM and FFM against the reference method (4C) were calculated.

5.2.5. Ethics

Ethical committees of all involved study centers: CEIC Hospital Universitari de Tarragona Joan XXIII, CEIC Hospital Universitari Sant Joan de Reus (29th January 2016, code 16-01-28/1ass2), CEIC IDIAP Jordi Gol (26th November 2015, code PI14/116) assessed and approved the protocol. If any amendments to the protocol were necessary, the Ethics Committees were notified. All parents or legal guardians signed informed consent prior to study enrollment. Children aged 12 years or above signed informed assent to participate in the study as well.

5.3. STUDY 3 METHODS

5.3.1. Study design

Study 3 was a cross-sectional validation study, secondary to the randomized clustered clinical trial OBEMAT2.0 (142). The method to be validated was the body composition measures from bioelectrical impedance in obese children, considering their specific FFM properties. The reference method of body composition used in this study was the 4C.

To perform the present validation study, we used the baseline body composition data of the participants enrolled in the OBEMAT2.0 clinical trial divided in two subsets: the train and the test subsets. We used specific D&BV FFM ($FFM_{D\&BV}$) and FM ($FM_{D\%BV}$) calculations from study 2 to generate the predictive equation in the train subset. Afterwards, the predictive equation was applied to the test subset to be validated against the 4 components model.

5.3.2. Study population

Data from 315 obese children (170 males; 145 females) aged 8 to 14 were obtained from the clinical trial OBEMAT2.0 at baseline following the same criteria as study 2 (see section 5.2.2).

5.3.3. Body composition measurements and outcome measures

Body composition was assessed by DXA, ADP and BIA in the overall sample of 315 subjects. From those, a subsample of 66 individuals (35 males; 31 females) were randomly selected to perform a TBW assessment by deuterium dilution analysis in study 2. Further, body composition by 4C was assessed in this subsample.

Body composition assessment techniques and protocols for **anthropometry**, **DXA**, **ADP** and **deuterium dilution analysis** have been described previously in section 5.2.3 (study 2).

BIOELECTRICAL IMPEDANCE ANALYSIS

BIA was measured by the octopolar TANITA BC–418MA (Tanita Corporation, Tokyo, Japan) device with a high-frequency constant current (50 kHz, 500 μA).

Subjects, wearing minimum clothing, stand barefoot on the metal footplates and to hold handgrips (2 electrodes in each handgrip, which are in contact with fingers and the thenar side of the hand, and 2 electrodes in each footplates to stand up, which are in contact with toes and heels). Measurements were taken twice and the average was then use in further calculations.

Outputs from the device were whole-body impedance (Z) and predicted FM, FFM and TBW by using manufacturer's internal equations. These algorithms were constructed using as reference the data acquired through DXA in Japanese and Western subjects and performing repeated regression analyses, including height, weight, age and impedance between the right hand and foot as variables (information provided by the manufacturer).

The assessment was performed by trained investigators

5.3.4. Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA). The sample was analysed separately as two sampled groups: the train sample (n=249) and the test sample (n=66). Kolmogorov-Smirnov test for normality was applied. Descriptive characteristics for the train, test and the overall sample are shown as means ± the standard deviation (SD). T-test

was performed to assess differences between sample groups (train sample against test sample).

A bootstrap linear regression analysis of 1000 sample replications from the train sample (n = 249) was undertaken to derive predictive equations for fat-free mass using impedance index (HT^2/Z) as an independent variable. Other values easily obtainable in clinics and research as age, gender, body weight and BMI were also included as independent variables to adjust the predictive models. The obtained predictive equation was applied to the test sample (n=66) to externally validate it.

We assessed the association between FFM and FM measurements from the new predictive equation and the reference method (4C) by Pearson correlation coefficients after applying Kolmogorov-Smirnov test for normality. Reliability was obtained from Cronbach's α analysis and concordance was given as intraclass correlation coefficient (ICC) with a confidence interval (CI) of 95%.

Bland and Altman plots were performed to assess agreement between methods, and the limits of agreement for FM and FFM against the reference method (4C) were calculated.

Percentage differences between methods and 4C are shown as mean ± SD with a 95% CI.

5.3.5. Ethics

Ethical committees of all involved study centers: CEIC Hospital Universitari de Tarragona Joan XXIII, CEIC Hospital Universitari Sant Joan de Reus (29th January 2016, code 16-01-28/1ass2), CEIC IDIAP Jordi Gol (26th November 2015, code PI14/116) assessed and approved the protocol. If any amendments to the protocol were necessary, the Ethics Committees were notified. All parents or legal guardians

signed informed consent prior to study enrollment. Children aged 12 years or above signed informed assent to participate in the study as well.

6. RESULTS

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

RESULTS OF THE STUDY 1

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

- *Description of the sample*

After screening for implausible values for hydration and density of the FFM, and accounting for missing data which prevented full calculation of the 4C model for hydration and density of the FFM (n=77 and n=105 respectively), a total of 936 data points for hydration and 905 for density of the FFM were analysed.

Table 11 shows a description of the characteristics of the sample stratified by gender. No differences were found in age between males and females (13.0 ± 4.1 and 13.4 ± 4.4 years old, respectively). Neither weight, height nor hydration of the FFM showed differences between genders.

Statistically significant differences were found for all other body variables. Girls showed greater BMI than boys ($p<0.001$). Females also 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 29**, showing wide variability of BMI at all ages. The distribution of age and gender by BMI groups is displayed in **Figure 21** (see section **5.1.2** of the methods of study 1). **Table 12** provides mean and SD of age, and the ratio of males to females, for each BMI category.

Table 11. Description of the sample.

	Males (n = 416)		Females (n = 520)		Whole sample (n = 936)	
	mean (± SD)	Range	mean (± SD)	Range	mean (± SD)	Range
Age (years)	12.9 (±4.1)	4.22 – 22.0	13.4 (± 4.4)	4.5 – 21.9	13.2 (± 4.3)	4.2 – 22.0
Weight (kg)	49.6 (± 20.8)*	15.2 – 111.3	52.8 (± 20.0)*	16.1 – 119.6	51.4 (± 20.4)	15.2 – 119.6
Height (cm)	153.2 (± 20.4)	102.5 – 194.7	151.8 (± 15.6)	103.9 – 185.4	152.4 (± 17.9)	102.5 – 194.7
BMI (kg/m2)	20.2 (± 5.2) †	13.0 – 40.6	22.2 (± 6.2) †	12.5 – 48.4	21.3 (± 5.9)	12.5 – 48.4
BMI SDS	0.45 (± 1.42) †	-3.09 – 4.74	0.79 (± 1.52) †	-3.33 – 4.46	0.64 (± 1.49)	-3.33 – 4.74
HT SDS	0.16 (± 1.05) ‡	-2.77 – 3.93	0.37 (± 1.08) ‡	-2.77 – 4.11	0.28 (± 1.08)	-2.77 – 4.11
WT SDS	0.43 (± 1.35) †	-3.09 – 4.84	0.84 (± 1.51) †	-4.02 – 4.77	0.66 (± 1.45)	-4.02 – 4.84
Fat Mass (kg)	12.1 (± 10.1) †	0.97 – 58.6	18.0 (± 11.9) †	2.3 – 67.6	15.4 (± 11.5)	0.97 – 67.7
Fat Free Mass (kg)	38.3 (± 14.4) ‡	12.8 – 72.2	35.7 (± 9.4) ‡	12.1 – 64.1	36.9 (± 12.0)	12.1 – 72.2

*P-value for differences between males and females: * p = 0.02; † p < 0.001; ‡ p<0.005.
Abbreviations: BMI = Body Mass Index; HT = height; WT = weight; SDS Standard deviation score; FFM = Fat-free mass; SD = Standard deviation.

Table 12. 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.

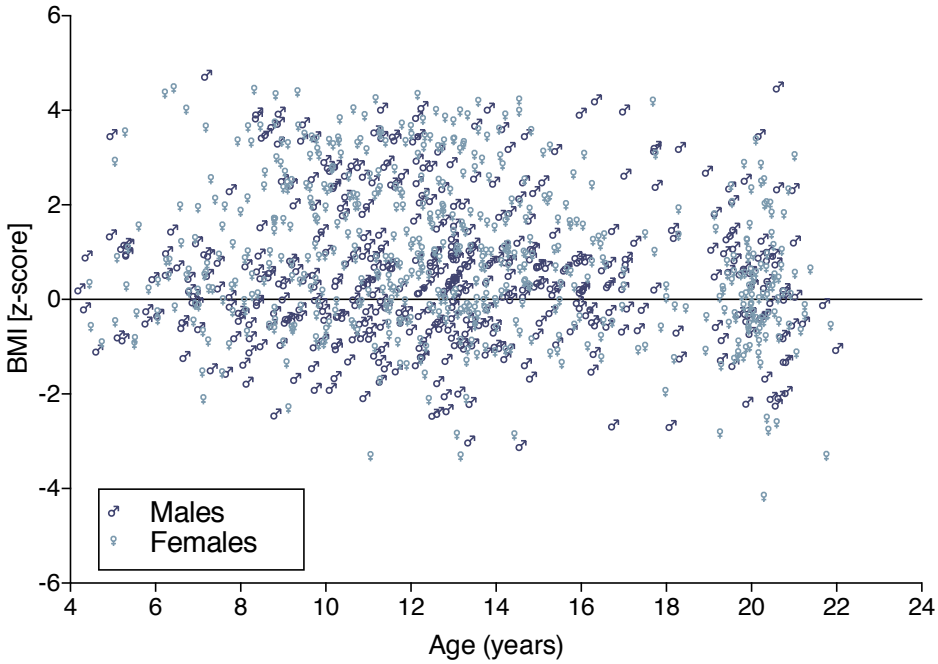

Figure 29. Distribution of the sample by body mass index z-scores and age in years.

- *Hydration of the fat free-mass*

Hydration of FFM values are illustrated in **Figure 30** and **Figure 31,** which show how hydration of FFM varies in association with body mass index categories (**Figure 30**) and age (**Figure 31**). Heavier groups (obese and severely obese) showed clearly higher hydration levels of FFM at all ages. Furthermore, hydration decreased with age in all BMI groups, but with different patterns (**Figure 31**)**.** While the decrease was marked in lower BMI groups, heavier groups showed a weaker decrease, trending to a plateau. Beyond these patterns, wide variability range of hydration values could be found within each BMI group.

All BMI groups showed statistically significant differences ($p<0.001$) in hydration of FFM except the two highest ones, with not statistically significant differences between obese and severely obese ($p=0.121$).

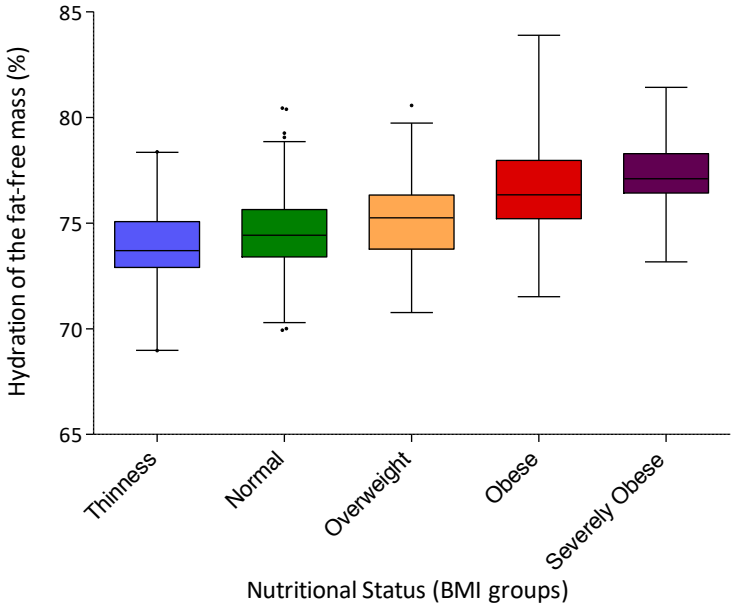

Figure 30. Graphic representation of the trend of the hydration of the fat-free mass according to nutritional status. All groups showed statistically significant differences between all groups ($p<0.001$) except between obese and severely obese groups, where differences were highly significant ($p = 0.121$).

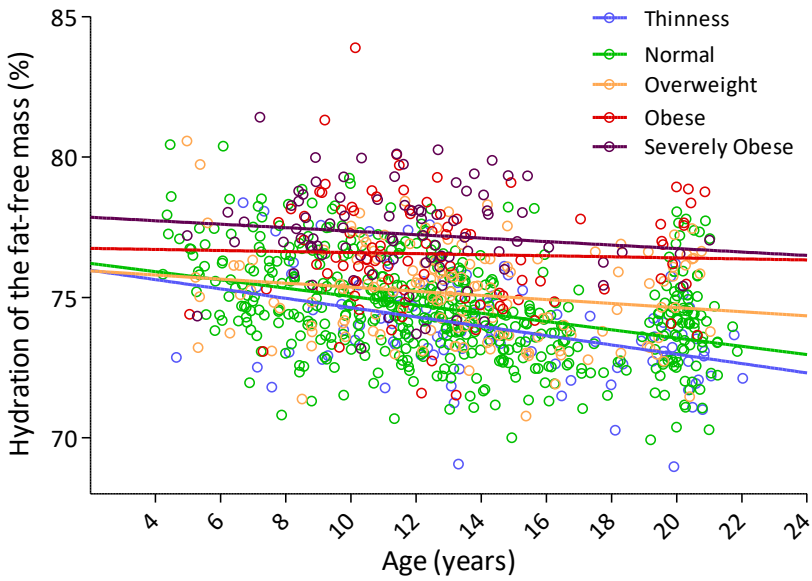

Figure 31. Graphic representation of the trends of the hydration of the fat-free mass according to age and stratified by nutritional status.

Table 13 displays prediction models of hydration. Age explained the 10% of the variability of the hydration of the FFM (**Table 13** – model 0). Around 30% of the hydration variability was then explained by both age and BMI (even as a continuous variable in model 1 or as index categories in dummy variables in model 2 or 3). Gender did not show improvement of the models and was not significant for these cases, thus, gender was not included in the models shown below.

Although interaction of age and BMI was individually statistically significant for overweight and obese groups when compared to the normal group (**Table 13**, model 3), BMI groups showed a significant interaction with age for hydration of the FFM ($p = 0.007$) when the general linear model univariate test was performed.

Table 13. Prediction of the hydration the fat-free mass.

HYDRATION						
		B	SE	p value	R^2	s.e.e
Model 0.	Constant	76.992	0.202	**<0.001**	0.101	1.906
	Age (years)	-0.150	0.015	**<0.001**		
Model 1.	Constant	74.611	0.231	**<0.001**		
	Age (years)	-0.124	0.013	**<0.001**	0.292	1.692
	BMI SDS (cont.)	0.596	0.037	**<0.001**		
Model 2.	Constant	76.212	0.186	**<0.001**		
	Age (years)	-0.124	0.013	**<0.001**		
	Thinness	-0.545	0.179	**0.002**	0.303	1.677
	Overweight	0.565	0.158	**<0.001**		
	Obese	1.976	0.189	**<0.001**		
	Severe Obese	2.495	0.197	**<0.001**		
Model 3.	Constant	76.514	0.229	**<0.001**		
	Age (years)	-0.147	0.016	**<0.001**		
	Thinness	-0.238	0.613	0.698		
	Overweight	-0.451	0.534	0.398		
	Obese	0.296	0.658	0.653	0.309	1.670
	Severe Obese	1.478	0.720	**0.041**		
	Int. age-thinness	-0.019	0.041	0.639		
	Int. age-overweight	0.076	0.038	**0.046**		
	Int. age-obese	0.130	0.049	**0.008**		
	Int. age-severe obese	0.084	0.059	0.152		

"Normal" group was chosen as the reference group. Abbreviations: BMI SDS = body mass index in standard deviation scores; cont. = continuous; Int. = interaction.

- *Density of the fat free-mass*

Density of FFM showed patterns with age and BMI that were broadly inverse to those for hydration of FFM (**Figure 32 and Figure 33**), though with a stronger overall age-association (the higher the hydration level, the lower the density). **Figure 32** shows the decrease of the density of the FFM with BMI-groups. The density of the FFM increased with age for all BMI groups (**Figure 33)** but this increase was more obvious in the non-obese groups. In addition, differences in density among lighter and heavier BMI groups seemed to be more striking with increasing age.

Further, no significant differences were found for density among thin, normal and overweight nutritional groups (P>0.05) but highly significant differences appeared between the three non-obese groups and the two obese ones (p<0.001). In addition, a highly significant statistical difference was observed between obese and severely obese groups (p<0.001).

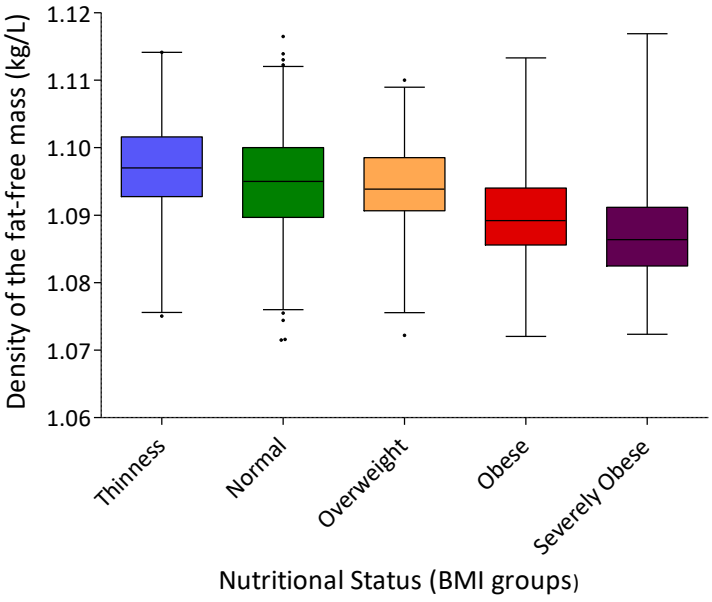

Figure 32. Trend of the density of the fat-free mass according to BMI groups. The three non-

obese groups (thinness, normal and overweight) did not show significant statistical differences between them; the differences between obese and severely obese groups where highly significant (p<0.001) as well as differences between the three non-obese groups and the two obese ones (p<0.001).

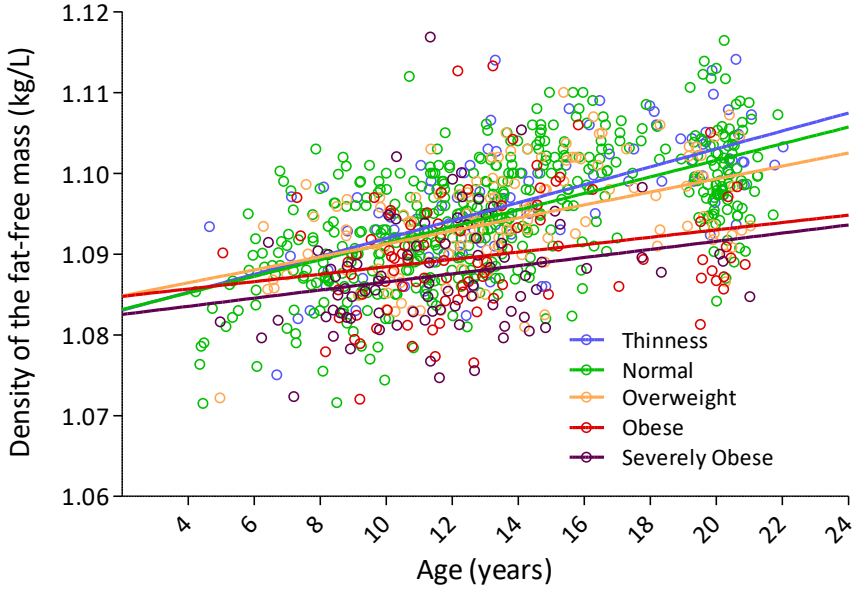

Figure 33. Graphic representation of the trends of the density of the fat-free mass according to age and stratified by nutritional status.

Prediction models of density are displayed in **Table 14.** These models showed that age, BMI and gender explained almost 40% of the density of the FFM variability, increasing the variability due to age (29 %) (**Table 14** - model 0).

According to the previous results, the interaction of age and BMI was individually statistically significant for the two obese groups when compared to the normal group and the overall BMI groups showed a significant interaction with age for density of the FFM (p = 0.014) when the general linear model univariate test was performed.

Table 14. Prediction of the density the fat-free mass.

DENSITY						
		B	SE	p value	R^2	s.e.e
Model 0.	Constant	1.080	0.001	**<0.001**	0.288	0.007
	Age (years)	0.001	<0.001	**<0.001**		
Model 1.	Constant	10.791	0.001	**<0.001**	0.375	0.006
	Age (years)	0.0009	<0.001	**<0.001**		
	Sex	0.0021	0.0004	**<0.001**		
	BMI SDS (cont.)	-0.0014	0.0001	**<0.001**		
Model 2.	Constant	10.793	0.0009	**<0.001**	0.378	0.006
	Age (years)	0.0009	0.0000	**<0.001**		
	Sex	0.0022	0.0004	**<0.001**		
	Thinness	0.0012	0.0007	0.066		
	Overweight	-0.0012	0.0006	0.050		
	Obese	-0.0048	0.0007	**<0.001**		
	Severe Obese	-0.0063	0.0007	**<0.001**		
Model 3.	Constant	10.782	0.0001	**<0.001**	0.385	0.006
	Age (years)	0.0010	0.0001	**<0.001**		
	Sex	0.0021	0.0004	**<0.001**		
	Thinness	0.0004	0.0023	0.850		
	Overweight	0.0015	0.0022	0.497		
	Obese	0.0024	0.0025	0.340		
	Severe Obese	-0.0001	0.0027	0.964		
	Int. age-thinness	-0.0001	0.0002	0.763		
	Int. age-overweight	0.0002	0.0002	0.201		
	Int. age-obese	-0.0005	0.0002	**0.003**		
	Int. age-severe obese	-0.0005	0.0002	**0.021**		

The nutritional group "Normal" has been chosen as the reference group for regressions. Abbreviations: BMI SDS = body mass index in standard deviation scores; cont. = continuous; Int. = interaction.

Given these results, we propose the following equation derived from model 2 to predict the density of the fat-free mass to be considered in body composition assessments when using 2C model-based techniques, especially in obese children:

$$D_{FFM} = 1.0791 + (0.009 \text{ x age}) + (0.0021 \text{ x gender}) - (0.0014 \text{ x BMISDS})$$

Figure 24. Density of the fat-free mass from study 1 predictive model. Age is given in years; gender 1 = male and 2 = female; BMISDS = body mass index in z-score.

RESULTS OF THE STUDY 2

A novel approach to assess body composition in obese children from density of the fat-free mass calculations

- *Description of the sample*

Physical characteristics of the sample are shown in **Table 15 and Table 16**. The average age of the sample was 10.7 ± 1.5. Although there were slightly more males than females, no statistically significant differences were found (p = 0.851). The average weight (WT) and height (HT) were 56.2 ± 11.2 and 146.9 ± 10.2.

Table 15. Description of anthropometry and body composition of the sample.

	Mean ±SD (n = 66)	Range
Age (y)	10.7 ± 1.5	8.0 - 13.3
Waist circumference (WHO) (cm)	84.1 ± 6.5	10.7 - 98.2
Weight (Kg)	56.2 ± 11.2	31.0 - 83.1
Height (cm)	146.9 ± 10.2	125.45 - 170.3
BMI kg/m2	25.9 ± 2.5	21.7 - 32.5
BMI SDS (WHO 2007)	2.62 ± 0.40	1.86 - 4.20
HT2/Z (m2/ohms)	33.9 ± 6.6	21.3 - 47.5
Body Volume (BOD POD - L)	56.0 ± 11.2	36.1 - 83.7
Density FFM predicted (kg/L)	1.088 ± 0.002	1.083 - 1.092
$FM_{D\&BV}$ (kg)	22.6 ± 5.6	11.2 - 38.2
$FFM_{D\&BV}$ (kg)	33.6 ± 6.9	20.2 - 53.7
FM_{BODPOD} (kg)	21.9 ± 5.6	11.4 – 38.4
FFM_{BODPOD} (kg)	34.4 ± 6.7	21.2 – 51.9
FM_{4C} (kg)	23.3 ± 5.8	11.6 - 38.7
FFM_{4C} (kg)	32.9 ± 6.7	20.3 - 50.6
Total Body Water (DD-kg)	24.8 ± 5.0	15.8 - 36.7

Table 16. Comparison of physical characteristics of the study sample between genders.

Significance: † p = 0.038; ‡ p < 0.001.

model; DD = deuterium dilution.

- *Agreement between methods*

Differences between FFM obtained from body volume measurements and $density_{FFM}$ calculations ($FFM_{D\&BV}$) and FFM from BOD PODS´s output (FFM_{BODPOD}) were analysed compared to the reference method (FFM_{4C}) (**Figure 34**). $FFM_{D\&BV}$ was overestimated by 0.71 kg (limits of agreement -1.08 kg, 2.51 kg) showing a mean difference of 2.80% ± 2.06% ($p<0.001$). On the other hand, FFM_{BODPOD} was overestimated by 1.50kg (limits of agreement -0.68kg, 3.63kg), showing a higher percentage of difference than $FFM_{D\&BV}$ when compared to 4C (4.87% ± 2.92%; $p<0.001$). However, bias of $FFM_{D\&BV}$ trended to increase when leaner, whilst FFM_{BODPOD}'s magnitude showed no trend.

Inversely, FM was underestimated by both techniques, predicted D&BV calculations ($FM_{D\&BV}$) and BOD POD's output (FM_{BODPOD}) by -0.71kg (limits of agreement 1.1kg, -2.5kg) and -1.4kg (limits of agreement 0.9kg, -3.6kg) representing a mean difference of 3.97% ± 2.92% ($p<0.001$) and 6.77% ± 3.77% ($p<0.001$), respectively, when compared to the reference method (FM_{4C}) (**Figure 34**) (**Table 17**). These biases trended to be higher when fatter.

A

B

C

D

Figure 34. Bland and Altman plots of the difference between fat-free mass (FFM) and fat mass (FM) (kg) as measured from ADP body volume measures (FFM_{ADP} and FM_{ADP}) and the reference method (FFM_{4C} and FM_{4C}) (A and C) and BODPOD'S fat-free mass (FFM_{BODPOD}) and fat mass (FM_{BODPOD}) outputs and the reference method (B and D), against the mean fat-free mass and fat mass (kg).

Table 17. Analyses of differences (%) between the body compositions calculated from D&BV, BOD POD and 4-component model.

	MEAN DIFFERENCE (95% IC; p-value)	SD	DIFF. MIN.%	DIFF. MAX.%
$FFM_{D\&BV}$	2.8% (2.29-3.3; p<0.001)	2.06	0.13%	8.91%
$FM_{D\&BV}$	3.97% (3.25-4.69; p<0.001)	2.92	0.26%	12.33%
FFM_{BODPOD}	4.87% (4.15-5.59; p<0.001)	2.92	0.22%	11.04%
FM_{BODPOD}	6.77% (5.6-7.45; p<0.001)	3.77	0.17%	14.59%

Correlations and reliability of both techniques are displayed in **Table 18**, showing that when body composition measurements are adjusted to FFM properties (density in this case) improves the correlation and reliability of the assessment when compared to the gold standard method.

Table 18. Correlations and reliability of fat-free mass measurements against 4C.

	Correlation coeff. (p-value)	Cronbach's α	ICC (IC 95% - p-value)
Density predictions	0.992 (p<0.001)	0.996	0.993 (0.967-0.997; p<0.001)
BOD POD	0.987 (p<0.001)	0.993	0.981 (0.640-0.995; p<0.001)

RESULTS OF THE STUDY 3

Validation of impedance analysis for body composition assessment in obese children aged 8-14

- *Description of the sample*

Table 19 shows the characteristics of the train and test samples. The average age of the train sample was 10.9 ± 1.6 and the test sample, 10.7 ± 1.5 without statistically significant differences (p = 0.275). Although there were more males than females in both samples, no statistically significant differences were found (p = 0.851). The average weight (WT) and height (HT) of the train sample were 58.5 ± 8.3 and 149.2 ± 10.6, respectively; the average for both measures of the test sample were 56.2 ± 11.2 and 146.9 (± 10.2). Despite train subjects where slightly heavier and taller than test subjects, these differences were not statistically significant (p = 0.188 for WT; p = 0.099 for HT). Neither BMI nor BMISDS showed statistically significant differences between groups (p = 0.879 and p = 0.636, respectively). BV from BOD POD and predicted density of the FFM neither presented any differences (p = 0.178 and p = 0.445, respectively). Finally, the train sample showed a slightly higher average of the FM and the FFM from ADP calculations (FM = 23.9 ± 7.1; FFM = 34.6 ± 7.7) than test sample (FM = 22.6 ± 5.6; FFM = 33.6 ± 6.9) but no statistically significant differences were found (p = 0.179 and p = 0.325, respectively). Hence, the test sample and the train sample were similar.

Table 19. Description of anthropometry and body composition of overall sample and train and test subsamples.

- *Construction of the new equation*

Differences between FFM obtained from predicted density of the FFM calculations ($FFM_{D\&BV}$) and FFM from BOD PODS´s output (FFM_{BODPOD}) were previously analysed compared to the reference method (FFM_{4C}) in a previous report [study 2].

Further, linear regression analysis was performed to predict FFM from HT^2/Z in the train sample (n=249) bootstrapping 1000 samples and using $FFM_{D\&BV}$ as the reference method (**Table 20**).

First, no additional adjustments were included in the regression. This model explained 85.7% of variance. Then, we tested different possible predictor variables to find the best predictive model, displayed in **Table 20**.

Although, model 4 seemed to be the best one to construct our predictive equation, when predictors correlations were analysed we found that weight was highly correlated with both impedance index and age (0.899; $p<0.001$ and 0.806; $p<0.001$) respectively, which could affect the model. Therefore, we chose model 5 instead, where BMI correlated weaker to impedance index (0.563; $p< 0.001$) and age (0.518; $p < 0.001$).

Table 20. Predictive models for the fat-free mass assessment derived from impedance index.

Therefore, the final FFM predictive equation derived from impedance index is shown in **Figure 35**.

$$\mathrm{FFM_Z} = -9.012 + \left(0.818 \text{ x } \frac{HT^2}{Z}\right) + (0.742 \text{ x age}) + (0.648 \text{ x gender}) + (0.235 \text{ x BMI})$$

Figure 35. New fat-free mass predictive equation from impedance index.
HT_2/Z was calculated from TANITA's Z raw output in m^2/Ω, age was given in years, gender 1 = male and female = 2, and BMI in kg/m^2. FM_Z was subtracted from total body weight (WT) and FFM_Z. The constructed FFM predictive equation (FFM_Z) was then tested in the test sample (n=66).

- *Agreement between methods*

Subsequently, FFM_Z and FM_Z results were compared to FFM_{4C} and FM_{4C}. FFM_Z showed a statistically significant overestimation of 0.8 kg (limits of agreement -2.54 kg, 4.16 kg) (**Figure 36**) with an average difference of 4.6% ($p<0.001$) (**Table 21**). Moreover, the prediction underestimated the FM_Z with an average difference of 6.38% ($p<0.001$) (mean difference -0.8 kg. limits of agreement -4.2 kg, 2.5kg).

Even though FFM_Z predictive equations showed statistically significant bias against FFM_{4C}, limits of agreement were narrower for both FFM and FM, than those obtained from TANITA's measurements (**Figure 36**). Comparison between TANITA's FFM and FM outcome and 4C calculations presented an average difference of 4.6 kg of FFM (limits of agreement 0.8kg, 8.3 kg) (**Figure 36-B**), overestimating the FFM 14.1% ($p<0.001$) (**Table 21**) and underestimating the FM 18.4% ($p<0.001$) (mean difference -4.3 kg; limits of agreement -0.6 kg, -8.0 kg) (**Figure 36-D**). Furthermore, FFM_Z predictive equation showed a clearly improvement of the agreement when compared to the reference method concerning TANITA's manufacturer's equations.

A

B

C

D

Figure 36. Bland and Altman plots of the difference between fat-free mass (FFM) and fat mass (FM) (kg) as measured by free mass (FFM_{TANITA}) and fat mass (FM_{TANITA}) output and the reference method (B and D), against the mean fat-free mass

Table 21. Analyses of differences (%) between the new equation, TANITA and 4C.

		MEAN DIFFERENCE (95% IC; p-value)	SD	DIFF. MIN.%	DIFF. MAX.%
Impedance	FFM	4.61% (3.80-5.44; p<0.001)	3.34	0.04	15.72
	FM	6.38% (5.34-7.42; p<0.001)	4.24	0.05	16.94
TANITA	FFM	14.05% (12.71-15.4; p<0.001)	5.44	1.26	29.34
	FM	18.39% (16.9-19.9; p<0.001)	6.06	1.01	29.32

Table 22 shows the correlations and reliability coefficients. Both new equation and TANITA measurements of FFM were highly correlated to 4C assessment and showed similar values of reliability. However, the FFM_Z show higher concordance than FFM TANITA with the gold standard.

Table 22. Correlation coefficients (Pearson) and reliability of fat free mass assessments against 4C.

	Correlation coeff. (p-value)	Cronbach's α	ICC (IC 95% - p-value)
FFM_Z	0.968 (p<0.001)	0.984	0.980 (0.957 - 0.989; p<0.001)
FFM_{TANITA}	0.969 (p<0.001)	0.982	0.886 (-0.101-0.973; p<0.001)

7. DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

DISCUSSION

This thesis provides new insights to properly assess body composition in obese children. Body composition techniques rely on assumptions about tissue properties, however, differences in properties of the FFM between the general and the obese populations had received little attention.

It was already known that FFM properties changed with age (25,114,143). Nonetheless, there is little evidence about the influence of nutritional status, specifically of the obese status, on these properties in adults (144) and there is a lack of evidence in children (93,145).

The present thesis confirms the hypothesis that FFM properties, both hydration and density, change with age and obesity. Moreover, this work showed that there were strong differences in properties of the FFM between obese and non-obese subjects. Further, this thesis proposes how to apply this knowledge to body composition techniques assessment to improve its precision when assessing obese children.

These findings may shed some light on why two-component based techniques for body composition assessment lose accuracy when evaluating obese subjects. Previous works have 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 (29,137,146).

To our knowledge, the **study 1** is the first to provide evidence of changes in hydration and density of the FFM due to an obesity status in a large population sample, with a wide BMI range, from childhood to young adulthood.

However, other researchers have previously found some evidence of these concerns. In 2005, Haroun *et al.* (93) investigated the composition of the FFM in 50 obese (n = 28) and non-obese (n = 22) British children using a matched case-control study. They found that obese children had higher BV, TBW, BMC, FM, FFM and also hydration levels of FFM and, consistently, they had lower levels of density of the FFM.

Previously, in 2001, Bray *et al.* analysed body composition data from 114 children set in USA in a longitudinal study (147). The follow-up measurements were separated by 2 years on average; the first measurement was taken at 10 years old on average and the second, at 12 years old. They found that hydration index was significantly higher in fatter children when compared to the leaner ones, but also that body density was higher in leaner subjects.

Therefore, our results were consistent to those found by Bray *et al*. and Haroun *et al.*

The reason of this phenomena remains poorly understood. However, some hypothesis has been published. Waki *et al.* suggested that extracellular water space might be relatively increased. They analysed extracellular fluid data of 39 obese and 26 non-obese adult women and found that there was an increase of extracellular water/intracellular water (ECW/ICW) ratio in the obese group. However, there was a wide variability of the ECW/ICW ratio within the obese group which suggested to be associated to subclinical complications of obesity i.e. oedema, pulmonary hypertension or cardiac dysfunction; but also to variability in hormone response due to excess of fat and its distribution (148).

On the other hand, Lichtenbelt and Fogelholm (149) found partially consistent results to those found by Waki but with new approaches. They analysed the ECW/ICW ratio of 30 obese women before and after 3 months' weight loss and after 9 months of weight maintenance. They found that ECW/ICW ratio was relatively high before weight reduction but after weight loss and maintenance this ratio increased. The hydration of the FFM level increased during weight reduction and remained high during the maintenance period. Thus, neither the ECW/ICW ratio nor hydration of the FFM normalized after weight loss.

Accordingly to Lichtenbelt, Leone *et al.* (150) compared two groups of 10 participants each group; post-obese group (after weight loss treatment) and never obese group. She compared body composition of the two groups and found that the post-obese group persisted overhydrated when compared to those never obese subjects.

The study of Haroun *et al.* mentioned above (93) also analysed the protein/mineral ratio in obese and non-obese groups. They found that mineral mass was significantly greater in the obese group, whereas protein mass did not show significant differences between the two groups. These differences in relative mineralisation between obese and control children contribute to the increased differences in hydration and density detected by the 4C model.

All these approaches lead to think that the expanded ECW space hypothesis and over-mineralization of obese subjects may be not the only mechanisms implicated in increased hydration levels of the FFM in obese patients and further research is needed in this field.

Our study goes further, by revealing interactions of BMI status with age, i.e. values change with age differently depending on BMI. For hydration of the FFM we showed that the combination of age and BMI group explained ~30% of variability. Thus,

hydration of the FFM models showed as expected decreasing values with age, but also interactions between BMI and age, with lower hydration reductions associated with obesity at older ages. Also, age-BMI interactions were significant only for overweight and obese subjects. On the other hand, density of the 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 density of the FFM.

These regression models proposed in our study can be used to predict individual hydration and density of the 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 hydration and density of the FFM cannot be explained by our predictors. Methodological error and other unknown biological properties are likely to contribute.

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 were weaker.

A limitation of the first study was the low number of participants between 17 and 20 years. However, the study has several strengths, as the big sample size, the wide age range and the use of the best available techniques used.

The knowledge acquired in study 1 is a gateway to the possibility of improving body composition assessment in obese children by 2C model-based techniques, i.e. the densitometry techniques like ADP.

ADP assumes constant values of the density of the FFM and, with **study 2**, we propose to consider that density of the FFM should be calculated instead of assuming the constant value in obese patients, due to the differences of density of the FFM between them and non-obese populations.

There are previously published data which suggest that assumptions of the properties of FFM could be the cause of some bias in the evaluation of FM by ADP. However, there are few studies which used a multi-compartment model to compare ADP and most of them are performed in adults.

Fields *et al.* (151) found differences in body fat percentage between BOD POD calculations based on Siri's equation and the 4C, showing that BOD POD underestimated %FM. However, both techniques were well correlated. They also investigated the relative hydration of the FFM as a possible explanation to the differences found. Hydration of the fat-free mass correlated the magnitudes of the difference between both techniques; when higher the hydration fraction was, the higher the differences. This study was performed with a sample of 42 British healthy adult females.

In a study performed with a sample of 50 young healthy adults (n males = 40) and mixed raced (n Caucasians = 35; n African-Americans =15) Millard-Stafford *et al.* (152), simultaneously and accordingly to Field, found that %FM calculations obtained from BOD POD D_b were underestimated when compared to the 4C and other methods as UW D_b or DXA. They proposed to consider the density of the FFM and its fractional components (i.e. mineral, water and protein), consistently to our findings.

To our knowledge, the only existing study comparing BOD POD ADP and other techniques with the four-component model was conducted by Fields and Goran (153) with 25 British healthy children. They did not found significant bias between ADP and 4C, but no BMI mentions were included in their analysis and the sample was homogenous in age and anthropometric measures.

Wells *et al.* (138) evaluated the ADP in 28 British healthy children aged 5 to 7 using the 3C model. They found a high accuracy of ADP when compared to 3C model body

composition measurements for groups but highlighted the need to improve biases in individuals, which could be due to methodological precision and biological variability in hydration. In addition, according to a previous finding, the same researchers reported (92) that the calculated density of the FFM was slightly increased for both sexes, but significant only for girls, when compared to reference data. Thus, they showed significant bias in %FM compared to 3C model when using predicted values for the density of the FFM. This implies that BOD POD calculations should be adjusted by specific FFM properties to increase its accuracy. Our studies 1 and 2 went further and included the nutritional status in the calculations.

Study 2 showed consistent results to those previously described by Wells *et al*. FM and FFM assessment had a narrower agreement with 4C model measurements when calculations are adjusted by specific density of the FFM than BOD POD outputs, which did not consider the nutritional status of the subjects and assumed a constant density of the FFM. These analyses were performed in a sample of more than 300 subjects, which might be considered a strength of the study. Thus, this study demonstrates and validates de use of the density of the FFM calculations together with body volume outcome from BOD POD and we encourage the manufacturers to improve their software calculations in order to get more precise and accurate results for a wide extent of patients with a broad range of BMI.

However, the use of ADP devices stills being limited to research and some sport institutions and it is usually unaffordable for clinical practice. Nonetheless, after analysing the results of the studies 1 and 2, we focused on transfer this knowledge to an applicable technique for measure body composition in obese children.

The third study of the present thesis focused on the need to find a body composition assessment technique suitable and affordable for both research and clinical practice with an acceptable accuracy and precision levels in obese individuals.

In **study 3**, we have proposed a method to improve the accuracy of body composition assessment using bioelectrical impedance.

BIA has been proposed by many investigators, as the feasible option to assess body composition when others of higher precision are not available, due to its advantages: it is relatively inexpensive, quick, easy, transportable, easy to store, it needs little operator training, and it is suitable for a large range of populations.

The main inconvenient is that BIA results are biased for those populations with altered hydration levels; pregnancy, fasting, exercise previous to the test, pharmacological treatments, diseases, obesity, pubertal stage, etc. are some of the conditions that may influence hydration levels. Actually, BIA has shown greater bias associated to higher BMI (135,154). Our work also identified TANITA's manufacturers equation biases when compared to the gold standard (4C), accordingly to previous studies performed with paediatric population (137,140,155–158). These BIA biases seemed to be due to the assumed constant values of the fat-free mass properties, more specifically, the water fraction of the FFM.

Published reference data for hydration (28) and density of the FFM (114) were obtained from general population studies. Thus, we found that body composition measurements in obese children from TANITA's outputs were highly biased when compared to body composition calculations with the 4C. These results were consistent to those found by other colleagues (146). In 1996, Deurenberg (159) discussed the validity of BIA in severely obese subjects and concluded that an increased relative amount of TBW will result in underestimations of %FM predicted by manufacturer's equations. In addition, he suggested that different body built due to differences in fat distribution between individuals would result in an underestimation of the %FM, especially in those subjects with severe abdominal

obesity, which have located a big proportion of fat and water in the trunk, part of the body which contributes poorly to impedance.

These findings are very important to consider when monitoring weight loss treatment or performing longitudinal studies where individual changes on body composition might not be reliably detected.

Therefore, many predictive equations for specific populations have been published, but not so many specifically for obese children, and few of them have been compared to multi-component models. The most recent approaches including BIA and obese children are Clasey *et al.* (2011), Haroun *et al.* (2009) and Seo *et al.* (2018).

Clasey *et al.* (156) published in 2011 a predictive equation obtained from a sample of 361 general (obese and non-obese) boys and girls aged 5 to 11 years and tested the equation in a sample of 75 children. The criterion method used was DXA. They obtained a high degree of agreement with DXA. They concluded that this equation was suitable for children, included those with obesity. However, they compared their results with DXA, which was the same technique chosen as the criterion method for the regression and a reduced obese sample was included in their analysis to confirm its validity in this specific population.

Haroun *et al.* (137) validated an impedance index predictive equation for obese children and adolescents. The train sample consisted on 77 (n males = 30) obese participants aged 5 to 22 years, and the test sample consisted in 17 obese children (n males = 5). They compared TANITA's body composition outputs to 3C model measurements and found that TANITA manufacturer's equations overestimated FFM, and thus underestimated FM. Then, they derived a predictive equation adjusted to impedance index which did not showed significant bias neither in FM nor FFM. Furthermore, they tested their equation in the follow-up measurements

and results did not show significant bias in changes when compared to 3C model body composition assessment. Thus, they concluded that their equation was reliable for longitudinal assessment in white obese children.

Recently, Seo *et al.* (160) have published a validation study of BIA against DXA and compared the agreement between both techniques depending on the degree of obesity. They included 316 obese participants from Korea aged 6 to 17 years (n males = 187) and classified them in two groups depending on the degree of obesity: mild to moderate obesity (n = 215) and severe obesity (n = 101). They found that both techniques have a good agreement and that this agreement was better in the group of severe obesity. Although BIA slightly overestimate %FM, there did not found statistically significant differences. Instead, based on the literature findings, DXA has some limitations when assessing obese subjects, both in adults (161,162) and children (163). Hence, the strength of the study 3 is that the agreement test has been performed comparing predictions made with the new equation against the gold standard method of body composition assessment, the 4C model.

Thus, our study created and validated a predictive equation in obese population to predict FFM using impedance measurement from TANITA's output. Our body composition results, compared to the gold standard method *in vivo* (4C), demonstrate the potential of BIA to predict body composition when population-specific equations are applied, explaining nearly 90% of the FFM variability. Our predictive equation presented higher agreement with the gold standard (4C) than TANITA's results and biases where clearly reduced.

To our knowledge, the present study is the first to deliver a valid predictive equation from BIA against FFM adjusted to density of the FFM in obese children and compared to the 4C model, which might be considered a strength of this work.

Relevance and Applicability of this research

The accurate determination of body composition usually requires expensive equipment, complex and time-consuming methods and even well trained technicians (e.g., DXA, MRI, ADP, UW, DD, etc.). These circumstances hinder the use of accurate methods to evaluate body composition in clinical practice and barely limit its use to research. Hence, clinicians use simpler and cheaper methods to evaluate body composition even though the precision is compromised as BMI and skinfolds thicknesses. BMI is widely used as an adiposity indicator for both children and adults and it correlates well with both fat and fat-free mass but cannot differentiate these body components. Thus, BMI is a weak predictor of relative body fat and has clear limitations when assessing body composition (44).

Some authors have proposed DXA as a criterion method to assess body composition (137,164,165). Despite DXA has been demonstrated to be a safe and accurate method to assess body composition, it is worth to be cautious when using DXA as a reference method in paediatric studies (166) and, in addition, DXA presents some limitations when assessing both children and adult obese patients (161,163,167) and longitudinal body composition changes (168). The main disadvantages of DXA are that DXA include a small amount of radiation and this limits its reproducibility in a short time for an individual, the equipment is very expensive and needs a large space to storage the device and requires a trained specialist technician to perform the test. Thus, DXA is barely limited to research studies and diagnosis related to bones health.

Although there was a lack of evidence about ADP accuracy in obese children and adolescents, some researchers had recently used ADP as the criterion method to validate BIA and other body composition measurements in children (169,170). ADP, performed by a BOD POD device, is an accurate, reliable and precise method (171)

and it can evaluate subjects with a relative big volume, which reduces biases caused by individual size. Despite this improvement, ADP shows some biases due to methodological precision but also due to biological variability (138) since ADP assumes constant density of the FM and the FFM (172). This assumption has been corrected in the present study using the predictive equation of density of the FFM previously reported by our group to calculate density of the FFM derived from BOD POD body volume measurement. Thus, the biases due to biological variability were reduced when calculating FM_{ADP} and FFM_{ADP}. However, BV obtained from BOD POD is used to calculate density of the FFM to adjust ADP body composition results and it is also included in the 4C model equation, which is compared to. This fact might be influencing the correlation between both equations and it could be considered as a limitation of this work.

The ADP is a quick, non-invasive, suitable for a wide range of populations and does not need expert technicians to perform the test. However, the BOD POD device is expensive, needs to be placed in a room with specific conditions of size and environment and it is only available in a few research facilities.

Hence, BIA has been proposed as a feasible method to assess body composition in children, considering our findings, when other techniques are not available for both research and clinical practice as described above.

However, although there is evidence of the adequacy of this technique, manufacturer's software from the devices, as well as other 2C model-based techniques, still relying on assumptions of the body composition properties, mainly FFM properties which influence accuracy and precision of body composition measurements in obese populations, as study 1 of the present thesis have shown. These findings have been applied on study 2 and 3 and showed consistent results to

those found in the first study, leading us to think that the assumption of constant properties of the FFM was the cause of biases in body composition analysis.

Finally, this thesis confirmed the three hypotheses raised with three robust studies, each providing substantial evidences of the body composition assessment in obese children, which confirm previous reports and highlights new approaches to body composition assessment.

Future research

The results of this thesis showed that avoiding the assumption of constant values for FFM properties it is possible to improve the precision of the body composition assessment in obese children using 2C model-based techniques like ADP and BIA.

In addition, we provided the technique to obtain the improved results suitable for both research and clinical practice in a cross-sectional analysis. This will be applicable for diagnosing obesity and the degree of obesity in children according to the definition of obesity (excess of fat) instead of BMI, which cannot differentiate whether the increases or the decreased BMI value is due to fat or lean mass.

Thereafter, the next step of this work will be to test this new approach in longitudinal assessments in order to verify whether this technique is suitable and precise to monitor body composition changes over time. This will be beneficial in clinics to monitoring obesity treatment either nutritional or surgical approaches. Likewise, this will be useful for monitoring other diseases progress which undergo with weight loss and weight gain due to the disease itself or heavy treatments in which body composition is compromised.

8. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

CONCLUSIONS BY STUDIES

STUDY 1

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.

This study suggests 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 highlight that reference data for FFM properties is needed to improve accuracy of body composition measurements in obese children, adolescents and young adults.

STUDY 2

The use of constant values for fat-free mass properties may increase bias when assessing body composition in obese children by two-component based techniques like air-displacement plethysmography.

Using specific calculations for the density of the fat-free mass reduces the bias in fat mass and fat-free mass measurements when compared to the reference method.

Specific equations for the density of the fat-free mass calculations are recommended to be included in the software of air-displacement plethysmography devices (BOD POD) to improve the accuracy of the measurements in individuals that deviate from the reference population as obese children. However, further studies are needed to consolidate these findings.

STUDY 3

The new predictive equation to assess body composition in obese children by using bioelectrical impedance analysis showed high precision.

The new predictive equation makes bioelectrical impedance suitable for body composition analysis both in research and clinics, due to the easiness and quickness of its calculation and no expensive equipment nor exhaustive training need.

Nonetheless, further studies are needed to evaluate the accuracy of the predictive equation in longitudinal studies and clinics follow-up to assess body composition changes in the short-to-mid-term.

OVERALL CONCLUSIONS

- The fat-free mass properties, hydration and density, are different between obese and non-obese subjects.

- The age-related changes on hydration and density of the fat-free mass are modulated by the nutritional status.

- Nutritional status should be considered when assessing body composition by two-component based techniques.

- The new predictive equation of the density of the fat-free mass is applicable in obese children.

- Density of the fat-free mass adjustments in body composition measurements improve the accuracy and the precision of the two-component based techniques, as air-displacement plethysmography, in obese children.

- The use of a new predictive equation for body composition assessment in obese children improve the accuracy and the precision of bioelectrical impedance analysis with the TANITA 418-bc device.

- The new predictive equation of the fat-free mass derived from impedance index is applicable in a population of obese children for research studies and clinical practice.

- New reference data for FFM properties is needed to improve accuracy of body composition measurements in obese paediatric patients.

9. FUNDING & CONFLICT OF INTERESTS

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FUNDING AND CONFLICT OF INTERESTS

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This thesis has nothing to disclose and declares no conflict of interests.

10. REFERENCES

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11. ADDENDUMS

UNIVERSITAT ROVIRA I VIRGILI
BODY COMPOSITION ASSESSMENT IN PAEDIATRIC PATIENTS. VALIDATION OF NEW METHODS OF BODY COMPOSITION MEASUREMENTS IN OBESE CHILDREN
Desirée Gutiérrez Marín

ADDENDUM NUM. 1

The Obemat2.0 Study: A Clinical Trial of a Motivational Intervention for Childhood Obesity Treatment

Veronica Luque, Albert Feliu, Joaquín Escribano, Natalia Ferré, Gemma Flores, Raquel Monné, Desirée Gutiérrez-Marín, Núria Guillen, Judit Muñoz-Hernando, Marta Zaragoza-Jordana, Mariona Gispert-Llauradó, Carme Rubio-Torrents, Mercè Núñez-Roig, Mireia Alcázar, Raimon Ferré, Josep M. Basora, Pablo Hsu, Clara Alegret-Basora, Francesc Arasa, Michelle Venables, Priya Singh and Ricardo Closa-Monasterolo

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Addendum 1

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The Obemat2.0 Study: A Clinical Trial of a Motivational Intervention for Childhood Obesity Treatment

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Abstract: The primary aim of the Obemat2.0 trial was to evaluate the efficacy of a multicomponent motivational program for the treatment of childhood obesity, coordinated between primary care and hospital specialized services, compared to the usual intervention performed in primary care. This was a cluster randomized clinical trial conducted in Spain, with two intervention arms: motivational intervention group vs. usual care group (as control), including 167 participants in each. The motivational intervention consisted of motivational interviewing, educational materials, use of an eHealth physical activity monitor and three group-based sessions. The primary outcome was body mass index (BMI) z score increments before and after the 12 (+3) months of intervention. Secondary outcomes (pre-post intervention) were: adherence to treatment, waist circumference (cm), fat mass index (z score), fat free mass index (z score), total body water (kg), bone mineral density (z score), blood lipids profile, glucose metabolism, and psychosocial problems. Other assessments (pre and post-intervention) were: sociodemographic information, physical activity, sedentary activity, neuropsychological testing, perception of body image, quality of the diet, food frequency consumption and foods available at home. The results of this clinical trial could open a window of opportunity to support professionals at the primary care to treat childhood obesity. The clinicaltrials.gov identifier was NCT02889406.

Keywords: motivational interview; childhood obesity; obesity treatment; primary care

1. Introduction

Childhood obesity treatment is challenging and not always cost-effective. Several interventions and programs to treat childhood obesity have been tested, but recent reviews showed a highly variable efficacy, with slight reductions in body mass index (BMI) [1,2].

Generally, treatment consists of promoting changes in lifestyle habits related to diet and physical activity. Although there exist strategies focusing only on physical activity or diet, multicomponent interventions consisting of dietary modification, physical activity, behavioral therapy, and education have shown to improve body mass index (BMI), blood pressure, and lipids profile [3,4]. A Cochrane review published by Mead and collaborators in 2017, reported that multi-component behavior-changing interventions considering diet and physical activity could be effective in achieving small, short-term reductions in BMI in children aged 6 to 11 years [2].

In a study in which health professionals were interviewed to identify the main barriers for effectiveness of the treatment, they considered that the lack of motivation of the adolescent patients as well as the lack of family engagement were the main reasons to not achieve the success of the intervention [5]. In this regard, a review by Poobalan in 2010 concluded that the most significant weight reductions were obtained by combining diet and exercise, but also motivational strategies [6].

The motivational interview has been used for achieving behavioral changes in different pathologies [7]. Miller defined it as direct assistance with a strong psychological basis and patient-focus; the aim should be to cause a permanent change in behavior, helping to resolve conflicting feelings about the same thing [8]. Motivation was defined as "brain processes that energize and direct behaviour" [9,10]. In an observational prospective study published by Feliu et al. in 2013 [11], a motivational therapy was highly effective in treating adolescent obesity, achieving a BMI z score reduction of 0.5 standard deviations [11].

There is scientific evidence from motivational strategies other than the motivational interview itself. For example, engaging obese children in group-based sessions may enhance continued adherence and cohesion to a lifestyle intervention program [12]. On the other hand, other motivational methods as the use of eHealth devices to self-monitor physical activity is currently under study. The use of wearable sensor monitors to increase physical activity has been tested in youth, delivering some preliminary data to suggest that these devices may offer the potential to increase activity levels [13,14], however evidence in children is still scarce [15].

Obesity treatment interventions in childhood and adolescence have been carried out in a wide range of settings, community-based, school-based, primary care, specialized in clinical settings interventions or internet-based programs, with varying results. In 2014, Kothandan concluded that interventions at family level were more effective in reducing weight, body mass index (BMI) and waist circumference than those made only by addressing the child or adolescent in the school environment [16]. Recently, the American Academy of Pediatrics outlined several stages of the treatment, from which the first two included the primary care support [17]. According to these statements, primary care providers should offer motivational interviewing to achieve healthy lifestyle modifications in family behaviors or environments, and children requiring the next level of obesity treatment, would benefit from receiving a "structured weight management", receiving additional support beyond the primary care provider (such as a dietitian, physical therapist, or mental health counsellor) [17].

The Obemat2.0 trial (clinicaltrials.gov identifier NCT02889406) was designed and conducted to implement and to test the efficacy of a structured multicomponent motivational therapy, coordinated between hospital dieticians and primary care providers to treat childhood obesity.

2. Study Hypothesis

The introduction of a family orientated multicomponent motivational intervention, with a coordinated approach between reference units specialized in clinics and primary care services from the same health region can be an effective tool for the treatment of childhood obesity.

2.1. Objectives

2.1.1. Primary Objective

To evaluate the efficacy of a multicomponent motivational intervention for the treatment of childhood obesity (change in BMI z score), coordinated between primary care and hospital specialized services, integrating motivational individual interviews, educational groups and eHealth tools (wearable), compared to the usual intervention performed in pediatrics.

2.1.2. Specific Objectives

To evaluate the efficacy of a multicomponent motivational intervention compared to usual intervention performed in regular pediatrics practice on

- Reducing BMI z score,
- improving metabolic control (insulin resistance and lipids profile),
- improving body composition (fat mass and lean mass z scores),
- increasing physical activity,
- acquiring a healthy eating pattern,
- reducing psychosocial problems,
- increasing the adherence to the obesity treatment program in children with obesity between 8 and 13 years old.

Secondary to the intervention clinical trial, as observational analyses, this study proposed the following secondary objectives:

- To assess the precision of body composition techniques (air-displacement plethysmography, dual X-ray absorptiometry, and biological impedance) to detect changes in body composition over time in children with obesity and to validate its use against the four component model
- To assess vascular function, by measuring the intima media thickness and estimating blood vessels properties (distension and rigidity), in relation to the obesity degree and metabolic profile
- To assess the respiratory function, and the association between the obesity degree and the degree of bronchial obstruction.

3. Materials and Methods

3.1. Study Design and Study Setting

Randomized clustered clinical trial, with a treatment on children with obesity lasting 12 (+3) months, with two arms: a control group following the usual recommendations in primary care and an intervention group receiving a structured motivation-based interview supported by educational materials, combined with group therapy and eHealth. The investigators registered the trial at clinicaltrials.gov as NCT03749200.

3.2. Context and Time Frame of the Study

The project, conducted in Spain, started in January 2016. Up to March 2016, we designed the structure of the intervention and conducted the first round of recruitment of pediatricians and pediatric nurses at primary care centers. In March 2016, pediatricians and pediatric nurses willing to participate in the study as therapists were trained. The recruitment of children with obesity started in June 2016 and ended in March 2018. The baseline and final assessment of participants were conducted at the reference hospitals of the area—Hospital Universitari de Tarragona Joan XXIII and Hospital Universitari Sant Joan de Reus—and the treatment of the obese participants took place in the primary care centers of the Tarragona region, all of that between June 2016 and June 2019.

3.3. Sample Size

We considered the number of individuals of a simple random design, multiplied by the design effect to calculate the sample size of each group. Assuming a standard deviation of 0.75 and a 30% lost to follow up, 98 children with obesity were required in each group to detect a difference $\geq$ 0.36 units of BMI z score (with and alpha risk = 0.05 and beta risk = 0.2 in a bilateral contrast) [18]. Calculations were performed using GRANMO v7.12 software [19]. Effect estimates of intracluster correlation coefficient in cluster randomized trials in primary care were generally lower than 0.05 [20]. The effect of the design corresponded to 1.7. Assuming these values, the final calculated sample size was 167 participants in each group (n = 334 overall).

4. Recruitment and Allocation

4.1. Randomization of Therapists

The investigators considered to randomize each child for allocation either in the intervention or control groups. However, children had a pediatrician and a nurse already assigned for regular health control. If all the pediatricians and nurses were responsible to perform motivational and control interventions, there was a high risk of contamination of the control intervention by motivational techniques. Therefore, to ensure no interference between the control and the intervention groups, and to avoid differences by socio-economical levels according to the location of centers, we performed randomization by clusters, where each primary care center was a cluster. In each primary care center, pairs of pediatricians and pediatric nurses worked as a team and were called a basic care unit (BCU), and this was considered as the unit of randomization. So, each team of pediatrician and nurse was randomized to be part of either the intervention or the control groups. Eight primary care centers were recruited in the first round (February 2016) which included 62 pediatricians and pediatric nurses. This means that the primary care centers had half of the BCU as control therapists and the other half as intervention therapists, when possible. In a second round, in January 2017, 7 additional primary care centers and their 20 pediatrician–pediatric nurse pairs were recruited and randomized by clusters to participate as a control or intervention BCU. Finally, there were a total of 82 pediatricians and pediatric nurses from 15 centers, distributed in 44 BCU taking part in the study as therapists (21 in the control group and 23 in the intervention group). Given the nature of the intervention, participants or researchers could not be blinded. Randomization was performed 1:1 with the EPIDAT 3.0 statistical Program [21].

4.2. Training of Therapists

All of the pediatricians and pediatric nurses received a 4-h training course during which they received information on the rationale and design of the project, good clinical practices and methods to assess the obese participants.

After this, only the intervention group therapists followed with the training on the Obemat2.0 motivational intervention, which consisted on 12 h of additional training (two sessions of six hours).

The first of these two dedicated sessions consisted of: 1h for theory of the motivational approach, the basis to apply it to childhood obesity treatment, and how to structure the interview (Figure 1). After this, the training consisted of a detailed explanation of the activities to be carried out at each visit during the motivation-based interviews with the patients (Table 1). This session was dedicated to the motivational interview ending with a role-play activity in which therapists acted as such, or as parents or as children with obesity during a motivational interview.

The last of the training sessions focused on the three workshops in which the children in the intervention group would take part: (1) Motivation to increase physical activity, (2) labeling of food products and recommended portions, and (3) cooking healthy recipes.

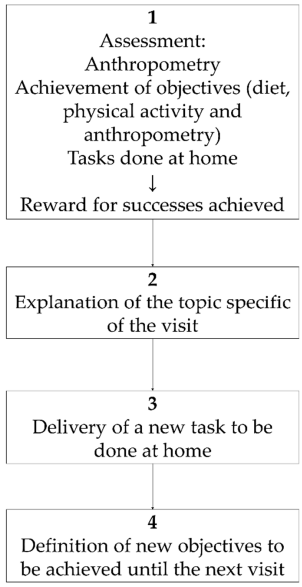

Figure 1. Obemat2.0 motivational interview structure within each visit.

5. Participants

Inclusion criteria: Age range between 8 and 13 years at enrolment and a BMI > 97th percentile of the Hernandez references from 1988 [22] as indicated by the Guidelines for Clinical Practice on the Prevention and Treatment of Childhood and Adolescent Obesity of the Spanish Health System [23] for childhood obesity diagnosis.

Exclusion criteria: Children with eating disorders, families not available to attend scheduled visits, simultaneous participation in another clinical trial, presence of endocrine disorders (growth hormone disorder, hypothyroidism, Cushing's disease, precocious puberty or other) and lack of command of local languages.

6. Recruitment and Follow-Up of Participants

The primary care centers of the study region are organized in BCUs, so that each BCU provide public health care to an assigned part of the population of the area. Therefore, pediatricians and/or pediatric nurses recruited children during their regular clinical practise at primary health centers, and the children with obesity enrolled in the study belonged to the control or intervention group depending on the BCU to which they belonged.

After checking the eligibility criteria, therapists gave written information to the parents or caregivers and adapted information to the children (if they were 12 years or older). When they agreed to take part, they gave the signed consent (parents) and assent (children aged 12 years or older) and were enrolled in the study. Baseline assessment took place at the reference Hospitals (Hospital Universitari de Tarragona Joan XXIII and Hospital Universitari Sant Joan de Reus) and included anthropometry, blood sample analyses, measures of body composition (i.e., fat mass, fat free mass, total body water, bone mineral content) using appropriate techniques as dual-energy X-ray absorptiometry (DXA), air displacement plethysmography (Bod Pod®), bioelectrical impedance, deuterium dilution (in a subsample), and blood pressure. A sociodemographic questionnaire, medical history, index of diet quality (KIDMED) [24], and a food frequency questionnaire [25] were completed during the interview. Furthermore, a physical activity questionnaire (PAQ-C) [26], sedentary activity

questionnaire (ASAQ) [27], a neuropsychological test, a perception of body image test, and a form designed to record all foods present at home were given to the patient and his/her family to be completed at home and returned at the next visit. Furthermore, the intervention group completed a short motivation test (Figure 2).

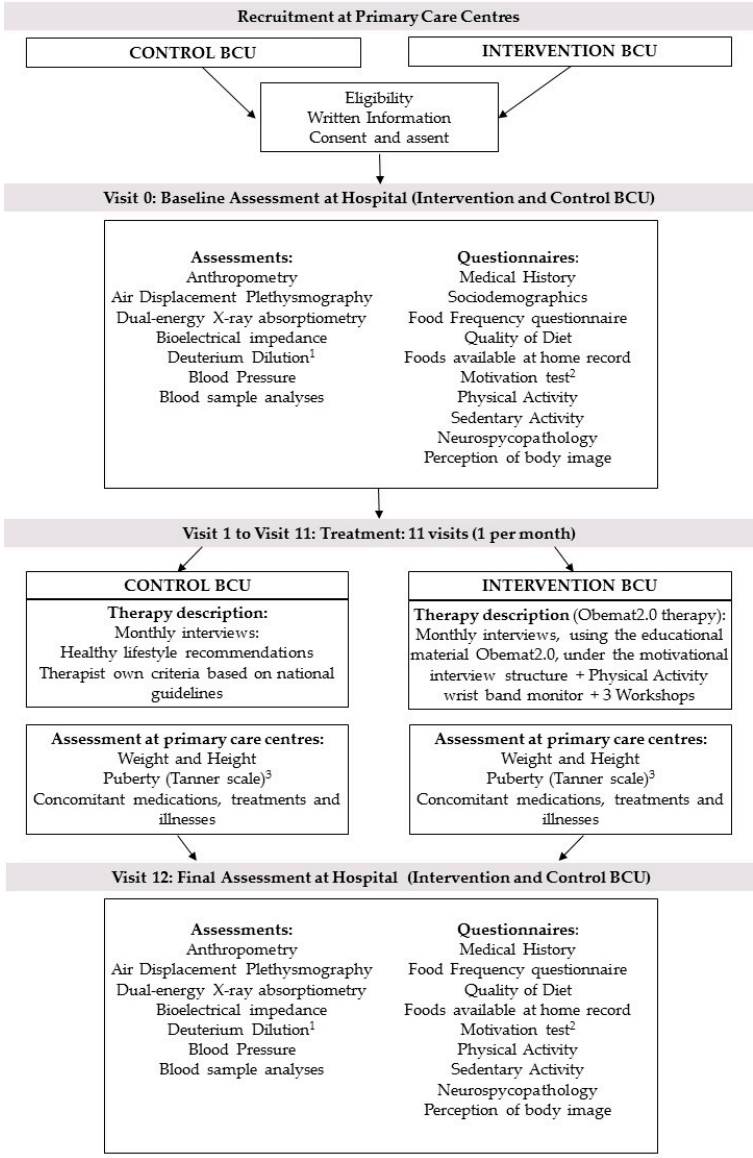

Figure 2. Flow diagram of recruitment, assessment per visit, and intervention. BCU: Basic Care Units; [1] Deuterium dilution analysis in a subsample; [2] Motivation test only in the intervention group; [3] anthropometry at primary care centers was performed on a monthly basis, except for the puberty development, that was assessed only at visit 1 and 11.

After the baseline assessment at the reference hospital, the participants started the treatment which was conducted by their regular pediatrician and/ or pediatric nurse at the primary care center, where they were visited monthly (11 visits in total). At all visits, children were weighed and height was measured. In addition, at visits 1 and 11, the pediatrician assessed pubertal development status (according to the Tanner Pubertal stages [28,29]), whether they showed acanthosis nigricans and/or there were any pathological result emerging from blood sample analyses.

After visit 11 was performed, children were again assessed at the reference hospital (visit 12) as done at the baseline visit (visit 0) (Figure 2).

The children nor their families in the intervention or the control groups received any incentive nor benefit or payment because of their participation in the study. On the other side, participants did not have to pay for the treatment or visits.

7. Protocol Variation

Although the overall participation of the children with obesity and their families was planned to last 12 months, delays (considered as normal within the clinical practise) lead to a variation of the protocol. Reasons for delay included changes in appointments for children's exams, family vacations or family problems, or delays caused by professionals' illnesses or vacations. With this protocol variation, children's follow-ups and final assessments could take place during up to 15 months.

8. Description of the Interventions

The treatment of both groups (control and intervention) took place in the primary care services by their usual pediatrician and/or pediatric nurse, along with the 11 monthly visits, each visit lasted approximately 20 min.

8.1. Usual Care (Control Group)

Children assigned to the control group received advice as recommended by the Guidelines for Clinical Practice on the Prevention and Treatment of Childhood and Adolescent Obesity of the Spanish Health System [23]. At each visit, the family received explanations about carrying out a balanced diet, divided into five meals, in order to provide a moderate energy intake reduction (assessed qualitatively, recommending exemption of energy-dense superfluous foods, restricting eating out of meals and reducing the size of the portions, if this was necessary). Specific dietary recommendations were four to five portions of fruits and vegetables per day, increasing wholemeal cereal products, avoidance of sugared beverages, cakes and pastries, junk food, fried food products, energy dense dairy desserts, and oil-based sauces. The pediatricians and/or nurses recommended preparing the same meals for the whole family and limiting the access of the child to energy dense food products. Therapists recommended an increase in physical activity, both in terms of leisure activity, and regular sports engagement. At monthly visits, therapists also measured weight and height, and assessed whether the family followed the recommendations. The pediatricians and/or nurses in the control group did not receive specific instruction to promote family tasks such as recoding food intake or physical activity; however, the therapists asked about these topics during the interviews in a non-structured way with the objective to reinforce compliance with recommendations.

8.2. Obemat2.0 Intervention

The intervention group followed the motivational interviewing schema described in Figure 1 during the 11 visits, took part in three workshops and participants got a wristband physical activity monitor (Figure 2). To guarantee the follow up of the motivation interview schema (Figure 1), printed material was designed and provided to the intervention BCU. That printed educational material was used with each child at every visit. It contained a section to ensure the revision of goals achievement, a section to explain the specific topic for each visit, a document to execute the task to be done at home

and a last section where the agreed objectives to be accomplished until the next visit were recorded. Table 1 shows the details of topics and tasks planned at each single visit.

Furthermore, during the first four to six months, children in the intervention group could participate in workshops where therapists taught and/or trained and tried to motivate them on three different topics:

Workshop 1: Increasing physical activity by using an eHealth monitor (Fitbit®).

During the session, the children received a wrist physical activity monitor (Fitbit®), were taught about using it and were motivated to increase the physical activity records by doing leisure time family activities, walking, playing outdoors, etc. The monitor, connected to an App, allowed several features to fieldworkers. On one hand, children attending the workshop (maximum eight children per session) could be part of a Fitbit App® group, so they were able to compete between them to increase their number of daily steps. Moreover, on the other hand, the fieldworker was able to download the time wearing the device and the daily steps of all participants to assess objectively their adherence to the treatment and the actual physical activity level. Each child in the intervention group received the wristband monitor by free.

Workshop 2: Food choices and balance.

The workshop had two different parts: at the beginning, children and their families received training to read labels of food products in order to discard energy dense food products.

During the second part, children and their families were trained to design a balanced diet and reduce portion sizes with the help of educational materials as plates and food models (from Portion Perfection (Great Ideas in Nutrition©, Tweed Heads, NSW, Australia).

Workshop 3: Using healthy cooking methods.

During the third workshop, children took part in a cooking session when they participated preparing healthy recipes.

In addition, at the end of the session, families received printed recommendations on preparing healthy foods, using low energy cooking methods.

The reason to conduct the workshops (when possible) during the first four to six months of treatment was preventing the loss of interest that could be produced after several months of participation in a childhood obesity program. In other words, the aim of doing the workshops during the first six months was contributing to the child's motivation.

Online Supplementary Table S1 provides the Template for Intervention Description and Replication checklist.

Table 1. Obemat2.0 intervention scheme.

Visit	Topic to Be Discussed	Task Proposed to Be Done
V1	General concept of obesity. Acknowledgement of the problem and self-willingness to change	The patient should complete a list with the pros and cons of following the treatment against obesity
V2	Recommendations for food shopping.	Parents should sign a compromise to follow the food shopping list recommendations
	Workshop 1: Strategies to increase physical activity by using an eHealth monitor	
V3	Dietetic balance Healthy Menu	To design a menu for the whole week which follows the healthy balance and is adapted to the family preferences
	Workshop 2: Food products labeling and recommended food portions	
V4	To explore daily physical activities that could be increased (such us walking to school, taking care of chores such as walking the dog)	To make a list of activities that the child could do to reduce sedentary behavior
	Workshop 3: Cooking methods. Workshop at the kitchen.	
V5	What can I do if I have "anxiety"? What to do, what kind of healthy snacks could I have?	To make a list of lifestyle behaviors the patient realize is doing properly and those that should be improved to treat his/her obesity
V6	Habits around the table: family meals at regular times without TV nor screens, avoiding conflicts about food during mealtime, table without any food not assigned to any member of the family (i.e., full piece of bread in the middle of the table, excess of cooked food available)	To set the rules and record the order and schedules of the family around meals
V7	Breakfast and mid-afternoon snacks	To keep a 7 day record of all breakfasts and mid-afternoon snacks eaten (the week prior to the next visit)
V8	Recommended portion sizes Preparing foods to avoid leftovers	To record during the next month how many days there is an excess of prepared food
V9	Different types of physical activity: sport, daily activities, daily displacements, family outdoors activities	To plan family physical activities such as biking, hiking, promenades, etc.
V10	To revise the recommended daily or weekly portions of the different food groups (fruits and vegetables 4–5 per day, pulses 2–3 per week, etc.). Distribution and balance in lunch and dinner within the same day	To do a 7 day food diary during the week prior to the next visit
V11	Food shopping: coming back to "avoiding the negative stimulus" and planning the shopping list Strategies to avoid the access to energy dense foods	Plan the family menu for the next week and plan the shopping list avoiding unnecessary energy dense foods

9. Outcome Measures

Children from both study groups were invited to take part in the same assessments at baseline and the end of the intervention (visits 0 and 12, except for vascular and the respiratory function tests, which were only assessed at the end) at one of the two reference Hospitals (but by the same research team). Between the two main visits, weight and height were recorded monthly, and other medical history aspects (described below) at visit 1 and visit 11 at the primary care centers as well.

9.1. Primary Outcome

The primary outcome was change in BMI z score between visit 0 and visit 12.

9.2. Secondary Outcomes

Secondary outcomes were changes in body composition (fat mass index, fat free mass index), systolic and diastolic blood pressure z scores, triglycerides mg/dL, HOMA-IR index and low density lipoproteins (mg/dL) between visits 0 and 12 (See the extended list of secondary outcomes in Table 2).

Table 2. Scheme of data collection per visit.

Parameter/Test	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Medical history: Birth characteristics, feeding in early life	*												
Sociodemographic questionnaire	*												
Medical examination: Tanner maturation stage, Acantosis Nigricans, systolic and diastolic Blood Pressure z score	*	*										*	*
Anthropometry: weight, height, body mass index z score	*	*	*	*	*	*	*	*	*	*	*	*	*
Anthropometry: waist circumference	*												*
Body composition: Bone mineral content and density, Fat Mass, Fat Free Mass, Total Body Water (all standardized as z scores for age and gender)	*												*
Blood sampling: HOMA-IR, Lipid metabolism, Liver enzymes and Thyroid Hormones	*												*
Neuropsychology, behaviour, and self-perception: BRIEF score, SDQ score, perception of body figure	*												*
Diet: Food Frequency Questionnaire, Quality of Mediterranean Diet in children (Kidmed) and pantry	*												*
Physical and Sedentary Activity questionnaires, daily steps [1]	*												*
Motivational interview [1]	*												*
Adherence to treatment: attendance to visits, attendance to workshops [1], use of the eHealth monitor [1]		*	*	*	*	*	*	*	*	*	*	*	*
Vascular function: Intima media thickness and vessels properties (ultrasound scan)													*
Respiratory Function (Easy Breathing Survey, forced spirometry and bronchodilator test)													*

[1] Only in the intervention group.

10. Outcomes and Variables

The Table 2 shows a summary of tests and measurements performed per visit.

10.1. Anthropometry

Trained study personnel measured weight (kg), height (m), and waist circumference (cm) at baseline and final visits using a SECA769 scale (precision 50 g), SECA 216 Stadiometer (precision 1 mm) and Holtain waist circumference non-extensible tape (precision 1 mm). In addition, pediatricians or pediatric nurses at primary health care centers measured weight and height at monthly visits. Waist circumference was measured at three different levels: at the iliac crest, at the mid-point between the iliac crest and the lower rib, and encompassing the maximum circumference. body mass index (BMI)

(kg/m^2) was calculated as $BMI\ (kg/m^2) = \frac{weight\ [kg]}{height\ (m)^2}$, and the z score of the BMI (BMI z score) for age and gender according to the World Health Organization (WHO) references [30] was calculated using the WHO software [31].

10.2. Body Composition

Body composition was assessed by means of several methods to obtain the gold standard measures of each component of the named "four components model" [32] at the beginning and the end of the treatment, always in fasted conditions.

Dual-energy X-ray absorptiometry (DXA): Bone mineral content (g) and density (g/cm^3) were obtained by means of Axial Lunar Prodigy Full Advance device and the Software EnCore 2014 v15.20.002 (GE Lunar Corporation, Madison, WI, USA). The patient lay in a supine position, in underwear without any metallic object, and the toes of the two feet set one beside the other. Output variables were bone mineral content (g), bone mineral density (g/cm^3), fat mass (kg) and fat free mass (kg) for the whole body, trunk and limbs.

Air displacement plethysmography (Bod Pod®): the child was assessed in duplicate by Bodpod instrumentation (Life Measurements, Concord, CA, USA) wearing tight-fitting underclothes or swimsuit and a swimming cap to discard the air existing between hairs. Results were the mean of two or three measures of body volume of 50 s lasting each one. If the two first measures were consistent (differences between measures <150 mL of body volume), a third measure was not necessary. Thoracic gas volume was predicted by the BodPod during normal tidal breathing [33] and subtracted from total body volume in subsequent calculations.

Based on the principles of densitometry, body density was calculated by following the general equation of densitometry

$$Body\ Density\ (\frac{kg}{L}) = \frac{Body\ Weight\ (kg)}{Body\ Volum\ (L)}$$

and then this body density was used to predict *Fat Mass* (%) from Lohman's equation [34]:

$$Fat\ Mass\ (\%) = \frac{C1}{Body\ Density - C2} \times 100$$

where *C1* and *C2* are constants based on age and gender.

Fat Mass was converted to kg

$$Fat\ Mass\ (kg) = \frac{Fat\ Mass\ (\%) \times 100}{Body\ weight\ (kg)}$$

Then, *Fat Free Mass* (kg) was obtained from subtracting *Fat Mass* (kg) from total *Body Weight* (kg).

$$Fat\ Free\ Mass\ (kg) = Body\ weight\ (kg) - \ Fat\ Mass\ (kg)$$

Deuterium dilution: deuterium dilution was used in a subsample ($n = 75$) as the gold standard measure for total body water (kg) determination [35]. This assessment was not performed in the overall sample because of budget constraints. Each subject provided a baseline urine sample prior to receiving a weighed oral dose of ^2H_2O. The dose was the equivalent of 70 $mg \cdot kg^{-1}$ body weight ^2H_2O. Parents got sample collection forms, written instructions (that trained study personnel delivered and explained) and the necessary material for urine collection. Instructions stated to collect samples of the child's urine for the following five days after dosing, at a similar time of day provided it was not the first void of the day, and to preserve them refrigerated. Urine samples were kept frozen until being shipped frozen to MRC Elsie Widdowson Laboratory, Cambridge, UK. For ^2H enrichment, samples of 0.4 mL were placed in 3.7 mL glass vials and flush-filled with hydrogen gas, and then equilibrated for six hours in the presence of a platinum catalyst. The headspace of the samples were then analyzed

using a continuous flow IRMS (Sercon ABCA-Hydra 20-22, Sercon Ltd, Crewe, UK). All measurements were made relative to V-SMOW (Vienna Standard Mean Ocean Water) using calibrated laboratory standards. Analytical precisions (SD) are better than ± 1.3 ppm for ^{2}H. TBW was calculated using the zero-time intercept of ^{2}H turnover and corrected for non-aqueous exchange within the body. Hydration factors [36] were used to derive fat free mass (FFM) (kg). Fat mass (FM) (kg) was calculated as the difference between body weight and FFM.

Bioimpedance: Total bioelectrical impedance (BIA) with a high-frequency constant current (50 KHz, 500 μA) was assessed in duplicate with the 8-electrode BIA using the Tanita BC-418 (Tanita corporation, Tokyo, Japan). The child stepped on the scale barefoot and dressed in underwear, and instructed to find the right position to get in contact with the foot electrodes. The palms of the hands were on top of the device handles, with the fingers touching the lower electrodes and the thumbs placed straight along the electrode at the top. Outputs were impedance (Ω), total body water (TBW) (kg), FFM (kg), and FM (kg) for the whole body and the segments (trunk and limbs).

As body composition changes with age as part of human development, all body composition variables were standardized as z scores according to age and gender [37].

Blood sampling: at baseline and final visits, a trained nurse extracted a blood sample from the child's in fasting conditions. Glucose (mg/dL), insulin (mIU), low density lipoproteins cholesterol (LDL) (mg/dL), high density lipoproteins cholesterol (HDL) (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), liver enzymes (Alanine trasnsaminase, Gamma-Glutamyl Transferase, Aspartate transaminase), thyroid hormone (TSH), blood cell count, iron, ferritin, transferrin, creatinine, urea, C-reactive protein, and vitamin D (mg/dL). Insulin resistance index (HOMA-IR) was calculated as

$$HOMA - IR = \frac{Insulin\left[\frac{\text{uU}}{\text{mL}}\right] \times Glucose\,[\text{mmol/l}]}{22.5}$$

Medical history: health records were consulted to obtain birth characteristics (delivery type, gestational age and birth weight, length and head circumference) and feeding type in early life (duration of breastfeeding and complementary feeding introduction).

Sociodemographic questionnaire: at the baseline interview, the study personnel performed the sociodemographic questionnaire including information on gender, date of birth, household income, level of education, occupation and employment of father and mother.

Medical examination: pediatricians or nurses explored Tanner maturation stage [28,29] and presence and placement of Acantosis Nigricans at visit 1 and 11. Trained study personnel measured systolic and diastolic blood pressure (mmHg) at baseline and final visit (at least 20 min after arriving to the study center) in duplicate (with a time slot of 5 min between measures) using a Dinamap Pro 100 device on the left arm, while the child remained sat down with the arm laying comfortably.

Diet: diet was assessed qualitatively by means of a short Food Frequency Questionnaire validated in a similar population [25] and a questionnaire to assess the adherence to Mediterranean Diet in children (Kidmed) [24] completed by parents with the support of the children. Food items from the food frequency questionnaire were translated to grams using the same food portions of the validation study and the kidmed score was calculated. Finally, the participant families were asked to record all the foods available in the pantry and fridge at home at the beginning and the end of the study.

Physical and Sedentary Activity: parents with the help of their children completed validated questionnaires to assess physical activity [26] and sedentary activities [27] at the beginning and the end of the treatment.

Neuropsychology, behaviour, and self-perception: the Behavior Rating Inventory of Executive Functions (BRIEF) [38] was completed by parents to get a score on the inhibitory control, shift and emotional control. The Strengths & Difficulties questionnaire (SDQ) [39] was completed by parents as well to obtain scores for emotional symptoms, conduct problems, hyperactivity/ inattention, peer-relation problems, pro-social behavior, and "total behavior difficulties score". Parents' [40] and child's self-perception of body figure and parents' perception of the child's figure were assessed by

means of printed figures [41]. Misperception of body figure was assessed by the difference between perceived BMI and actual BMI; discomfort with body figure was assessed by the difference between perceived and desired images.

Motivational interview: the children in the intervention group (at the beginning and the end of the treatment) completed a questionnaire in which they should state several personal thoughts as: (1) the degree of willingness to lose weight, (2) the reason why they would like to lose weight, (3) which positive things they would get from becoming thinner, and (4) the negative aspects of following the dietary recommendations and physical activity advice.

10.3. Vascular Function

To measure carotid intima media thickness (cIMT), we used a My Lab 50 X-Vision sonographer (Esaote SpS, Genova, Italy) with a linear array ultrasound probe small parts broadband transducer (5-12MHz). We identified the far wall of the common carotid artery (1cm proximal to the bifurcation), the bifurcation, and the internal carotid artery pf the left and right carotid arteries. Measurements of cIMT were performed in vivo at the predefined points using QIMT© radiofrequency image processing software (Esaote SpA, Genova, Italy). We defined pathological cIMT as the 75th percentile of cIMT values in the general population banded with respect to age and sex, and plaque as a focal structure encroaching into the arterial lumen by at least 0.5 mm or 50% of the surrounding IMT value, or a thickness >1.5 mm.

Arterial stiffness expressed by the pulse wave velocity (m/s), carotid distensibility (μm), and augmentation index (%) was measured directly at both common carotid arteries using the ultrasound linear probe (5-12 MHz) as a tonometer and analyzed in vivo by the QAS© radiofrequency software (Esaote SpA, Genova, Italy). Maximum and minimum carotid diameters (μm) were acquired using the attained distension curves, and vascular stiffness parameters were calculated after calibration for blood pressure. Carotid distensibility was the change in diameter of the carotid artery secondary to intravascular volume expansion caused by the left ventricle systole. The pulse wave velocity was obtained from brachial blood pressure and the accurate measurements of diameter and change in diameter of carotid arteries. Augmentation index was measured by the pulse wave analyses and local pressure. To reduce observer variability, a single operator obtained and measured the images. Final values were the mean measurements of the right and left carotid arteries.

10.4. Respiratory Function

With the aim to assess the degree of bronchial obstruction in these children with obesity, patients and their families were asked to complete the Easy Breathing Survey (EBS) test [42]. Children with a previous diagnosis of asthma or an EBS score $\geq$1 performed a forced spirometry and bronchodilation test. The spirometer used was a Sibelmed W20S BETA® (Sibel S.A., Barcelona, Spain) and its software (511-BL0-MU1 Rev). Main outcome measures were forced vital capacity FVC (%), forced expiratory volume in one second (FEV_1) (%), its ratio ($\frac{FEV1}{FVC}$) and Forced Expiratory Flux between the 25% and 75% of the FVC ($FEF_{25\%–75\%}$). After the administration of a bronchodilator, FEV_1 increment ($FEV_1\Lambda$) was assessed [43]. All the measures were standardized as z scores according to the multi-ethnic reference values of the Global Lung Function Initiative and the European Respiratory Society [44] by means of the GLI2012® desktop software [45].

10.5. Adherence to the Intervention

To assess the adherence to the therapy, investigators recorded overall attendance to visits and attrition rates in both the intervention and control groups, as secondary outcomes. Furthermore, within the motivational program, investigators recorded the attendance to group sessions and the time using the wearable physical activity monitor (minutes/ day, days/ month) to assess the efficacy of the different parts of the intervention.

11. Conditions for Discontinuation of Participation in this Study and Follow-Up Actions

Conditions for discontinuation of participation in this study were illnesses diagnosis that could bias the study (such as neuropsychological, endocrine diseases considered as exclusion criteria) or that could affect the participant's safety, such as eating disorders (for example, a pathological behavior to lose weight).

12. Follow-Up of Adverse Events

This study consisted of the implementation of a motivational program to promote behavioral changes for a healthier lifestyle. The professionals providing the advice are registered pediatricians, nurses and registered dieticians working on pediatric clinics. There is little likelihood of any health hazards. If any serious adverse event occur, it would be reported in line with the Consort guidelines [46]. Until now, no hazards have been reported nor identified.

13. Statistical Analysis Plan

The statistical plan was testing the effect of the motivational intervention through a two-way ANOVA model to find differences in BMI z score increments (baseline to final visit) between groups (control vs. intervention); we considered adjustment by possible confounders such as gender, age diet, physical and sedentary activity, feeding in early life and nutritional status at birth.

The statistical plan also included similar models to test the secondary outcomes such increments (baseline to final visit) in systolic blood pressure, diastolic blood pressure, FM and FFM from a four-component model, triglycerides, LDL, HOMA-IR, and psychosocial problems. Specific models to assess the factors to adhere to the treatment were planned as well. These models had as dependent variables attendance (number) to interviews, and included the intervention vs. control group as a main factor; parents' BMI, parents' education level, baseline perception of body image; gender and age were possible modulators. The statistical plan was included as well the analysis of the extent in which the different motivational tools could influence BMI z score, by using as independent variables the attendance to individual interviews, to group sessions and the use of the physical activity monitor.

14. Ethics

The study followed the rules of the Declaration of Helsinki [47]. The ethical committees holding the activity of all the involved study centers approved the protocol: CEIC Hospital Universitari de Tarragona Joan XXIII (2 March 2016, code CP.OBEMAT2.0-C.I.01p/2016), CEIC Hospital Universitari Sant Joan de Reus (29 January 2016, code 16-01-28/1ass2), CEIC IDIAP Jordi Gol (26 November 2015, code PI14/116). If any amendments of the protocol were made, the Ethics Committees were notified as necessary. All parents or legal guardians signed informed consent prior to study enrollment. Children aged 12 years or above signed informed assent to participate in the study as well.

15. Study Status

The recruitment started (first patient-first visit) in June 2016. The fieldwork is expected to end in June 2019.

16. Discussion

The study will assess whether a multicomponent motivational program, including a bundle of motivational strategies (such as motivational interview supported by educational materials, a wearable physical activity monitor and group sessions) conducted in primary centers by therapists with 12 h of specific training could be more effective than usual care.

The motivational interview relies on the basis that the child and the family should feel by themselves the motivation to improve their lifestyle, and to achieve that, clinicians should adopt a consultative role through which families have the responsibility to be active in their care [48].

The clinician is encouraged to have a non-judgmental position, including understanding resistance to change and trying to drive the patient to resolve ambivalences [49]. The motivational interview as way of inducing to changes in health care settings has been the focus of recent reviews providing insights into its usefulness [50].

To our knowledge, motivational-based interventions to lose weight have been conducted in young adults, with positive effects on weight control, cardiovascular risk factors and self-esteem, as reported by a systematic review published in 2009 by Poobalan et al. [6].

In children, a review published in 2015, found three studies reporting on positive effects of motivational interview on BMI and other obesity-related behavior outcomes [51]. The authors concluded that motivational interviewing might be applicable in childhood obesity, but there was lack of research on this specific sample. A more recent randomized clinical trial on motivational interview counselling to treat childhood obesity concluded that therapists providing motivational interview and registered dieticians were able to achieve significantly greater BMI reductions compared to other health care providers and interventions [52]. The authors suggested that further, large-scale research was necessary, as is training of pediatricians and registered dieticians. The present clinical trial has provided a short period of training to a full set of pediatric professionals from primary care settings of a province. Therefore, the study will have the possibility to show whether this kind of training was effective in improving childhood obesity treatment at a wide scale.

There is a strong body of scientific literature supporting the use of dynamic groups to treat childhood obesity, as a method to make the children feeling engaged with their peers and the therapy, usually combined with personal interviews [12].

From the eHealth perspective, data supporting its use in childhood obesity is scarce [15]. In young adults, several works have reported no effect of wearable devices [14]. In children, the use of an electronic device could be considered more attractive and support self-monitoring to increase physical activity [13].

Among the barriers to treat childhood obesity, there might be some coming from the patient and his/her family (as lack of motivation to lose weight, non-healthy food preferences, etc.). However, some barriers may come from the health professionals. In a study assessing the self-efficacy of the pediatricians to treat childhood obesity, most pediatricians reported feeling ineffective in their ability to treat obesity and welcomed clinical resources for obesity management as practice-based tool kits [53]. One of the main strengths of the present study is that educational kits for children with obesity and their families have been designed; these kits will be available for use by health care providers once the intervention is finished.

17. Limitations and Strengths

The first limitation was that the nature of the intervention did not allow blinding the participants nor the therapists, which may affect the results. Furthermore, we acknowledge that randomizing the therapists but not the patients could be a limitation; and the recruitment of patients by the therapists could introduce certain degree of bias. Another possible limitation of this study is that given the combination of motivational strategies within the intervention program, we will not be able to determine which of the strategies (the motivational interview, the use of the eHealth device, the group sessions or the supporting educational materials) could have a more powerful effect on lifestyle changes and subsequent BMI improvement separately. Multivariate models will consider the adherence to the different strategies (i.e., attendance to visits and workshops) to assess, at least in part, its usefulness.

One of the most important strengths of this study is its randomized clinical trial design; to our knowledge, this is the first randomised trial to assess the effect of a structured motivational interview established in the primary care to promote behavior changes in children with obesity within this age range.

Another relevant strength of the Obemat2.0 study is that the protocol was designed based on positive results obtained in a prior observational study using a similar intervention structure [11]. Furthermore, the homogeneity of the intervention was facilitated by providing printed educational materials to be given to families, specific for each visit (something that has previously been reported by pediatricians to be needed to overcome barriers in the treatment of childhood obesity [53]). A possible limitation of the study was that the actual fidelity of the therapists in the intervention group to follow the instructions could not be documented. However, we think that this could reinforce the robustness of the results, since this might reflect real clinical practise.

Last, but not least, one of the main interests of this study is that the intervention program did not focus on a specific highly trained clinic or team but has expanded to the full set of professionals in a multicenter primary care area. Establishing programs, which are feasible and can be widely spread in health systems as primary care, might be useful to arrive to the highest proportion of the obese population. This clinical trial should allow for testing the feasibility and efficacy of a program to treat children with obesity in primary care. Thus, a little improvement in a wide area would account for an overall great success.

In summary, we expect that this clinical trial could open a window of opportunity to support professionals at the primary care level to treat childhood obesity.

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Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/2/419/s1, Table S1: TIDieR-PHP items to appropriately report the study design.

Author Contributions: V.L. drafted the article, V.L., A.F., J.E., N.F., G.F., R.M., N.G., R.F., J.M.B., P.H., C.A.-B., F.A., M.V., and P.S., R.C.-M. contributed to the design of the project and the intervention, D.G.-M., J.M.-H., M.Z.-J., C.R.-T., M.N.-R., M.A., R.F., P.H., F.A. collected data. All the authors revised and agreed the content of the article.

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Addendum 1

ADDENDUM NUM. 2

STUDY 1

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

Desirée Gutiérrez-Marín, Veronica Luque, Natalia Ferré, Mary Fewtrell, Jane Williams, Jonathan CK Wells

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Addendum 2

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 from 4 to 22 years old were available from different data bases from the Childhood Nutritional Research Centre (UCL Institute of Child Health, London, UK) (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 after recruitment, and obese children taking part in weight-loss trials (14,16), however other smaller studies were also incorporated (10,17). 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. The 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 the whole 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 the 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:

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

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

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

D_{FFM} was then calculated as follows:

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

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 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.

Table 1 shows a description of the characteristics of the sample stratified by gender. No differences were found in age between males and females, the means being 13.0 and 13.4 years old respectively. Neither weight, height nor H_{FFM} showed differences between genders.

Statistically significant differences were found for all other body variables. Girls showed greater BMI than boys ($p<0.001$). Females also 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.

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.

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 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 persists 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 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.

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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347 **Figure legends**

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

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

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

Table 1. Description of the sample.

	males (n = 416)		females (n = 520)		whole sample (n = 936)	
	mean (± SD)	Range	mean (± SD)	Range	mean (± SD)	Range
Age (years)	12.9 (±4.1)	4.22 – 22.0	13.4 (± 4.4)	4.5 – 21.9	13.2 (± 4.3)	4.2 – 22.0
Weight (kg)	49.6 (± 20.8)	15.2 – 111.3	52.8 (± 20.0)	16.1 – 119.6	51.4 (± 20.4)	15.2 – 119.6
Height (cm)	153.2 (± 20.4)	102.5 – 194.7	151.8 (± 15.6)	103.9 – 185.4	152.4 (± 17.9)	102.5 – 194.7
BMI* (kg/m2)	20.2 (± 5.2)	13.0 – 40.6	22.2 (± 6.2)	12.5 – 48.4)	21.3 (± 5.9)	12.5 – 48.4
BMI SDS*	0.45 (± 1.42)	-3.09 – 4.74	0.79 (± 1.52)	-3.33 – 4.46	0.64 (± 1.49)	-3.33 – 4.74
HT SDS*	0.16 (± 1.05)	-2.77 – 3.93	0.37 (± 1.08)	-2.77 – 4.11	0.28 (± 1.08)	-2.77 – 4.11
WT SDS*	0.43 (± 1.35)	-3.09 – 4.84	0.84 (± 1.51)	-4.02 – 4.77	0.66 (± 1.45)	-4.02 – 4.84
Fat Mass* (kg)	12.1 (± 10.1)	0.97 – 58.6	18.0 (± 11.9)	2.3 – 67.6	15.4 (± 11.5)	0.97 – 67.7
Fat Free Mass *(k	38.3 (± 14.4)	12.8 – 72.2	35.7 (± 9.4)	12.1 – 64.1	36.9 (± 12.0)	12.1 – 72.2

*Significant difference between males and females at $p<0.05$.
(Abbreviations: BMI = Body Mass Index; HT = height; WT = weight;
SDS Standard deviation score; FFM = Fat-free mass; SD = Standard deviation.)

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 $p<0.05$.

Figure 1

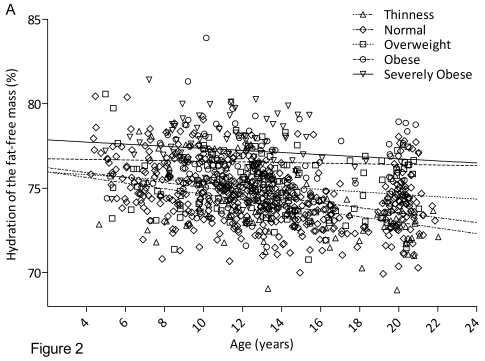
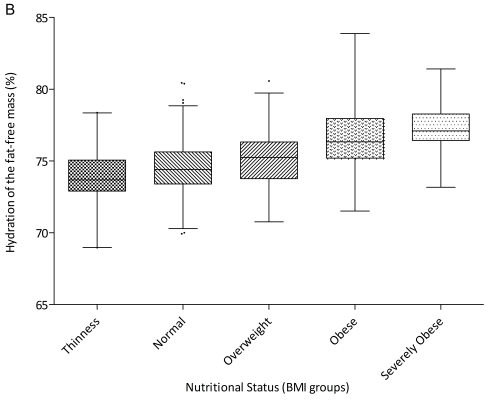


Figure 2



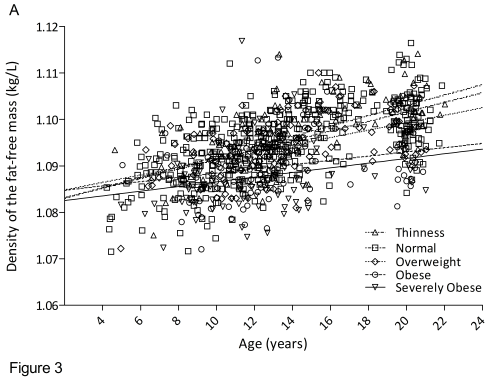
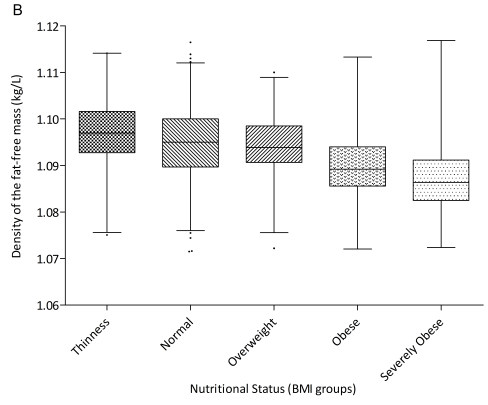


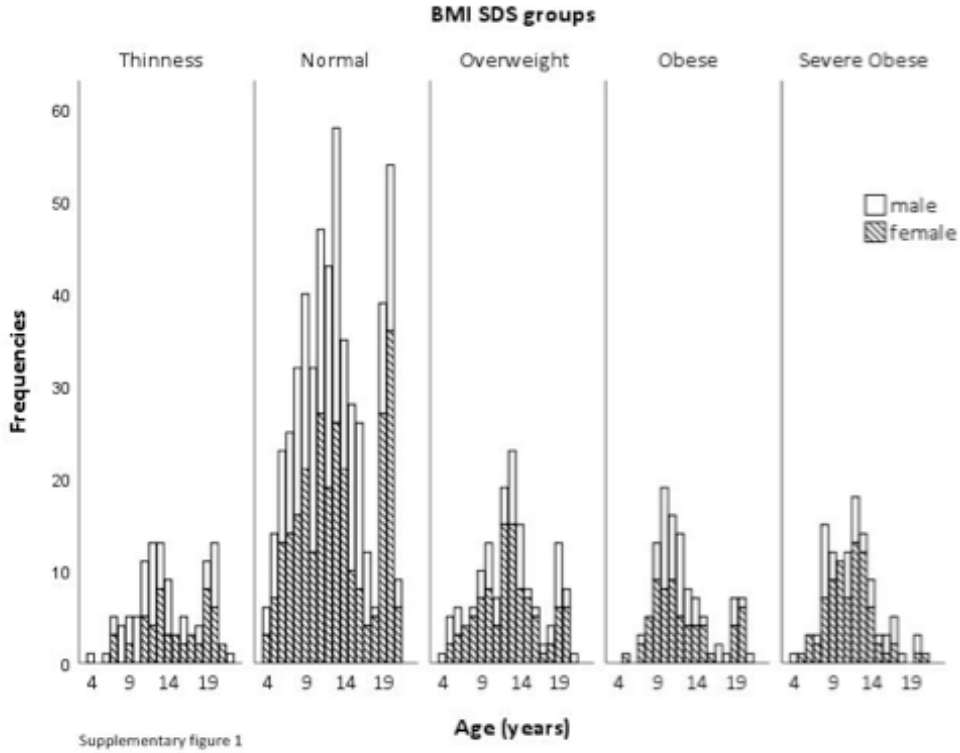
Figure 3



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