USING ULTRASOUND TO ASSESS NORMAL WEIGHT OBESITY

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Abstract. Normal weight obesity (NWO) is a condition encountered in people whose body mass index (BMI) is in the normal range (18.5–24.99 kg/m^2), but their body fat percentage (%BF) is higher than a certain limit established in large-scale population studies (23.1 % for men and 33.3 % for women). NWO is associated with insulin resistance, metabolic syndrome, and increased risk of cardiovascular events. Therefore, it is desirable to detect NWO in a routine clinical investigation. Nevertheless, techniques of body composition assessment that are considered to be accurate require expensive apparatus and dedicated laboratories. Portable instruments are increasingly used for this purpose, but their accuracy needs to be established in various classes of subjects. Amplitude A-mode ultrasound (US) was found to be highly reliable, affordable and user-friendly, attracting much attention in recent years. This work evaluates the potential of A-mode US to detect NWO. Here, US is compared with air displacement plethysmography (ADP) – a reference method of body composition analysis. We found that US has a tendency to underestimate %BF, especially in subjects of high adiposity. Therefore, US proved ineffective in evaluating NWO defined in terms of fixed %BF cutoff values. When cutoff values were defined as relative quantities, such as the median of %BF within the ranges of BMI used in the classification of nutritional status, US was useful for sorting our sample into expanded NWO categories. Although its validity needs to be improved for certain categories of subjects, A-mode US is a promising technique for the study of NWO.

Key words: Body fat percentage, body mass index, BodyMetrix, BOD POD, air displacement plethysmography.

INTRODUCTION

What does obesity really mean? Does it mean that you have too much body fat and too low self-esteem, and your doctor keeps telling you to change your eating habits? Obesity is much more than that. We may be at risk without knowing it, even if we are young. Studies performed on college students observed weight gain, change in body mass index, defined as body mass (kg) divided by height

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squared (m^2), waist circumference, and body fat percentage (%BF), defined as 100 times fat mass divided by body mass. The students were evaluated at the beginning of their college studies and four years later [12].

Body mass is determined by an interaction between genetic, environmental and psychosocial factors. Evidence indicates that genetics also plays an important role [7].

Obesity is a condition that leads to hypertension, dyslipidemia, coronary heart disease, heart failure, gallbladder stones, obstructive sleep apnea syndrome, asthma, psychiatric disease, including depression, polycystic ovary syndrome, nonalcoholic fatty liver disease, osteoarthritis, cancers (postmenopausal breast, endometrial cancer, prostate and colorectal cancer), diabetes, and deep vein thrombosis. Obese patients had higher 30-day mortality due to myocardial infarction [6].

Obesity also boosts insulin resistance, sympathetic nervous activity, and increases fatty acid turn-over. It is associated with increased leptin levels and high values of hs-C-reactive protein (CRP). It is also correlated with decreased levels of 25-hydroxivitamin D [11].

Impaired adipocyte proliferation or differentiation brings to adverse endocrine and immune responses leading to metabolic disease. Adipose tissue is an active secretory organ sending out and responding to signals that modulate appetite, insulin sensitivity, endocrine system, inflammation and immunity. Adipokines (produced by the adipose tissue) do not only act as autocrine/paracrine regulators. They also reach target organs through the systemic circulation. This phenomena is known as "outside to inside" cellular cross-talk [7].

The parameter that is usually taken into account to define obesity is BMI [29]: people with BMI < 18.5 kg/m^2 are regarded as underweight, those with BMI in the range 18.5–24.99 kg/m^2 are characterized as normal weight, those with BMI in the interval 25–29.99 kg/m^2 are deemed overweight, whereas persons whose BMI ≥ 30 kg/m^2 are considered obese.

BMI, however, fails to discriminate between body fat and lean tissue. Moreover, BMI cannot be used to characterize fat distribution. This is an important shortcoming because adipose tissue located between internal organs (visceral fat) is a risk factor for many diseases [10]. Although BMI's specificity of detecting obesity is quite good, its sensitivity is low. In a study of 6123 subjects, 29 % of the individuals classified as lean and 80 % of those classified as overweight according to BMI were found to be obese according to their %BF measured *via* air displacement plethysmography (ADP) [11].

A vast literature indicates that %BF carries important information about our health [20]. Obesity has been classified recently by distinguishing four phenotypes: normal weight obese, metabolically obese normal weight, metabolically healthy obese, and metabolically unhealthy obese. It is important to mention that sarcopenic obesity (high %BF and low muscle mass) was related to all phenotypes [7].

Metabolically obese normal weight people have normal BMI, but they do have a cluster of metabolic disturbances that characterize a typical obese person, of BMI > 30 kg/m^2: low insulin sensitivity, high amount of visceral fat, and ectopic liver fat.

Metabolically healthy obese people exhibit an obese phenotype in the absence of any metabolic abnormalities. Nevertheless, studies with long follow-up periods demonstrated that such individuals were at higher risk for major cardiovascular events compared to metabolically healthy, normal weight individuals.

Metabolically unhealthy obese people have high body fat percentage and suffer from metabolic syndrome [7].

Normal weight obesity (NWO) is a condition of subjects with normal BMI (18.5–24.99 kg/m^2) but excess body fat, defined by the highest sex-specific tertile of %BF. Based on this definition, a study of over 6000 normal weight subjects established the lower limits of body fat percentage of people that suffer from NWO as 23.1 %BF in men and 33.3 %BF in women [24].

NWO is associated with significant cardiometabolic dysregulation, including metabolic syndrome and cardiovascular risk factors. It also increases cardiovascular mortality [1]. Furthermore, NWO appears to be associated independently with increased cardiovascular mortality in women [24], highly prevalent among them, suggesting that maybe sex hormones do play an important role in this condition [10]. The Third National Health and Nutrition Examination Survey demonstrated that short term mortality was higher in women, while long-term mortality was higher in men [1].

Body fat percentage proved to be a better indicator for ischemic heart disease than waist circumference. Patients with NWO may or may not have changes in other anthropometric parameters, such as waist circumference, waist-to-hip ratio, waist-to-high ratio and percent of android or gynoid fat [8, 10]. Prevalence of hypertension is higher, the pulse wave velocity is higher, fasting glucose levels and lipid profiles are worse. A very important aspect is that NWO was considered an independent risk factor for developing soft coronary plaques, which implies a very high risk for having a myocardial infarction [10].

The concept of NWO has been generalized (expanded) to consider categories of high body fat and low body fat within each BMI interval employed in the conventional classification of obesity [29], except for underweight. It has been demonstrated that high body fat is associated with insulin resistance [21]. In this study, body fat percentage was determined by DEXA and insulin resistance was evaluated *via* the homeostatic model assessment for insulin resistance (HOMA-IR), defined as fasting insulin (μU/mL) × fasting glucose (mg/dL)/405. Higher levels of %BF were associated with higher levels of HOMA-IR [21]. Other investigations, which used ADP to determine %BF, also concluded that NWO is a predictor of higher HOMA-IR levels [11].

Individuals with NWO tend to develop low-grade proinflammatory status (high CRP level) and increased oxidative stress. Oxidative stress interferes with both pancreatic secretion of insulin and glucose uptake by muscle and adipose tissue [10]. It promotes damage to cell membranes, proteins and DNA and it damages cellular components, especially the mitochondria [4].

Hyperhomocysteinemia, an important cardiovascular risk factor, is also linked to %BF. When its concentration in the blood is high, homocystein is oxidized, leading to the increase in the concentration of prooxidant substances in the blood. Consequently, low-density lipoprotein particles are built, which lower the antioxidant activity of the endothelium and the bioavailability of nitric oxide [10]. Inflammation and oxidative stress status develops long before the development of the metabolic syndrome [9].

Adipocytes produce proinflammatory cytokines [7, 10]. Women with NWO had higher levels of interleukins (IL-1, IL-6, IL-8) and tumor necrosis factor, TNF-α. Besides total adiposity, IL-6 and TNF-α concentrations were associated with fat mass distribution. IL-8 is known to be implicated in the pathogenesis of atherosclerosis [2, 14]. The proinflammatory cytokines could be regarded as prognostic indicators of the risk of obesity.

NWO patients have higher degree of vascular inflammation, compared to normal weight lean people. Inflammation influences atherosclerotic progression, being major determinant of plaque rupture. The degree of subclinical vascular inflammation was evaluated using the mean and maximum target-to-background ratios of the carotid artery, which were measured by ^{18}F-fluorodeoxyglucose (^{18}F-FDG)-PET/CT (a noninvasive tool for assessing vascular inflammation). The ^{18}F-FDG uptake correlates with macrophage infiltration [19].

Taken together, the studies of NWO indicate that excessive body fat is a risk factor that is worth monitoring. Accurate techniques of body composition analysis require expensive equipment and adequate space [15]. Less expensive, portable instruments would be desirable for assessing %BF in a clinical setting. Such instruments, however, need to be validated for various categories of subjects. Therefore, the present study aims to evaluate the accuracy of a handheld A-mode ultrasound (US) device in a heterogeneous sample of healthy adults and test its ability to identify subjects with NWO. We compared the US measurement results with those obtained *via* ADP – a well-established technique of body composition assessment. To further improve the reliability of the reference technique, we conducted the ADP measurements according to the repeated measures protocol proposed by Tucker *et al.* [27].

MATERIALS AND METHODS

STUDY POPULATION

This study was conducted on a sample of 200 healthy adults (105 men and 95 women). Each participant provided a written informed consent. Performed in accord with the Declaration of Helsinki, this investigation was approved by the Committee of Research Ethics of the “Victor Babeş” University of Medicine and Pharmacy Timişoara.

REPEATED MEASUREMENTS OF AIR DISPLACEMENT PLETHYSMOGRAPHY

ADP assessments of %BF were conducted using a BOD POD® Gold Standard Body Composition Tracking System (COSMED USA, Inc., CA, USA), with BOD POD software version 5.3.2. Scale calibration and system quality check were carried out on a daily basis.

Subjects were asked to refrain from alcohol consumption and intense exercise for at least 12 hours before the test. ADP trials and US measurements were performed on the same day, after at least of 4 hours of fasting; neither food nor drinks were consumed by the subject during this period. Before being tested, each subject was asked to visit the restroom if she/he has not done so during the last 30 minutes. Body mass was measured to the nearest 10 grams using the scale connected to a BOD POD Gold Standard Body Composition Tracking System (COSMED USA, Concord, CA, USA). We measured height to the nearest 0.5 cm using a wall mounted tape measure (GIMA 27335, GIMA, Gessate, Italy). During an ADP trial, the subject wore a swim cap and form-fitting swimsuit. She/he adopted a precise position in the BOD POD chamber, with hands resting on the knees and straightened back without leaning on the backrest of the seat, thereby avoiding variability related to subject positioning [23]. We used the BOD POD software to predict thoracic gas volume and to calculate %BF using the Siri formula [25].

For each subject, at least two complete ADP trials were conducted and the resulting %BF values were compared. If they differed by at most 1 %BF, their mean value was computed and reported as the result of the ADP assessment. If the outputs of the first two trials differed by more than 1 %BF, we performed a third trial and took the mean of the two closest %BF values. This repeated measures protocol, proposed by Tucker *et al.*, was found to be more reliable than individual ADP tests [27].

A-MODE US MEASUREMENTS

Amplitude A-mode US measurements were performed using a BodyMetrix™ BX2000 instrument (IntelaMetrix, Livermore, CA, USA) working at a frequency of 2.5 MHz.

We first created a new client profile for each subject in the BodyView™ software (v5.7.11043). We introduced name, age, gender, height, weight and athletic type. The athletic type qualifier was set to ”Athletic” for underweight and normal weight subjects (BMI < 25 kg/m^2) and ”Non-Athletic” for overweight and obese ones.

Following the manufacturer's recommendations [5], we measured the thickness of the subcutaneous adipose tissue at the anatomical locations considered by the 7-site Jackson and Pollock formula (JP7) [16, 17]: triceps, chest, scapula, axilla, waist, hip, and thigh. The operator placed about 0.25 cm^3 of US conductive gel on the transducer. During the measurement, the transducer was moved back and forth, about 0.5 cm above and below the chosen site, while exerting a steady inward force of about 1 N on the transducer. The small force assured good contact between the transducer and the body surface, but did not cause a significant deformation of the underlying fat layer. The continuous movement of the transducer was necessary for smoothing the recorded signal. Once the measurement was completed at all the anatomic sites of the JP7 model, %BF was displayed by the BodyView software on the basis of a proprietary formula (a modified version of the JP7 formula from anthropometry [16, 17]).

STATISTICAL ANALYSIS

Data analysis was performed in MATLAB 7.13 (The MathWorks, Natick, MA, USA). Linear regression analysis was employed to evaluate the accuracy of %BF measurement using A-mode US. The line of regression was represented on the scatter plot of %BF measured *via* US *versus* %BF measured *via* ADP according to the repeated measures procedure devised by Tucker *et al.* [27]. We computed Pearson's product-moment correlation coefficient, R, and its square, R^2, the so-called coefficient of determination. The latter is a measure of the proportion of the variance in the data that is described by the regression equation.

RESULTS

The investigated sample comprised 200 healthy adult volunteers. To test the validity of A-mode US for a diverse population in what concerns nutritional status, this study was conducted on a heterogeneous sample (see Table 1 for its descriptive statistics).

Table 1

Characteristics of the study population (mean values ± standard deviation (SD))

Subjects	**All** ($n = 200$)		**Men** ($n = 105$)		**Women** ($n = 95$)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Age (years)	31.4±10.4	19–66	30.9±9.7	20–66	32.0±11.2	19–62
Height (m)	1.71±0.1	1.49–1.96	1.78±0.07	1.55–1.96	1.63±0.06	1.49–1.80
BM (kg)	76.6±20.0	38–160.5	84.7±19.6	55–160.5	67.6±16.4	38–115.5
BMI (kg/m^2)	26.0±6.0	16.6–47.9	26.6±5.6	17.0–47.9	25.4±6.4	16.6–45

Before turning to the study of NWO, we asked the question whether A-mode US is an accurate technique for evaluating human body composition. To investigate this problem, we compared the %BF values provided by the BodyMetrix US device *via* the JP7 formula with the results given by ADP.

Figure 1 presents the results of the linear regression analysis of %BF$_{JP7}$ *vs.* %BF$_{ADP}$. If US would be highly accurate in comparison with ADP, the regression line (dashed line) would coincide with the line of identity (solid line) and the experimental points would be evenly distributed on both sides of that line; moreover, the residuals would be relatively small compared to the measured value.

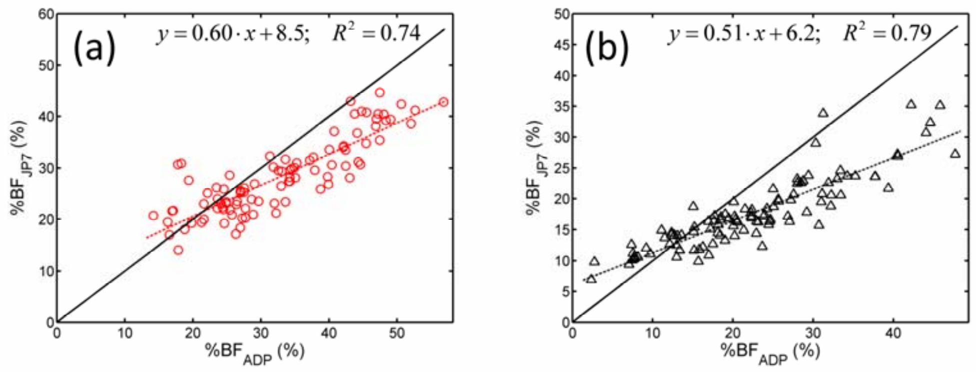

Fig. 1. Scatter plots and linear regression analysis of body fat percentage (%BF) measured using A-mode US and the 7-site Jackson and Pollock (JP7) formula *vs*. %BF measured *via* ADP in the case of women (a) and men (b). In both panels, the dotted line plots the linear regression formula displayed in the upper portion of the respective panel, whereas the thick solid line is the line of identity (the plot of $y = x$); R^2 is the coefficient of determination.

The regression lines of Fig. 1 indicate that A-mode US underestimates %BF for most subjects of both genders. In the case of women (Fig. 1a), US is most accurate in the range of 20–25 %BF; below this range, it has the tendency to overestimate the subject's adiposity. For men (Fig. 1b), US is most valid in the range of 12–15 %BF, whereas below this range it gives higher values than ADP, just as in the case of women. The slopes of the regression lines are less than 1 (see the regression equations displayed on Fig. 1), showing that the tendency of US to undervalue the adiposity of the subject is largest at high %BF.

For both genders, the coefficient of determination, R^2, shows that a large part of the variance of the dependent variable (%BF$_{JP7}$) is predictable from the independent variable (%BF$_{ADP}$) by using the regression equation (74 % in the case of women and 79 % in the case of men).

To study the ability of A-mode US to detect NWO, we selected the normal weight subjects from our sample (i.e. subjects whose BMI was in the range of 18.5–24.99) and represented scatter plots of %BF$_{JP7}$ *vs*. %BF$_{ADP}$ for each gender. In each plot, we represented the gender-specific lower limit of a subject's %BF for being classified as suffering from NWO (23.1 % BF for men and 33.3 % BF for women) [24]. The vertical and horizontal lines that plot these lower limits divide the plot in four quadrants.

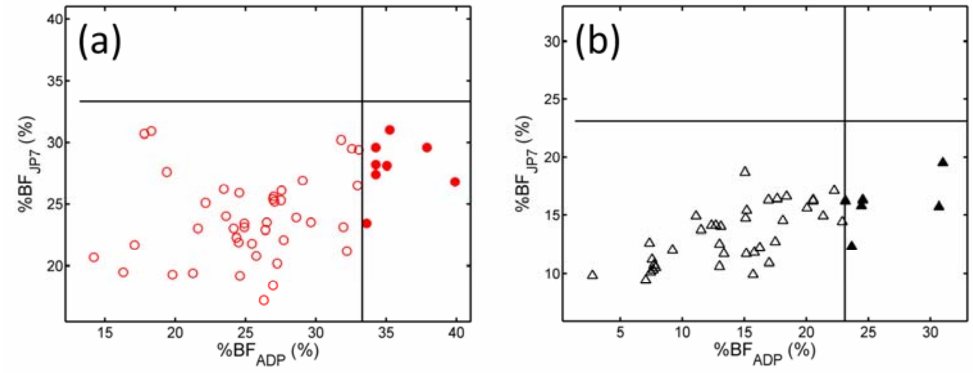

Fig. 2. Scatter plot of %BF measured using A-mode US *vs*. %BF measured using ADP for normal weight women (a) and men (b). In each plot, the vertical (horizontal) line represents the lower limit of %BF of NWO subjects assessed *via* ADP (US). A female subject is deemed to have NWO if her %BF measured by ADP exceeds 33.3 % (panel (a), solid disks). A male subject is considered to suffer from NWO if his %BF measured by ADP exceeds 23.1 % (panel (b), solid triangles) [24]. If also US would evaluate these subjects as having NWO, the solid markers would be located in the top-right quadrant.

The bottom-left quadrant contains the data points of subjects deemed without NWO using both techniques, the bottom-right quadrant contains data points of subjects that have NWO according to ADP, but not according to US, the top-right quadrant corresponds to subjects evaluated by both techniques as suffering from NWO, whereas the top-left quadrant would contain data points of subjects appreciated by US as having NWO, but not by ADP. Figure 2 shows that A-mode US was ineffective in detecting NWO, presumably because it underestimated the adiposity of the subjects.

Figure 3 represents scatter plots of %BF measured using ADP and BMI for women (panel a) and men (panel b). The horizontal axis refers to the subjects' BMI; it is divided in 4 intervals by dashed vertical lines, according to the classification of nutritional status adopted by the WHO [29]: underweight, normal weight, overweight, and obese - in the order of increasing BMI. Within each BMI range, we computed the median of %BF and represented it as a horizontal solid line, thereby dividing subjects in two equal-sized groups: low body fat (data represented by empty markers) and high body fat (solid markers). Hence, Fig. 3 represents %BF *vs*. BMI for all the categories of expanded normal weight obesity (eNWO) defined by Martinez *et al*. [21]. More precisely, these authors did not divide underweight (BMI < 18.5 kg/m^2) subjects into two categories because their sample, just as ours, contained relatively few underweight subjects.

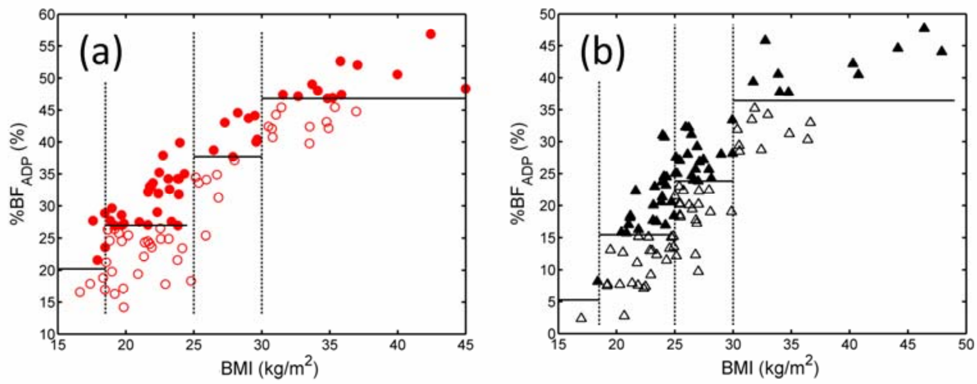

Fig. 3. Scatter plot of %BF measured using ADP *vs*. BMI, plotted for women (a) and men (b). Vertical dashed lines delimit the BMI categories designated as underweight, normal weight, overweight and obese in the conventional classification adopted by the World Health Organization (WHO). Horizontal solid lines represent the median of %BF of subjects from each BMI category. Subjects whose adiposity exceeds the median of her/his BMI category are considered to have eNWO [21] and the corresponding data points are represented by solid markers - disks for women (a) and solid triangles for men (b).

Figure 4 displays the scatter plots of %BF given by US *versus* BMI. In these plots, vertical dashed lines delimit again underweight, normal weight, overweight, and obese subjects [29], whereas %BF values are given by the JP7 formula from the BodyView software based on subcutaneous fat layer thicknesses measured using the BodyMetrix instrument at 7 anatomic sites [16, 17].

In Figure 4, solid markers situated above the median line correspond to subjects classified by both techniques as having high body fat; solid markers located below the median line correspond to subjects of high body fat according to ADP but not according to JP7 (*i.e.* subjects whose potentially risky level of high body fat remained undetected when the evaluation was done by A-mode US). Empty markers located above the median line refer to subjects deemed to have low body fat according to ADP and high body fat according to JP7 (*i.e.* subjects who would be mistakenly classified as having high body fat). Due to the definition of the cutoff, as being the median of the %BF values measured by the given instrument, the number of undetected cases is equal to the number of false-alarm cases. Table 2 summarizes the analysis of adiposity categories within normal weight, overweight and obese subjects.

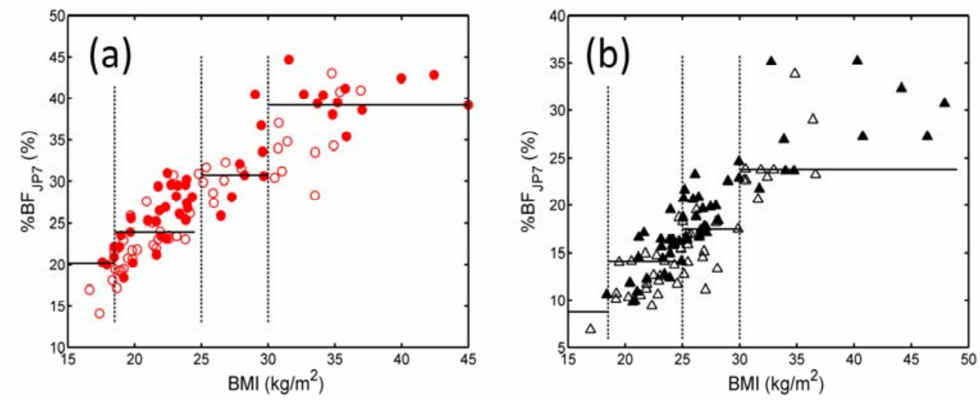

Fig. 4. Scatter plot of %BF measured using US and the JP7 formula *vs*. BMI for women (a) and men (b). Solid markers represent the data points of subjects identified by ADP as suffering from eNWO (*i.e.* %BF exceeding the median of the %BF of all the subjects from the same BMI category). Vertical dashed lines delimit BMI categories, whereas horizontal solid lines represent the median of %BF values obtained *via* JP7 within each BMI category.

Table 2

Comparison of ADP and A-mode US (using the JP7 formula) for establishing expanded NWO categories: low fat subjects and high fat subjects within BMI intervals designated as normal weight (NW), overweight (OW) and obese (OB) in the WHO classification of nutritional status [29].

	Women			**Men**		
BMI category	**NW**	**OW**	**OB**	**NW**	**OW**	**OB**
Median of %BF$_{ADP}$ (%)	27.0	37.7	46.9	15.5	23.8	36.5
Median of %BF$_{JP7}$ (%)	23.9	30.7	39.2	14.1	17.5	23.8
Number of high fat subjects	25	8	12	22	20	10
Detected high fat subjects (%)	72.0	62.5	75.0	72.7	75.0	70.0

Although the US underestimated the adiposity of most subjects, resulting in smaller values of the medians that separated the categories of low body fat and high body fat within each BMI class (compare the first two rows of Table 2), it proved rather effective in establishing the right hierarchy of %BF within the investigated sample. Indeed, US was able to detect more than 70 % of the men (and 62.5 % of the women) with high body fat (Table 2).

DISCUSSION

Relying on repeated ADP measurements as reference values of %BF, we evaluated the accuracy of A-mode US as well as its ability to identify subjects who might be at health risk because of NWO. Our study demonstrates that US can be used to assess expanded NWO, but it is of limited use for studying NWO defined in terms of fixed lower limits of %BF.

A-mode ultrasound is not the only technique of body composition analysis using cost-effective instruments. An investigation of the impact of elevated %BF on lung function in NWO subjects relied on multi-frequency bioelectrical impedance measurements [3].

The result of the linear regression analysis is in agreement with the work of Smith-Ryan *et al*. [26] regarding the validity and reliability of A-mode US in comparison with a 3-compartment model based on ADP and bioelectrical impedance spectroscopy. Investigating a sample of 47 overweight and obese subjects, these authors found that the sample mean of %BF given by the US instrument applying the JP7 formula was 4.7 % lower than the one given by the 3-compartment model; this difference was statistically significant (P < 001). For overweight subjects, US gave 27.1±5.7 %, whereas the 3-compartment model gave 31.3±6.2 %. In our study, at the reference value of 31.3 % (based on ADP) the difference between the line of identity and the line of regression was 4.0 % for women and 9.1 % for men. It is not clear why in our study the underestimation of %BF by A-mode US was more pronounced for men than for women.

A study of college-aged subjects (22.9±1.35 years) [18], revealed excellent agreement between %BF evaluated using the BodyMetrix instrument (15.7±5.14 %) and the BOD POD (15.5±5.83 %). At the ADP value of 15.5 %, our study revealed a difference between the line of identity and the line of regression of 2.3 % for women and −1.1 % for men, showing a good agreement with ref. [18], conducted on 18 men and 8 women. Indeed, the weighted average of the differences observed in our study is 2.3 % × 8/26 − 1.1 % ×18/26 = −0.05 % comparing well with the difference of 0.2 %BF observed by Johnson *et al*. between US and ADP [18]. Also, these authors obtained a Pearson product-moment correlation coefficient of 0.879 between the indications of the two instruments, comparable to ours (0.888 for men and 0.857 for women).

In their study performed on 31 young adults (26.7±3.9 years) with 17.6±6.9 %BF, Hendrickson *et al.* [13] found no systematic disagreement between ADP and A-mode US based on the 3-site Jackson and Pollock (JP3) formula [16, 17]. At a

%BF of 17.6 %, our work indicates an overestimation by 1.5 % for women and an underestimation by 2.1 % for men; computing the weighted average for 21 men and 10 women (see ref. [13]) leads to an underestimation of %BF by 0.94 %. This value is smaller than the technical error of measurement of ADP (1.07 %BF) [22].

Wagner *et al*. [28], on the other hand, observed an overestimation of %BF by A-mode US by 3 % in comparison to ADP. These authors applied the JP3 formula from BodyView to compute %BF on the basis of US measurements. The discrepancy between the results of our study and those of ref. [28] might stem from differences between the investigated samples or from differences between US measurement procedures. Further research is necessary to elucidate them.

CONCLUSIONS

A-mode ultrasound was found accurate in the present study in the case of lean subjects, whose body fat percentage (%BF) was similar to elite athletes. At larger levels of %BF, the higher was the subject's adiposity, the larger was the difference between the %BF given by air displacement plethysmography and %BF given by ultrasound. The discrepancy between the two techniques was larger for men than for women. Our work suggests that body composition assessments using A-mode ultrasound are affected by a systematic underestimation of percent body fat in subjects with average to high adiposity. Therefore, ultrasound was incapable of identifying subjects with normal weight obesity in our sample. By contrast, ultrasound was effective in the investigation of expanded normal weight obesity, which is based on classifying subjects into low body fat and high body fat categories within standard intervals of body mass index.

The accuracy of A-mode ultrasound needs to be improved for certain categories of subjects. Nevertheless, the reliability, portability, and affordability of this technique are likely to motivate further progress in the field of ultrasound-based body composition assessment, turning it into a promising tool of clinical relevance.

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REFERENCES

1. BATSIS, J.A., K.R. SAHAKYAN, J.P. RODRIGUEZ-ESCUDERO, S.J. BARTELS, V.K. SOMERS, F. LOPEZ-JIMENEZ, Normal weight obesity and mortality in United States subjects > 60 years of age (from the third national health and nutrition examination survey), *Am. J. Cardiol.*, 2013, **112**, 1592–1598.
2. BRUUN, J.M., S.B. PEDERSEN, K. KRISTENSEN, B. RICHELSEN, Opposite regulation of interleukin-8 and tumor necrosis factor-alpha by weight loss, *Obes. Res.*, 2002, **10**, 499–506.

3. CHEN, Y.Y., T.W. KAO, W.H. FANG, C.C. WANG, Y.W. CHANG, Body fat percentage in relation to lung function in individuals with normal weight obesity, *Scientific Reports*, 2019, **9**, 3066, 1–7.
4. CHOI, K.M., Sarcopenia and sarcopenic obesity, *Endocrinology and metabolism* (Seoul, Korea), 2013, **28**, 86–89.
5. DA SILVA, L., *An Introduction to Ultrasound and the Bodymetrix System*, IntelaMetrix, Livermore, California, 2010.
6. DE LORENZO, A., S. GRATTERI, P. GUALTIERI, A. CAMMARANO, P.BERTUCCI, L. DI RENZO. Why primary obesity is a disease?, *J. Transl. Med.,* 2019, **17**, 169, 1–13.
7. DE LORENZO, A., L. SOLDATI, F. SARLO, M. CALVANI, N. DI LORENZO, L. DI RENZO, New obesity classification criteria as a tool for bariatric surgery indication, *World J. Gastroenterol.*, 2016, **22**, 681–703.
8. DERVAUX, N., M. WUBULI, J.L. MEGNIEN, G. CHIRONI, A. SIMON, Comparative associations of adiposity measures with cardiometabolic risk burden in asymptomatic subjects, *Atherosclerosis*, 2008, **201**, 413–417.
9. DI RENZO, L., F. GALVANO, C. ORLANDI, A. BIANCHI, C. DI GIACOMO, L. LA FAUCI, R. ACQUAVIVA, A. DE LORENZO, Oxidative stress in normal-weight obese syndrome, *Obesity (Silver Spring)*, 2010, **18**, 2125–2130.
10. FRANCO, L.P., C.C. MORAIS, C. COMINETTI, Normal-weight obesity syndrome: Diagnosis, prevalence, and clinical implications, *Nutr. Rev.*, 2016, **74**, 558–570.
11. GÓMEZ-AMBROSI, J., C. SILVA, J.C. GALOFRÉ, J. ESCALADA, S. SANTOS, D. MILLÁN, N. VILA, P. IBANEZ, M.J. GIL, V. VALENTI, F. ROTELLAR, B. RAMIREZ, J. SALVADOR, G. FRÜHBECK, Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity, *Int. J. Obes.*, 2011, **36**, 286–294.
12. GROPPER, S.S., K.P. SIMMONS, L.J. CONNELL, P.V. ULRICH, Changes in body weight, composition, and shape: A 4-year study of college students, *Appl. Physiol. Nutr. Metab.*, 2012, **37**, 1118–1123.
13. HENDRICKSON, N., J. DAVISON, L. SCHILLER, M. WILLEY, Reliability and validity of A-mode ultrasound to quantify body composition, *J. Orthop. Trauma*, 2019, **33**, 472–477.
14. HENRICHOT, E., C.E. JUGE-AUBRY, A. PERNIN, J.C. PACHE, V. VELEBIT, J.M. DAYER, P. MEDA, C. CHIZZOLINI, C.A. MEIER, Production of chemokines by perivascular adipose tissue: A role in the pathogenesis of atherosclerosis?, *Atheroscler. Thromb. Vasc. Biol.*, 2005, **25**, 2594–2599.
15. HEYMSFIELD, S., T.G. LOHMAN, Z.-M. WANG, S. GOING, eds, *Human Body Composition*, 2nd edition, Human Kinetics, Champaign, IL, 2005.
16. JACKSON, A.S., M.L. POLLOCK, Generalized equations for predicting body density of men, *Br. J. Nutr.*, 1978, **40**, 497–504.
17. JACKSON, A.S., M.L. POLLOCK, A. WARD, Generalized equations for predicting body density of women, *Med. Sci. Sports Exerc.*, 1980, **12**, 175–181.
18. JOHNSON, K.E., I.A. NACCARATO, M.A. CORDER, W. REPOVICH, Validation of three body composition techniques with a comparison of ultrasound abdominal fat depths against an octopolar bioelectrical impedance device, *International Journal of Exercise Science*, 2012, **5**, 205–213.
19. KANG, S., C. KYUNG, J.S. PARK, S. KIM, S.P. LEE, M.K. KIM, H.K. KIM, K.R. KIM, T.J. JEON, C.W. AHN, Subclinical vascular inflammation in subjects with normal weight obesity and its association with body fat: An 18 F-FDG-PET/CT study, *Cardiovasc. Diabetol.*, 2014, **13**, article number 70, 1–12.

20. KAPOOR, N., J. FURLER, T.V. PAUL, N. THOMAS, B. OLDENBURG, Normal weight obesity: An underrecognized problem in individuals of south asian descent, *Clin. Ther.*, 2019, **41**, 1638–1642.
21. MARTINEZ, K.E., L.A. TUCKER, B.W. BAILEY, J.D. LECHEMINANT, Expanded normal weight obesity and insulin resistance in US adults of the National Health and Nutrition Examination Survey, *Journal of Diabetes Research*, 2017, **2017**, 9502643, 1–8.
22. NOREEN, E.E., P.W.R. LEMON, Reliability of air displacement plethysmography in a large, heterogeneous sample, *Med. Sci. Sports Exerc.*, 2006, **38**, 1505–1509.
23. PEETERS, M.W., Subject positioning in the Bod Pod® only marginally affects measurement of body volume and estimation of percent body fat in young adult men, *PLoS One*, 2012, **7**, e32722.
24. ROMERO-CORRAL, A., V.K. SOMERS, J. SIERRA-JOHNSON, Y. KORENFELD, S. BOARIN, J. KORINEK, M.D. JENSEN, G. PARATI, F. LOPEZ-JIMENEZ, Normal weight obesity: A risk factor for cardiometabolic dysregulation and cardiovascular mortality, *Eur. Heart J.*, 2010, **31**, 737–746.
25. SIRI, W.E., Body composition from fluid spaces and density: Analysis of methods, In: J. Brozek, A. Hanschel, (Eds.), *Techniques for measuring body composition*, National Academy of Sciences, Washington, DC, 1961, pp. 223–244.
26. SMITH-RYAN, A.E., S.N. FULTZ, M.N. MELVIN, H.L. WINGFIELD, M.N. WOESSNER, Reproducibility and validity of A-mode ultrasound for body composition measurement and classification in overweight and obese men and women, *PLoS One*, 2014, **9**, e91750.
27. TUCKER, L.A., J.D. LECHEMINANT, B.W. BAILEY, Test-retest reliability of the Bod Pod: The effect of multiple assessments, *Percept. Mot. Skills*, 2014, **118**, 563–570.
28. WAGNER, D.R., D.L. CAIN, N.W. CLARK, Validity and reliability of A-mode ultrasound for body composition assessment of ncaa division i athletes, *PLoS One*, 2016, **11**, e0153146.
29. WHO, CONSULTATION, *Obesity: Preventing and Managing the Global Epidemic*, World Health Organization, Geneva, Switzerland, 2000.