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# BODY FAT CUTOFFS MEASURED BY ISOTOPIC DILUTION WITH DEUTERIUM OXIDE IN ADOLESCENTS.


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## ABSTRACT

**Objective:** To establish nutritional diagnosis and provide a series of reference body fat percentiles for age and sex in adolescents using isotopic dilution with deuterium oxide, a method considered the gold standard for assessing body composition. **Method:** After obtaining data on weight and height, among participants aged 10 to 14 years, the Body Mass Index (BMI) was calculated, used to obtain the nutritional diagnosis. The analysis of body composition was performed on a saliva sample collected before and after the administration of a dose of Deuterium Oxide (D2O, at a dose of 0.5g/kg (maximum 30g), using the technique of Infrared Spectrometry by Transform of Fourier (FTIR), following the methodology described by Slater et al., (2005). Study approved by the Institutional Research Ethics Committee. **Results:** The sample consisted of 390 participants, 233 (59.8%) girls, with a mean age of 12.7 ±1.1 years. The percentage of body fat by D2O showed a parametric distribution between males and females, with a mean of 21.7±9.4%BF and 28.7±8.1%BF, respectively. **Conclusion:** The prevalence of overweight was 15.6% and 1.5% with obesity. There is a trend towards an increase in %BF in female quartiles and a reduction in %BF in all male quartiles. Girls enter adolescence (10 years old) with a statistically lower amount of body fat than in the intermediate period of this phase. This did not occur in boys, who remained with the same absolute value of body fat. Girls are classified as overweight when they reach 1/3 of their weight consisting of fat.


**KEYWORDS :** Body composition, Cutting points, Reference Value, Deuterium Oxide

## INTRODUCTION

Even though the prevalence of overweight and obesity multiples between adolescence and young adulthood, there be quite no conflict about the exact age and the suitable metrics used to distinguish when excess weight develops. The Body Mass Index (BMI) in men and the percentage of body fat (%BF) in women are the metrics commonly used for early identification of excess weight[1].

Despite that, it is known that BMI is a measure of relative weight and not adiposity, and it is widely recommended for children and adolescents to determine excess weight[2].

The BMI cut-off values for global estimates of overweight prevalence from the World Health Organization are ≥25 kg/m2 for overweight and ≥30 kg/m2 for obesity, as reported by age and sex, with no consideration of the diversity of ethnicity and gender, and this is mainly significant in obesity prevention strategies[3]. For adolescents aged between 10 and 19 years, the Ministry of Health's recommendation was followed, which recommends the use of the WHO reference curves, from 2007[4].

Even though the conventional use of BMI to identify obesity, only from time to time it is possible for the method can distinguish and account for visceral fat from the rest of body fat[5]. Furthermore, it is recognized that the use of BMI cutoff points to diagnose obesity and body fat needs to take into consideration the potential impact of ethnicity, age group, gender and associated diseases, when in comparison with other assessment methods, in particular DEXA and isotopic dilution with deuterium oxide[6,7].

However, muscle mass differs with sex, height and weight and 8 many adjusted muscles mass indices have been suggested . There is evidence of a new finding putting forward that lean mass independent of body fat is closely related to a combined risk of cardiovascular disease in girls, further indicating that BMI is a good risk index for cardiovascular disease but a poor index for adiposity[9].

In addition, BMI leans to underestimate or overestimate body fat in children of different ethnicities, identified in Asia, Africa and New Zealand[10,11]. Therefore, in the last decade, various techniques have arisen for the evaluation of body composition in children, although equivalent data are still lacking for some age groups by virtue of the low adherence to most of these techniques, which remains to be the limitation for the comparability between young and old, as a result of the variation in the hydration coefficient[12].

This study has as objective to evaluate the nutritional status and provide a series of reference body fat percentiles for age and sex in adolescents through isotopic dilution with deuterium oxide, the gold standard method for assessing body composition.

## METHOD

For the purpose of measuring body weight, a portable digital scale, brand Tanita®, with a maximum capacity of 150 kg and graduation of 0.1 kg, was emplyed. Height was measured using a mobile stadiometer, brand Alturaexata®, with a maximum length of 214 cm and 0.1 cm accuracy. The procedures for obtaining anthropometric measurements were carried out according to the recommendations of the World Health Organization[13]. From the measurements of weight and height, the body mass index (BMI) was calculated by applying the equation: BMI = Weight (Kg)/Height (m)².

The BMI for age (BMI/A) was employed for nutritional status classification, in a z- score, considering the following cutoff points: marked thinness (< z-score -3); thinness (≥ z score -3 and < z score -2); eutrophy (≥ z score -2 and ≤ z score +1); overweight (> z score +1 and ≤ z score +2); obesity (> z score +2 and ≤ z score +3); severe obesity (> z score +3)[14].

The height-for-age index (H/A) was determined as very short height for age (< z score -3); short stature for age (≥ z score -3 and < z score -2); and height adequate for age (≥ z score -2)[14]. To recognize the z-score of BMI/A and H/A, the WHO AnthroPlus software version 1.0.39 was used[15]

The isotopic dilution technique with deuterium oxide has been utilized in nutrition studies for over 50 years. Deuterium (2H) is a stable, non-radioactive isotope of hydrogen. Administered orally, it quickly mixes with body fluids, being eliminated by saliva, urine, sweat and breast milk. The technique is precise, highly accurate and allows the evaluation of body composition in accordance with the total body water content (TCA), in a safe and innocuous way for humans16. Deuterium oxide (D2O) was measured using the Fourier Transform Infrared Spectrometry (FTIR) technique and the entire methodology described by Slater et al.[17] was followed, by one data recorded in a specific form.

The rules to calculate the ACT and, accordingly, the body composition were made by isotopic dilution with deuterium oxide. All analyzes were executed at the EMESCAM Stable Isotopes Laboratory, by a biochemist trained in the IAEA Reference Laboratory, at the Centro de Investigación em Alimentación y Desarrollo A. C. (Mexico). Analyzes were carried out in the SHIMATZU FTIR IRAfinit-1 ® equipment, using 99.99% D2O from Sigma Aldrich® and the software IRsolution. Previous criteria for performing the procedures were: (a) two-hour fasting; (b) urinate before testing; (c) Collection of a basal saliva sample, as stated in a standardized protocol; (d) Administration of the dose of D2O (0.5 g/kg body weight), in agreement with a standardized protocol, maximum 30g, and (e) collection of a post-dose saliva sample 3 hours after administration of D2O, according to a standardized protocol.

### Statistical analysis

Descriptive and analytical statistical techniques were employed. Descriptive results were acquired through measures of central tendency (mean and median), dispersion (standard deviation and data amplitude). For the purpose of calculating the percentiles of age and %F used in both sexes, the values were sorted in ascending order and calculated (R=p/100)(n+1), where "R" is the classification of the percentile, " p" is the percentile and "n" is the sample size. The SPSS version 20.0 program was used to perform the calculations. The normality of the data was checked using the Kolmogorov-Smirnov test.

Body fat percentage z-scores (%BF) utilizing the deuterium oxide dilution technique for overweight and obesity were determined to identify cutoff points for normal weight, overweight, obesity and severe obesity. Each z-score is considered (overweight above one standard deviation z+1, obesity z+2 and severe obesity z+3, or even considering a cutoff point for "overweight" (cutoff above z+1 ).

Correlation analyzes were executed between the %BF obtained by deuterium and the age of the sample, in both sexes. Tests (parametric and non-parametric) were used to verify differences in means/medians of %F between quartiles.

## RESULTS

The sample composed of 390 participants, with 157 (40.2%) boys and 233 (59.8%) girls, with a mean age of 12.5±1.0 years and 12.7 ±1.1 years. The prevalence of overweight was 15.6% and 1.5% with obesity.

The sample %BF through D2O presented a parametric distribution for both sexes, in agreement with the Kolmogorov-Smirnov test, at a significance level of $\alpha=0.05$ ($p<0.05$), with a mean of 21.7±9 .4%BF among boys and 28.7±8.1%BF among girls. The summarized description of the data is presented in TABLE1.

Table 1: Descriptive statistics sample of adolescents between 10 and 14 years old of Vitória – Espírito Santo, n=390

| | N | Mean± DP | Median (Range) | Normality of data† |
|---|---|---|---|---|
| Female | | | | |
| 1° age quartile | 55 | 11,1 ± 0,3 | 11,2 (10,3-11,7) | O,045* |
| 2° age quartile | 60 | 12,2 ± 0,3 | 12,3 (11,8-12,7) | 0,017* |
| 3° age quartile | 57 | 13,2 ± 0,2 | 13,2 (12,8-13,5) | 0,000* |
| 4° age quartile | 61 | 14,1 ± 0,4 | 14,0 (13,6-14,9) | 0,060 |
| GC 1° quartile | 55 | 13,6 ± 7,5 | 12,1 (3,8-45,2) | >0,10 |
| GC 2° quartile | 60 | 15,4 ± 7,5 | 14,3 (5,2-37,5) | 0,078* |
| GC 3° quartile | 57 | 16,8 ± 8,1 | 16,1 (3,0-37,1) | >0,10 |
| GC 4° quartile | 61 | 17,6 ± 7,3 | 17,5 (4,1-33,6) | >0,10 |
| %GC 1° quartile | 55 | 27,3 ± 8,6 | 27,2 (11,4-48,0) | >0,10 |
| %GC 2° quartile | 60 | 28,8 ± 7,7 | 28,7 (13,8-47,6) | >0,10 |
| %GC 3° quartile | 57 | 28,9 ± 8,8 | 28,7 (7,0-46,3) | >0,10 |
| %GC 4° quartile | 61 | 29,6 ± 7,3 | 30,4 (10,4-41,7) | 0,026* |
| %GC Total | 233 | 28,7 ± 8,1 | 29,1 (6,9-48,0) | >0,10 |
| Male | | | | |
| 1° age quartile | 38 | 11,2 ± 0,4 | 11,3 (10,4-11,7) | >0,10 |
| 2° age quartile | 35 | 12,0 ± 0,1 | 12,0 (11,8-12,2) | 0,005* |
| 3° age quartile | 40 | 12,6 ± 0,3 | 12,6 (12,3-13,1) | 0,001* |
| 4° age quartile | 44 | 13,8 ± 0,5 | 13,7 (13,2-14,9) | 0,000* |
| GC 1° quartile | 38 | 11,4 ± 5,8 | 10,7 (1,5-24,9) | >0,10 |
| GC 2° quartile | 35 | 10,2 ± 6,4 | 8,0 (1,9-28,7) | 0,042* |

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|---|---|---|---|---|
| GC 3° quartile | 40 | 11,2 ± 6,5 | 9,8 (2,8-27,6) | 0,075 |
| GC 4° quartile | 44 | 13,0 ± 8,9 | 12,3 (1,5-40,4) | 0,062 |
| %GC 1° quartile | 38 | 24,7 ± 8,9 | 24,4 (5,1-42,3) | >0,10 |
| %GC 2° quartile | 35 | 21,4 ± 9,1 | 19,5 (6,2-40,6) | >0,10 |
| %GC 3° quartile | 40 | 20,8 ± 9,0 | 20,3 (7,8-38,7) | >0,10 |
| %GC 4° quartile | 44 | 20,1 ± 10,2 | 18,3 (3,3-45,1) | >0,10 |
| %GC Total | 157 | 21,7 ± 9,4 | 21,6 (3,3-45,1) | >0,10 |

Caption: N, sample number; SD, standard deviation; †, Second Kolmogorov-Smirnov Test; *, non-parametric data distribution (α=0.05); GC, body fat.

The correlation between age and body fat in the female sample was statistically significant and positive, whose Pearson coefficient was r = 0.2, with straight line plotting shown in FIGURE 1.

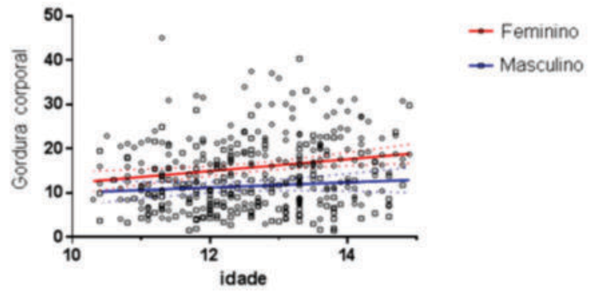


**Figure 1:** Sample correlation graph between body fat percentage and age in both sexes. Vitória, Espírito Santo. N=390.

The considerable differences between the means and medians of the variables age, body fat and %BF between the age quartiles were assessed. There was a statistically substantial difference between the mean values of body fat between quartiles 1 and 4 in females, in line with the Tukey-Kramer multiple comparisons test. The results are shown in TABLE 2.

Table 2- Analysis of variance between the quartiles of body fat and percentage of body fat in the sample composed of adolescents between 10 and 14 years old, Vitória, Espírito Santo. N=390

| | p-value† | Significant differences |
|---|---|---|
| Female | | |
| Body Fat | 0,031* | 1° e 4° quartiles** |
| % Body Fat | 0,471 | |
| Male | | |
| Body Fat | 0,542 | |
| % Fat | 0,141 | |

Caption: N, sample number; †, according to ANOVA Oneway or Kruskall-Wallis Test; *, statistically significant (α=0.05); ** second Tukey-Kramer Test (α=0.05)

The cutoff points in both sexes, categorized every 0.5 years, that is, 10.5 years, 11 years, 11.5 years, 12 years, 12.5 years, 13 years, 13.5 years, 14 years and 14.5 years are shown in TABLE 3. The cutoff points were considered using z-scores, above the mean, being overweight between a standard deviation z+1 and z+2, obesity between z+2 and z +3, and severe obesity above z+3.

Table 3- Cutoff points* for % Body fat, according to sex, according to the deuterium oxide dilution method in a sample of adolescents between 10 and 14 years old, Vitória, Espírito Santo. N=390

| | Eutrophy | Overweight | Obesity | Severe obesity |
|---|---|---|---|---|
| Female | -2 a +1 | +1 a +2 | +2 a +3 | >+3 |
| 10,5 years | <35,33 | ≥35,33 e <44,21 | ≥44,21 e <53,15 | ≥53,15 |
| 11,0 years | <35,84 | ≥35,84 e <44,41 | ≥44,41 e <53,06 | ≥53,06 |
| 11,5 years | <36,23 | ≥36,23 e <44,56 | ≥44,56 e <53,00 | ≥53,00 |
| 12,0 years | <36,54 | ≥36,54 e <44,70 | ≥44,70 e <52,94 | ≥52,94 |
| 12,5 years | <36,80 | ≥36,80 e <44,81 | ≥44,81 e <52,88 | ≥52,81 |
| 13,0 years | <37,01 | ≥37,01 e <44,91 | ≥44,91 e <52,83 | ≥52,83 |
| 13,5 years | <37,18 | ≥37,18 e <44,99 | ≥44,99 e <52,78 | ≥52,78 |
| 14,0 years | <37,32 | ≥37,32 e <45,06 | ≥45,06 e <52,73 | ≥52,73 |
| 14,5 years | <37,20 | ≥37,20 e <45,12 | ≥45,12 e <52,68 | ≥52,68 |
| Male | | | | |
| 10,5 years | <34,57 | ≥34,57 e <42,97 | ≥42,97 e <50,61 | ≥50,61 |
| 11,0 years | <33,26 | ≥33,26 e <41,91 | ≥41,91 e <50,23 | ≥50,23 |
| 11,5 years | <32,21 | ≥32,21 e <41,11 | ≥41,11 e <49,93 | ≥49,93 |
| 12,0 years | <31,37 | ≥31,37 e <40,50 | ≥40,50 e <49,68 | ≥49,68 |
| | <30,69 | ≥30,69 e <40,03 | ≥40,03 e <49,47 | ≥49,47 |
| 13,0years | <30,13 | ≥30,13 e <39,66 | ≥39,66 e <49,30 | ≥49,30 |
| 13,5years | <29,67 | ≥29,67 e <39,37 | ≥39,37 e <49,16 | ≥49,16 |
| 14,0years | <29,28 | ≥29,28 e <39,14 | ≥39,14 e <49,04 | ≥49,04 |
| 14,5years | <28,96 | ≥28,96 e <38,95 | ≥38,95 e <48,94 | ≥48,94 |

Caption: *, normal weight defined as < z-2 score, overweight between one standard deviation z+1 and z+2, obesity between z+2 and z+3, and severe obesity above ten+3; N, sample number.

## DISCUSSION

This study is one of the few to present a set of percentile references for body fat in boys and girls, analyzed by isotopic dilution with Deuterium Oxide in adolescents aged 10 to 14 years. The method utilized is considered the gold standard for assessing body composition, being an alternative to the limitations of analyzes that use the body mass index (BMI). The performance of the method is similar to indexes evaluated by other methods. Thus, it has being preferred because of its reliability and facility of calculation[18], an innovation despite the possibility of being used for more than half a century.

The body mass index (BMI) is conventionally used to diagnose obesity. Despite its numerous benefits, there is concern that not all individuals at risk for obesity-associated diseases are being identified, as the percentage of body fat (%BF) and visceral adipose tissue mass are not fully explained by assessment of BMI, therefore not identifying individuals who would benefit most from the therapeutic intervention[5,19]. There was a Gaussian distribution of %BF in both sexes and indicated a trend towards an increase in %BF in females, from 11 years of age, confirmed even by the positive correlation analysis, although small, presented in Figure 1 and reduction of %BF with male quartiles.

This study demonstrated that there was a Gaussian distribution of %BF in both sexes and indicated a trend towards an increase in %BF in females, from 11 years of age, confirmed even by the positive correlation analysis, although small, presented in Figure 1 and reduction of %BF with male quartiles.

It was observed a trend towards an increase in the %BF in females, confirmed even by the positive correlation analysis, although small, shown in Figure 1 and a reduction in %BF with the quartiles in males. The study also showed that girls enter adolescence (10 years) with an absolute amount of body fat statistically lower than in the intermediate period of this phase. This did not occur in boys, who remained, statistically, with the same absolute value for body fat. It was found that girls were diagnosed with overweight, when the %BF was greater than 1/3 of their weight in body fat. The cutoff points of %BF, in girls, tend to increase in each quartile, whereas in boys, they tend to decrease. Dong et al. (2020)20, in a sample of 12,790 Chinese children and adolescents, reported an increase in body fat in boys up to 10 years of age and a reduction between 11 and 14 years, and among girls the increase in %CG was kept relatively constant, a similar result to the one found in our study.

Furthermore, greater adiposity in pre-adolescence is associated with greater height, although accelerated growth in childhood may, by itself, be a risk factor for obesity later in life[21].

It's possible to observe that there is a positive and significant secular trend of increase in %BF and body adiposity, for most age groups, especially in the last three decades[22]. The concern with the increase in body fat in adolescents is justified by its association with the origin of type 2 diabetes mellitus and cardiovascular diseases, although there is great variability in risk prediction, considering that thin individuals may be affected by chronic diseases[23].

The prevalence of overweight was 15.6% and 1.5% with obesity found in this study was the same as in a previous study in the region of Vitória (ES)[24] and in other states in the southeastern region of Brazil[25].

## CONCLUSION

As reported by the established cutoff points, The prevalence of overweight was 15.6% and 1.5% with obesity individuals. There is a trend towards an increase in %BF in the female quartiles and a reduction in %BF in all male quartiles. Girls enter adolescence (10 years old) with a statistically lower absolute amount of body fat than in the intermediate period of this phase. This did not occur in boys, who remain with the same absolute body fat value. The results also indicate that girls are classified as overweight, when they reach 1/3 of their weight is constituted by fat. The cutoff points for girls tend to increase with each quartile, while for boys tend to decrease.

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