

Diagnostic accuracy of anthropometric indices in predicting excess body fat among seven to ten-year-old children

Acurácia diagnóstica de índices antropométricos na predição do excesso de gordura corporal em crianças de sete a dez anos

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ABSTRACT: *Purpose:* To evaluate the accuracy of the body mass index (BMI), waist circumference (WC) and waist-to-height ratio (WHtR) in detecting excess body fat among schoolchildren in Florianópolis, Santa Catarina. *Methods:* Cross-sectional study with 2,772 schoolchildren aged seven to ten years. *Receiver Operating Characteristic (ROC) curves* were used to compare the diagnostic accuracy of the BMI, the WC and the WHtR in identifying children with excess body fat (defined as values that were equal to or higher than the 90th percentile of the standardized residuals obtained from the sum of the four skinfolds thickness). Likelihood ratio estimates were used to select, for each anthropometric index, the cut-off points that presented the highest association with excess body fat. *Results:* The BMI, WC and WHtR performed well in detecting excess body fat, indicated by areas under the ROC curve (AUC) close to 1.0, with slightly higher AUC for the BMI in comparison to the WC and the WHtR concerning both sexes. Highly sensitive and specific cut-off points were derived for the three anthropometric indices. Sensitivity ranged from 85.7 to 92.9% for the BMI, from 78.6 to 89.7% for the WC, and from 78.6 to 89.2% for the WHtR. Specificity ranged from 83.2 to 91.4%, from 75.0 to 90.7%, and from 77.4 to 88.3% for the BMI, the WC and the WHtR, respectively. *Conclusion:* BMI, WC and WHtR can be used as diagnostic tests to identify excess body fat in children from seven to ten years of age.

Keywords: Child. Adiposity. Body Mass Index. Waist circumference. Abdominal obesity. Test validity.

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RESUMO: *Objetivo:* Avaliar a acurácia do índice de massa corporal (IMC), circunferência da cintura (CC) e razão cintura-estatura (RCEst) no diagnóstico do excesso de gordura corporal em escolares de Florianópolis, Santa Catarina. *Métodos:* Estudo transversal com 2.772 escolares de sete a dez anos. Curvas ROC (*Receiver Operating Characteristic*) foram utilizadas para comparar a acurácia diagnóstica do IMC, CC e RCEst na identificação de crianças com excesso de gordura corporal (definido como valores iguais ou superiores ao percentil 90 do resíduo padronizado do somatório de quatro dobras cutâneas). A estimativa da razão de verossimilhança foi utilizada para selecionar, em cada índice antropométrico, o ponto de corte de maior associação com o excesso de gordura corporal. *Resultados:* O IMC, a CC e a RCEst obtiveram bom desempenho na detecção do excesso de gordura corporal, indicado por áreas sob a curva (ASC) ROC próximas a 1,0, com uma ASC um pouco maior para o IMC do que para a CC e RCEst em ambos os sexos. Para os três índices antropométricos, pontos de corte altamente sensíveis e específicos foram derivados. A sensibilidade variou de 85,7 a 92,9% para o IMC, de 78,6 a 89,7% para a CC e de 78,6 a 89,2% para a RCEst. A especificidade variou de 83,2 a 91,4%, de 75,0 a 90,7% e de 77,4 a 88,3% para o IMC, CC e RCEst, respectivamente. *Conclusão:* O IMC, a CC e a RCEst podem ser usados como teste diagnóstico para identificar o excesso de gordura corporal em crianças de sete a dez anos.

Palavras-chave: Criança. Adiposidade. Índice de Massa Corporal. Circunferência da cintura. Obesidade abdominal. Validade dos testes.

INTRODUCTION

Adequate diagnoses of weight excess in children and adolescents and early intervention are important aspects in reducing the risk of diseases related to obesity in adulthood. Since obesity is linked to adiposity as well as to risks to health¹, the precision of anthropometric adiposity indices must be evaluated based on their capacity to detect excessive body fat or harmful effects to an individual's health. Although the adverse effects of weight excess on health are considered as resulting from excessive body fat, due to the easiness to conduct this measurement and the simplicity to obtain its components, simple and reproducible techniques — such as the body mass index (BMI) and circumference indices — have been extensively utilized as the proxy for adiposity. The most adequate methods to measure body fat, such as dual-energy X-ray absorptiometry (DEXA) and hydrostatic weighing, are very complex and expensive, in addition to being generally limited to small-scale studies². For this reason, in some population studies, body mass has been measured by skinfold thickness and used to validate the capability of simpler anthropometric indices (for instance, BMI and waist circumference [WC]) to diagnose body adiposity.

Systems of classification of the nutritional status of children and adolescents have been recommended by the International Obesity Task Force (IOTF)³ and by the World Health Organization⁴, based on the BMI for each age range. National systems are also noteworthy, such as the one devised

for the Brazilian population⁵. One of the limitations of these BMI-based systems is the fact that this index is an indirect measurement of adiposity that expresses the sum of all body weight components (lean body mass, bones, adipose tissue), without distinction between lean and fat mass⁶. Furthermore, the BMI varies according to age, sex, body composition and sexual maturity stages⁷.

Owing to the limitations of the BMI, the use of WC and waist-to-height ratio (WHtR) has been suggested to evaluate adiposity. In studies with children and adolescents, authors have observed a significant association between cardiovascular risk factors and WC^{8,9}. The latter has been defended as a more precise indicator of visceral adipose tissue and of health risks related to obesity in comparison to the BMI¹⁰. However, because there are no specific critical values for the Brazilian population or internationally accepted values to classify abdominal adiposity in children and adolescents, the use of WC and its recommendation in public health have been limited.

WHtR incorporates WC as a measurement of abdominal adiposity and adjusts it to the individual's size by dividing it by his/her height¹¹. In studies carried out with children, WHtR has been indicated as a better risk predictor than BMI or WC for health outcomes^{8,12}. In spite of the large number of studies published on the prevalence of excess weight among children and adolescents, there are still controversies about the best indicator of excess body fat to be used.

In the present study, our purpose is to analyze the diagnostic accuracy of the BMI, WC and WHtR in tracking excess body fat determined by skinfold thickness in seven to ten-year-old children. In addition, we identified the cut-off points of the three anthropometric indices that presented a more marked association with body fat excess.

METHODS

SAMPLE

This is a cross-sectional population-based study conducted from September to November, 2002. We used data containing the complete information of a probabilistic sample of seven to ten-year-old students, enrolled in elementary schools in the city of Florianópolis, Santa Catarina. Details pertaining to the sample are available in other publications^{13,14}. In summary, a representative sample of students enrolled in the first to fourth grades of elementary schools in the city in question was selected from a sample stratified by clusters. The information included anthropometric data (body mass, height, skinfolds and arm, waist and hip circumference) and familial socioeconomic data. The present study only addresses measurements of the children's body mass, height, WC, and four skinfolds (triceps, subscapular, supriliac and medial calf).

Out of the 3522 children enrolled from the first to the fourth grades in the elementary schools selected, we excluded the data of 209 who were outside the age range delimited in the study (< 7 or > 10 years) and of 377 due to lack of information (the child was either absent or ill on the day of the anthropometric assessment, or refused to participate in the study). The parents signed the Informed Consent form so that their children could participate in

this study, which was approved by the Ethics Committee of Universidade Federal de Santa Catarina (report number 037/02).

ANTHROPOMETRIC MEASUREMENTS

The administrative department of each school provided information about the children's age and sex. The BMI, WC, WHtR and the sum of the four skinfolds thickness (SFT) were selected to assess the participants' nutritional status. The anthropometric measurements were taken in the schools selected by two Physical Education teachers who were trained by an experienced anthropometrist so as to ensure technique standardization. The anthropometric measurements were conducted with the children barefoot and wearing light clothes, using standardized techniques recommended by Lohman et al.¹⁵. Their body mass was measured by means of a portable digital scale with capacity for up to 180 kg (MARTE[®], model PP), and the height was taken with a measuring tape attached to a wall without a baseboard. The BMI was calculated as body mass (in kg) divided by squared height (in meters). WC (cm) was measured at the middle point between the last rib and the upper edge of the iliac crest (natural waist) with a non-elastic tape. WHtR was determined by the ratio between WC (cm) and height (cm).

The skinfolds were measured in four locations of the individuals' bodies: triceps, subscapular, suprailiac and medial calf using a skinfold compass (CESCORF[®], *Scientific Sports Equipment*, Porto Alegre, Rio Grande do Sul, Brazil) on the right side of the body. The measurements were taken twice and a third time when the skinfolds differed in more than 1mm. The average of the measurements in each location or of the two most approximate readings was used in the analysis.

Due to the importance of age in body fat variation, SFT values were modeled as a polynomial function of age (age in completed years, age squared and age cubed) in linear regression models weighted by the inverse of the variance. Using the model with the highest Pearson's coefficient (R^2), we estimated the standardized residuals (variation of the skinfolds, regardless of the linear effect of age) for each sex. Values that were higher than ± 4 standard deviations of the standardized residuals were excluded (73 boys, 91 girls), resulting in a final sample of 2722 children (51,6% boys). The non-conventional value of ± 4 standard deviations was chosen to preserve the sample's heterogeneity as much as possible. The values from the sum of skinfolds (standardized residuals) that were equal to or higher than the 90th percentile were adopted as the reference measurement to classify excess body fat. The absolute values of the BMI, WC and WHtR were standardized according to the individuals' sex and age, and converted into Z scores so as to be utilized in the analyses as diagnostic tests.

STATISTICAL ANALYSIS

The differences between the sexes regarding the averages of the anthropometric variables were analyzed by means of Student's *t* test for independent samples. To evaluate

the differences of the areas under the curve (AUC) *Receiver Operating Characteristic* (ROC) among the anthropometric indices, we observed the superposition of confidence intervals of 95% (95%CI).

The analysis of the ROC curve was conducted to evaluate the overall performance of the BMI, WC and WHtR in detecting excess body fat. The AUC was used as a global measurement of accuracy of the BMI, WC and WHtR in screening excess body fat. AUCs may vary between 0 and 1; values higher than 0.5 indicate that the selected tool is better than random classification, and 1 implies a perfect performance.

In order to determine the BMI, WC and WHtR values that were more closely associated with excess body fat, referred to as optimal cut-off points from this point on, we calculated positive [sensitivity/(1-specificity)] and negative likelihood ratio [(1-sensitivity)/specificity] of these anthropometric indices for each age and sex. Likelihood ratios (LRs) shows how many times it is more (or less) probable that a test result will be found in sick people compared to individuals who do not present the same illness. In the context of the present study, LRs indicate how many times the results of the BMI, WC and WHtR are more likely to be found in individuals with excess body fat in relation to those without it.

Positive likelihood ratio (+LR) higher than 10 and negative likelihood ratio (-LR) lower than 0.01 have yielded diagnoses with convincing evidence, whereas values above 5.0 and below 0.2 indicate moderate diagnosis evidence^(17,18). In the present study, the selection of optimal cut-off points was carried out in search of an average value for the BMI, WC and WHtR among participants who showed +LR between 5 and 10, and -LR between 0.1 and 0.2 — according to age and sex — in other words, cut-off points that would potentially yield moderate diagnoses of excess body fat.

The probability of identifying excess body fat in children diagnosed as being or not being excess weight (according to optimal BMI cut-off points) and as having or not having excessive abdominal adiposity (according to optimal WC and WHtR cut-off points) was calculated through the equation¹⁸ Post-test odds = Pre-test odds x Likelihood ratio (+LR or -LR), in which Pre-test odds = Prevalence/1-Prevalence.

To calculate the Pre-test odds, we used the prevalence of excess body fat based on the sample's SFT 90th percentile (standardized residuals), according to sex and age. Finally, the values of the Post-test odds were converted into probabilities: Post-test probability = Post-test odds/1 + Post-test results.

The diagnostic accuracy of the anthropometric indices in identifying excess body fat was determined by the ROC AUC, and the diagnostic accuracy of the optimal cut-off points of these indices was determined by sensitivity, specificity, +LR, -LR, positive post-test probability (PPP) and negative post-test probability (NPP).

The significance level was $p < 0.05$. We conducted the statistical analyses by using the SPSS 15.0 (*Statistical Package for Social Sciences*) and the Stata 10.0 (*Stata Corporation, College Station, TX 10.0*).

RESULTS

The sample's anthropometric characteristics are displayed on Table 1. Compared to the girls, the boys were significantly taller, heavier, and presented higher averages of BMI, WC and WHtR. On average, the girls presented higher statistically significant SFT values than the boys.

Figure 1 shows the performances of the ROC curves concerning the BMI, WC and WHtR of the boys (a) and girls (b). All anthropometric indices obtained good performances in identifying excess body fat, as indicated by AUC values that are equal to or higher than 0.90. Although the 95%CI did not show statistically significant differences among the indices in regards to the AUC, the BMI performed better in identifying children of both sexes who had excess body fat.

The optimal cut-off points of the BMI, WC and WHtR and their respective +LR and -LR values, as well as sensitivity, specificity and post-test probability of excess body fat are presented on Table 2. All cut-off points chosen through analyzing the LR showed a balance between diagnosis sensitivity and specificity. However, the optimal cut-off points of the BMI presented better indices of diagnostic accuracy in identifying excess body fat than the WC and the WHtR. For instance, the data displayed on Table 2 show that a seven-year-old boy with excess body fat is 10.36 times more likely to be classified as being excess weight by the optimal cut-off points established for the BMI than a boy of the same age without excess body fat. On the other hand, a seven-year-old boy without excess body fat is only 0.08 times more likely to be classified as being excess weight in comparison to a boy of the same age with excess body fat.

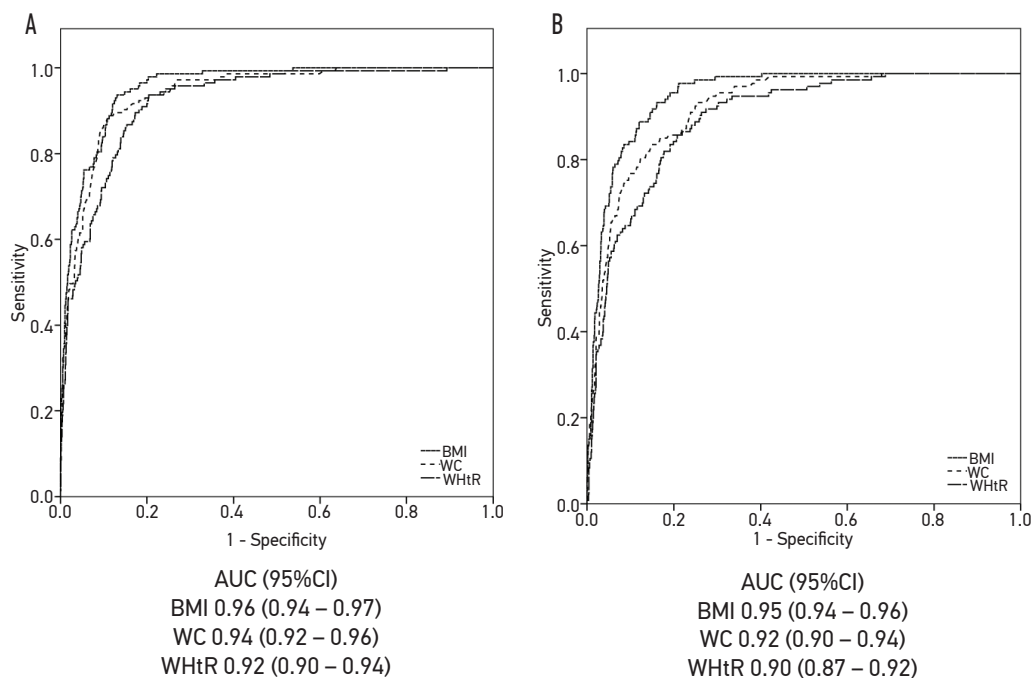
Although we adopted +LR values between 5 and 10, and -LR values between 0.1 and 0.2 when choosing the optimal cut-off points, this combination was not possible for the WHtR in

Table 1. Anthropometric characteristics of the children according to sex. Florianópolis, 2002.

	Boys (n = 1431)	Girls (n = 1341)	p-value
	Mean (SD)		
Age (years)	8.6 (1.1)	8.5 (1.1)	0.407*
Body mass (kg)	31.1 (7.0)	30.4 (6.9)	0.006*
Height (cm)	134.0 (8.4)	133.2 (8.9)	0.024**
BMI (kg/m ²)	17.1 (2.3)	16.9 (2.4)	0.019*
WC (cm)	59.3 (6.0)	57.4 (5.8)	< 0.001*
WHtR	0.44 (0.0)	0.43 (0.0)	< 0.001*
SFT (mm)	37.3 (19.1)	44.2 (18.8)	< 0.001*

*Student's *t*-test for equal variances; **Student's *t*-test for different variances.

SD: standard deviation; BMI: body mass index; WC: waist circumference; WHtR: waist-to-height ratio; SFT: sum of the thickness of four skinfolds.



AUC: area under the curve; BMI: body mass index; WC: waist circumference; WHtR: waist-to-height ratio.

Figure 1. Receiver Operating Characteristic curves for body mass index, waist circumference and waist-to-height ratio in boys (a) and girls (b) and their corresponding area under the curve.

the girls' case. Although the +LR and the -LR of the WHtR cut-off points chosen girls were more modest — 3.48 and 0.28 respectively — they were sufficiently strong to suggest that this cut-off point can be useful in identifying a child with excess body fat.

Overall, the post-test probability of a child classified as not being excess weight or without abdominal adiposity presenting excess body fat — as per the optimal BMI, WC and WHtR cut-off points — dropped from 10% (pre-test probability) to 2.2% at the most among the boys, and to 2.8% among the 10-year-old girls. When the test result was positive (a child classified as being excess weight or having abdominal adiposity according to optimal BMI, WC and WHtR cut-off points), the probability of a child presenting excess body fat rose to over 36.0% among the boys and to 23.5% among the 10-year-old girls (Table 2).

DISCUSSION

In this study, we compared the diagnostic accuracy of the BMI, WC and WHtR in identifying children with excess body fat (expressed through the sum of each individual's skinfolds) in a sample of seven to ten-year-old students in the city of Florianópolis. Furthermore, we

Table 2. Cut-off points for body mass index, waist circumference and waist-to-height ratio that presented a more marked association with excess body fat, according to sex and age. Florianópolis, 2002.

	Age	Boys						
		Cut-off point [#]	Se%	Sp%	+LR	-LR	NPP (%)	PPP (%)
BMI (kg/m ²)	7	17.5	92.9	91.0	10.36	0.08	0.8	50.0
	8	17.7	89.2	91.4	10.35	0.12	1.4	54.0
	9	18.6	89.7	88.2	7.62	0.12	1.3	45.0
	10	19.9	89.7	90.9	9.87	0.11	1.4	55.5
WC (cm)	7	59.1	89.3	90.7	9.59	0.12	1.2	48.1
	8	60.5	83.8	84.0	5.24	0.19	2.1	37.3
	9	63.2	89.7	88.8	7.99	0.12	1.3	46.2
	10	66.4	89.7	90.6	9.53	0.11	1.4	54.6
WHtR	7	0.46	85.7	88.3	7.31	0.16	1.5	41.4
	8	0.46	86.5	85.5	5.98	0.16	1.8	40.4
	9	0.45	84.6	83.8	5.23	0.18	1.9	36.0
	10	0.46	84.6	86.7	6.36	0.18	2.2	44.5
Girls								
BMI (kg/m ²)	7	17.1	89.7	88.0	7.50	0.12	1.2	44.1
	8	18.2	90.0	88.7	7.93	0.11	1.3	49.2
	9	18.7	91.9	90.1	9.28	0.09	1.1	52.4
	10	18.5	85.7	83.2	5.11	0.17	1.6	33.3
WC (cm)	7	56.8	82.8	85.9	5.86	0.20	2.1	38.1
	8	59.0	87.2	85.0	5.80	0.15	1.8	41.5
	9	61.5	89.2	88.5	7.76	0.12	1.4	47.9
	10	61.1	78.6	75.0	3.14	0.29	2.8	23.5
WHtR	7	0.45	82.8	80.4	4.23	0.21	2.2	30.7
	8	0.45	82.1	82.2	4.61	0.22	2.6	36.1
	9	0.44	89.2	85.6	6.20	0.13	1.5	42.4
	10	0.43	78.6	77.4	3.48	0.28	2.7	25.3

Se: sensitivity; Sp: specificity; +LR: positive likelihood ratio; -LR: negative likelihood ratio; NPP: negative post-test probability; PPP: positive post-test probability; BMI: body mass index; WC: waist circumference; WHtR: Waist-to-height ratio.

[#]Optimal cut-off points determined by LR analysis.

determined the cut-off points of the indices which presented a more marked association with excess body fat. The analytical procedures employed enabled us to establish estimates of the probability that the children classified as being excess weight or not, or as having abdominal adiposity or not, would present excess body fat.

The variation of AUC values (0.90 – 0.96) found in this study indicated that the three anthropometric indices in question achieved good performances in diagnosing excess body fat in children.

In other studies, some authors have suggested that central adiposity measurements are the best tools to identify children and adolescents with cardiovascular risk factors^{8,19,20}. In a study conducted with French children (6.8 – 11.8 years of age), WC was more consistent in discriminating obesity (determined by measuring skinfold thickness) than the BMI or WHtR²¹. However, other authors have reported minimal differences in the capability of the BMI, WC and WHtR to identify children and adolescents with cardiometabolic risk markers^{22,23}.

The diagnostic accuracy of the BMI, WC and of the waist-to-hip ratio (WHR) in screening obesity was evaluated in 17-year-old Swedish teenagers using plethysmography as a reference measurement. The results showed that the BMI and the WC had better diagnostic performances in screening obesity among the teenagers than the WHR²⁴.

The association between BMI, WC and body composition indices (total and truncal fat and lean mass) was evaluated in black prepubescent children in South Africa. The results showed that the BMI presented a statistically higher significant correlation to body composition than WC, and that this association was significantly more marked in relation to fat mass than lean mass, thus suggesting that the BMI is a better instrument to screen body fat than WC²⁵. The authors explained this result through the fact that the centralization of body fat occurs after puberty, and for this reason it was not expected that WC would reflect fat centralization prior to the beginning of puberty.

When selecting the best anthropometric index as a tool to triage excess body fat, other authors have also utilized ROC curve analyses, including when determining the cut-off points more closely associated with this outcome^{21,24,26,27}. In these studies, the choice of indices was made considering calculations of sensitivity and specificity, and the optimal cut-off points were selected based on anthropometric indices that either yielded equal estimates of sensitivity and specificity or minimized classification errors. The analytical difference of the present study in comparison to others published on the topic in question is that, after calculating the sensitivity and specificity for all standardized values of the BMI, WC and WHtR, we estimated the +LR and -LR that aided in choosing cut-off points that presented a closer association between body fat and excess weight (using BMI) or between excess body fat and central adiposity (using WC or WHtR). One of the advantages of the LR is that it is conducive to the description of the accuracy of a test of numerical results at several cut-off points. In this sense, analyzing the LR allowed us to choose a value for each index (according to sex and age) that would yield evidence-based results for moderate diagnoses, which can be considered the best scenario for interventions in public health. With exception of the WHtR in the girls' case, our selection method allowed all chosen cut-off points presented

the same diagnostic accuracy, differently than what occurs when one opts for analyzing sensitivity and specificity maximization for each sex and age.

In the present study, the optimal cut-off points were selected based, firstly, on the chance that a child who was classified as overweight or as having excessive abdominal adiposity would also present excess body fat; in other words, true positive cases, as tests with high +LR values provide strong evidence for a diagnosis when positive. We also took into consideration a potential low frequency of false negative cases (in the case of the BMI's cut-off points, this refers to the children classified as not being overweight, but who had excess body fat nonetheless), as tests with low -LR values are the best to exclude the possibility of illness. In other studies, different cut-off points can be chosen, depending on whether minimizing the number of false positive cases in relation to false negatives is deemed more important than the opposite scenario.

In this study, the BMI's optimal cut-off points for classifying excess weight in children from seven to ten years of age presented lower values than the international recommendation³ for both sexes and lower values than the Brazilian proposal⁷ in the case of the boys. This result may indicate that estimates of overweight prevalence based on the onset of illnesses in adults (exactly like the critical values proposed by Cole et al.³, and Conde e Monteiro⁵) tend to be more conservative than the outcomes based on anthropometric indices that point out body fat values of children within a self-same sample.

Children-focused WC reference values are scarce, and this hinders comparisons to the cut-off points determined in this study. In England, McCarthy et al.²⁸ identified WC cut-off points among seven to ten-year-old boys and girls that are similar to the ones found in this study. However, the same WC cut-off points indicated in the present study were approximately 4.2 cm smaller for the boys and 6.3 cm smaller for the girls, all between seven and ten years of age, than the average cut-off values suggested in a study carried out with New Zealander children²⁶. Compared to the study conducted with French children ranging between 6.8 and 11.8 years of age²¹, the optimal WC cut-off points to identify excess body fat in the present study were, on average, 5.2 and 9.9 cm smaller in boys and girls, respectively. Among the reasons for the differences in cut-off values of the anthropometric indices in question found in other studies are the diversity of methods chosen to measure body fat (for instance, skinfolds, bioimpedance, plethysmography, and DEXA); the methods selected to express excess body fat (for instance, predictive equations of body fat percentage, such as those devised by Lohman²⁹ and Slaughter et al.³⁰, as well as percentiles of skinfold sums); and also how anthropometric data are treated. Regarding this latter reason, we highlight that, in this study, the SFT values were analyzed so as to yield the values of the residuals (body fat values independent of the effect of the children's age). We can also mention issues related to ethnicity and puberty stages, as well as differences in the prevalence of excess weight and abdominal adiposity among the populations studied, and the fact that these research studies were conducted at different moments.

Among the relevant aspects of this study, we highlight the large size of the sample along with the use of LR and post-test probability analyses, which aid in refining the results of diagnostic tests, a step forward in relation to analyses of sensitivity and specificity, widely utilized in other studies. Moreover, we must also mention that we did not use any sophisticated reference measurement

for body fat due to the fact that this is a population-based study. Skinfold measuring has been used in several population-based studies to estimate the individuals' body fat^{27,31,32}. The use of the 90th percentile based on a sample of absolute measurements of the skinfolds (standardized residuals) as the cut-off point for excess body fat was arbitrary in the present study. However, several authors have used prediction equations to calculate body fat percentage (BF%), indicating fixed percentages (for instance, BF > 25% for boys and BF > 30% for girls) to define excess body fat in children^{21,33}. This proposal, however, is questionable because predictive equations are susceptible to errors, as they are specific to the population in which they were developed^{34,35}. Furthermore, the quantity of body fat can vary according to age and sex³⁶.

Our data indicate that the diagnostic accuracy of the BMI, the WC and the WHtR was similar, although the BMI is more consistent in identifying children with excess body fat in relation to individuals without this condition. Our results also showed that optimal BMI, WC and WHtR cut-off points can also be used to effectively predict excess body fat, as expressed through skinfold measurement, in seven to ten-year-old children in Florianópolis. These cut-off points must be compared to health biological markers in future studies.

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