ACCEPTED version by the Nutrition in Clinical Practice after peer review

Finding the best waist circumference measurement protocol in patients with Non-alcoholic Fatty Liver Disease

Short title: Waist circumference in Liver Disease

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Keywords: Waist Circumference; Fatty Liver; Body Composition; Body Fat; Anthropometry; body circumferences; DXA; Hepatic Steatosis

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Abstract

Background

Central fat accumulation is important in Non-alcoholic Fatty Liver Disease (NAFLD) etiology. It is unknown weather any commonly used waist circumference (WC) measurement protocol (mp), as whole and central fat accumulation marker, is preferable for patients with NAFLD. The present study sought to find a preferable WC mp to be used in patients with NAFLD, based on three-fold criterion.

Material and methods

Body fat (BF) was assessed through Dual Energy X-ray Absorptiometry (DXA) in 28 patients with NAFLD (19 males, 51 + 13 yrs, and 9 females, 47 + 13 yrs). WC was measured using four different WC mp (WC1- narrowest torso, WC2- just above iliac crest, WC3- mid-distance between iliac crest and last rib and WC4- at the umbilicus).

Results

All WC measurements were highly correlated particularly with central BF depots, including trunk BF (r=0.78; r=0.82; r=0.82; r=0.84; respectively for WC1, WC2, WC3 and WC4) abdominal BF (r=0.78; r=0.78; r=0.80; r=0.72; respectively for WC1, WC2, WC3 and WC4) and central abdominal BF (r=0.76; r=0.77; r=0.78; r=0.68; respectively for WC1, WC2, WC3 and WC4), controlling for age, sex and body mass index. There were no differences between the correlation coefficients obtained between all studied WC measurements and each whole and central analyzed BF variable.

Conclusion

All studied WC mp seem suitable for use in patients with NAFLD, particularly as central BF clinical assessment tool, though not interchangeably. Hence biological and precision criteria alone did not sanction the superiority of any WC mp. Practical criteria may endorse WC measured at the iliac crest.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is a rising recognized condition that has caught growing attention. In an advanced stage NAFLD can ultimately lead to advanced fibrosis, cirrhosis, liver failure and death [1,2]. NAFLD is also associated with increased risk of cardiovascular events [3]. Both central body fat (BF) and insulin resistance have been found to increase the risk of NAFLD [4], furthermore whole and particularly central BF may also increase the risk for NAFLD by its strong association with insulin resistance [5,6]. Excess both whole and central BF accumulation is also a known cardiovascular risk factor [7,8]. These evidences arise the importance, particularly in this higher risk sub-population, of finding risk related clinical body composition surrogates, and potential therapy targets.

Waist circumference (WC) measurement is widely used in different settings and populations [9–11], including the sub-population of patients with NAFLD [12]. WC has been considered a proper surrogate of body composition, particularly when focusing on body fat distribution [9,13,14], and a risk factor for NAFLD [15]. In patients with NAFLD WC has been found to be associated with several metabolic impairments including insulin resistance [5,16] as well as liver fat [12] and NAFLD severity [17]. Even though widely used, there is currently no optimal and uniquely proposed WC measurement protocol (WCmp) to be used in clinical practice, either in the general population as in specific higher risk sub-populations. Several WCmp have been suggested but scientific rational is lacking to recommend one single protocol [10,18]. Suggested protocols differ mainly on the anatomical landmarks and correspondent measuring sites. The most commonly used WC measurement sites are the midpoint between the lowest rib and iliac crest, the umbilicus and the minimal waist, still a fourth measurement site has also been used and endorsed, which is at the superior border of the iliac crest [9,10]. Nevertheless several other measuring sites have been sparsely used [10].

To our knowledge this is the first study to look into the usefulness of commonly used waist circumference measurements as surrogates of whole and central body fat content in patients with NAFLD. Therefore the aim of the present study was to find which of the most used WCmp is the best for use in clinical practice in patients with NAFLD, considering a threefold criteria: the WC most closely associated with whole and central BF content in patients with NAFLD; the most precise WCmp; the most practical WCmp to use in clinical practice.

Materials and methods

Subjects:

This study was conducted at Exercise and Health Laboratory, from the Interdisciplinary Centre for the Study of Human Performance (Faculty of Human Kinetics, Technical University of Lisbon, Portugal). To be selected for the present study subjects had to be over 18 years of age, diagnosed with NAFLD, without history of hepatotoxic substances intake (eg. steroids) and tobacco consumption. Exclusion criteria included alcohol consumption over 20 gr/day; the presence of other possible causes for fatty liver disease, including hepatitis, self-immune disease and others; any physical and/or mental disabilities or any condition that constituted an absolute restriction from exercise, or diseases, other than metabolic (insulin resistance, hypertension or dyslipidemia), with mandatory specific pharmacologic therapy (eg. Asthma or other). We studied 28 patients with NAFLD (19 males, 51 + 13 yrs, and 9 females, 47 + 13 yrs) who were diagnosed through liver biopsy or ultrasound. Subjects were recruited from the outpatient periodical medical consultations in Santa Maria Hospital and Curry Cabral Hospital; 59 consecutive patients were selected based on selection criteria; 37 of the selected subjects accepted to participate and 28 were found eligible to enter the study after exclusion criteria was considered. Subjects were taking one or more of the following medication: platelet inhibitors, angiotensin-converting enzyme inhibitors, nitrates, statins, ezetimibe, nicotinic acid and biguanides with similar use among both genders. All participants signed an informed consent before being included in the present study and undergoing any study procedure. All methods used in the present study complied with good ethics and Portuguese laws and were approved by both the Portuguese Foundation for Science and Technology and Faculty of Human Kinetics institutional review boards.

Body composition:

Body composition was assessed using Dual Energy X-ray Absorptiometry (DXA) (Explorer W, Hologic; Waltham, MA, USA; Fan bean mode) whole body scans and anthropometric measurements. Repeated measurements in 18 young adults showed a coefficient of variation (CV) of 1.7% for total BF mass and 1.5% for total %BF. All scans were made in the morning after an overnight 12-hour fast. Quality control with spine phantom was made every morning, and with step phantom every week. By default DXA software (QDR for windows, version 12.4) estimates the head, trunk, arms and legs, both left and right, regions fat content, according to a three-compartment model (fat mass, lean tissue and bone mass). Whole BF, sometimes just referred to as BF, includes the fat mass of the whole body, as opposed to central BF, which refers to the trunk BF, abdominal BF and central abdominal BF variables, as analyzed by DXA regions of interest. The trunk region of interest (ROI) (CV = 0.5%) includes chest, abdomen and pelvis, to provide estimates of trunk BF and trunk %BF. All scans were submitted to additional analysis by ROI to assess absolute a relative fat content of the abdominal (Abd BF and Abd %BF) and central abdominal regions (CAbd BF and CAbd %BF) (Fig. 1) (CV = 1.0 %). The upper and lower limits of the abdominal and central abdominal ROI were determined as the upper edge of the second lumbar vertebra to the lower edge of the fourth lumbar vertebra, respectively [19–21]. The sides' limits of the abdominal ROI were determined as to include all trunk length, but exclude any upper limb scan area [20,21], whereas the vertical sides of central abdominal ROI were the continuation of the lateral sides of the ribs cage, as to exclude the lateral subcutaneous fat of the trunk, including the anterior and posterior subcutaneous abdominal fat, as well as the intra-abdominal fat [19], as seen in figure 1. Absolute and relative BF content results were registered to the nearest 0.01kg and 0.1%, respectively.

Anthropometric measurements consisted of weight, height and body mass index (BMI) as well as WC. Some standardization procedures were taken into account, as proposed by Agarwal and colleagues [22] to avoid any bias in the measurements, therefore all WC measurements were made with subjects in a standing comfortable position, in their underwear, in a 12-hour fasting state. All WC measurements were made by the same technician, who was a trained level 2 technician, certified by the International Society for the Advancement of Kinanthropometry, using an inelastic flexible metallic tape (Lufkin - W606PM, Vancouver, Canada) parallel to the floor after a tidal expiration, to the nearest 0.1cm. The WC measurement sites in the present study were the narrowest torso (WC1) [23,24], also called minimal waist[10], superior border of the iliac crest (WC2) [18,25], midpoint (WC3) [26] and umbilicus (WC4) [27,28], as detailed in table 1, and represented in figure 2. These are the most commonly used protocols endorsed by sound authorities in this field [9,10]. Time length of each WC measurement, including all procedures, from brief initial instruction results registering, was recorded in seconds, to the nearest 1 second, with a standard watch chronometer (model RS800, Polar, Oulu, Finland). Body weight was measured to the nearest 0.1kg, and height was measured to the nearest 0.1 cm, on a scale with an attached stadiometer (model 770, Seca; Hamburg, Deutschland), according to standard protocol [23]. Both weight and height were used to calculate the subjects' BMI, by dividing the weight, in kg, by the squared height, in meters (BMI = weight [kg] / height [m]^2). All anthropometric measurements were repeated two times, and if the second differed more than 1cm (for waist and height measurements) or 0,5kg (for weight measurement) from the first measurement, a third measurement was carried out. We always considered the result obtained in the second measurement unless a third measurement was carried out. When a third measurement was taken we considered the mode or, if mode was absent, the median value of all three measurements. By using this procedure we sought to always use the most suitable value that was actually measured on the subjects (instead of mean values).

Statistical methods:

Descriptive statistics are presented as mean ± sd and range for all analyzed variables. The Gaussian distribution of the data was assessed with the Shapiro-Wilk goodness-of-fit test. Levenne test was used for assessing sample variance homogeneity. Paired-samples T test was used to compare WC results obtained with different WCmp. When homogeneity of variance was not present, the corrected significance values of T test were used. The association between whole and central BF and the results obtained with each WCmp was assessed using partial as well as part, also called semipartial [29], correlations, controlling for age and sex and BMI. Both partial and semipartial correlation techniques allow controlling for confounders but, while partial correlation remove from the analysis the confounders-related variation of both dependent and independent variables, the semipartial technique only removes from the correlation analysis the confounder-related variation of the independent variable [29]. In order to accomplish a statistical power of 80% (β = 0.20) at a statistical significance level of 5% (α = 0.05), only coefficients of correlation equal or superior to 0.5, corresponding to a large effect size, were considered significant. This is in accordance with Cohen and Cohen [29] to assure that results are unexposed to type I and II errors, despite an rather modest sample size. Pairs of correlation coefficients obtained between each WC with each dependent variable were compared, using Z statistic, to find if any WC was more closely associated with whole and central BF. To evaluate the precision of the studied WCmp we calculated coefficients of variation for the repeated measurements, and compared them between WCmp using paired-samples T test. To respond to the third criteria, comprised in the aim of the present study, paired-samples T test was used to compare the time consumption of WC measurement between different measurement protocols. Statistical calculations were performed using the IBM SPSS Statistics version 19 (SPSS, inc, Chicago, IL), except for z statistic which was performed using Medcalc version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Mean values for all studied variables are presented in table 2. From among the 28 studied patients with NAFLD obesity was present in 9 subjects (3 were female), according to BMI classification, yet mean BMI showed no differences between sexes (p=0.075 on independent samples t test) and was considered to be in the overweight category for the whole studied sample. BMI was also not related with subjects' age (r= -0.222; p=0.266 on Pearson correlation). Results for WC measurements were considered to be different between all studied WCmp, for instance WC4 showed the highest values whereas WC1 showed the lowest, and WC3 was smaller than WC2, as shown in table 3.

Table 4 shows the results for partial and semipartial correlations between each WC and each whole or central studied BF depot controlled for sex, age and BMI. All WC results were somewhat correlated with the overall studied DXA assessed BF depots, controlling for age, sex and BMI, often showing correlation coefficients above 0.5. WC4 was correlated with both absolute and relative whole BF, while WC1, WC2 and WC3 were correlated only with the absolute values of whole BF, but only in partial correlations. All studied WC were correlated with absolute and relative trunk BF, in partial correlations, however, in semipartial correlations, this was true only for the absolute values of trunk BF. WC 1, WC2 and WC3 were associated with absolute a relative values of abdominal fat depots, except for WC4 which was only correlated with Abd BF and Cabd BF, in partial correlations. Semipartial correlations only confirmed the correlation with Abd BF and CAbd BF, for all WC.

Table 5 show the results for the comparison between the correlation coefficients listed in table 4. Comparisons were made between pairs of competing WC correlations results with each dependent variable. No differences were found between all performed correlations with each studied BF depot.

Table 6 shows both the coefficients of variation for the WC measurements according to the studied protocols, as well as the mean±sd time spent for WC measurements using each of the studied WCmp. Coefficient of variation was not different irrespectively of the WCmp used. Time spent in each measurement was longer for WC3 as compared to all others. WC2 was more time consuming than both WC1 and WC4. Both of the latest showed no differences between their mean time lengths of measurements. In summary time length of measurement of the studied protocols was as follows: WC1 and WC4 < WC 2 < WC3.

Discussion

Even though WC has been a widely used measure in the sub-population of patients with NAFLD [12,30], to our knowledge this is the first study to focus on the comparison of different WCmp based on a scientific and practical rational. The prevalence of high levels of BMI, including obese and morbidly obese patients, in the present sample was expected since obesity, along with insulin resistance, have been identified among the strongest risk factors for NAFLD, and therefore highly prevalent in this sub-population [31–34]. WC results were also quite high which was consistent with BMI levels. The magnitudes of WC mean values were different according to the protocol in use, as has been previously reported in different populations [35–37], meaning they are not interchangeable. This has imperative implications advising the consistent use of one single protocol to avoid misinterpretations either when monitoring longitudinal data as when using cut-off for classification of WC values and subjects subject's risk level appraisal [35,38,39]. Based on the small or absent differences reported, particularly in men, between measurements of WC2 (at the superior border of the iliac crest) and WC3 (at the midpoint between lowest rib and iliac crest) [35–37], it was proposed that current WC thresholds, generalized using WHO WCmp (WC3), could be applied to measurements using WC2 [10]. Our data do not support the mentioned generalization for patients with NAFLD, yet this analysis was not in the aim of the present study, and additional research is needed to look into sex differences and other possible influencing variables. These results reinforce the necessity of searching for the most useful, consensual and standardized WCmp for use in clinical practice with patients with NAFLD.

In the present sample of patients with NAFLD WC was highly associated with whole and central BF, adjusted for age, sex and BMI. The association of WC with BC, particularly with central BF has long been reported in diverse populations [40–42]. Wang and colleagues (2003) had already found stronger associations between WC and absolute central BF, as opposed to either whole BF or any relatively expressed BF depot, assessed by DXA. In the present report, the results obtained in partial correlation may suggest that WC1, WC2 and WC3 are more consistently associated with central BF, and therefore seem better markers of a more hazardous fat accumulation in the studied sample of patients with NAFLD, as opposed to WC4 which seems more consistently associated with Whole BF. When the results obtained in semipartial correlations were taken into consideration, all studied WC were similarly only correlated with central BF accumulation, namely trunk BF, Abd BF and CAbd BF, controlled for age, sex and BMI, revealing a cognate association pattern among different WCmp. Central BF accumulation has been found highly important in NAFLD etiology (Park et al. 2007) and is also a risk factor for cardiovascular diseases incidence and outcomes [7,8], which is increased in NAFLD (Targher et al. 2005). The present results support the use of WC measurement in patients with NAFLD, as a cost-effective screening procedure, to assess hazardous body composition phenotypes, namely central BF accumulation, in routine clinical appraisals, irrespective of the WCmp in use.

Comparisons between pairs of competing WC correlations results with each dependent variable showed no differences meaning all WC results are similarly associated with the analyzed BF depots, irrespectively of the WCmp used, in the studied sample of patients with NAFLD. Never the less, conflicting results can be found in general population [36,37]. A report focusing on different WC measuring sites, including at the superior border of the iliac crest (WC2) and also at the midpoint between lowest rib and iliac crest (WC3), found differences between correlation coefficients, in partial correlations, with abdominal adipose tissue assessed with magnetic resonance imaging, in women but not in men [37]. In agreement with the present report, Wang and colleagues [36] found WC correlations to be stronger with trunk BF, regardless of sex and WCmp. The present results showing a preferable association between WC and preferably central BF, regardless of WCmp, together with the well-established recently reported [10] relationships between WC and morbidity of cardiovascular disease and diabetes and with cardiovascular and all-cause mortality, also rather unaffected the use of different

WCmp, settle both the importance of WC measurement in the screening of patients with NAFLD as well as the usefulness of WC measurement regardless the WCmp in use.

In the absence of biological support for the use of one preferable WCmp, additional criteria have been suggested in the attempt to substantiate the choice of one particular WCmp, including the use of bony landmarks and ease of measurement [9,10,18]. It was argued that the use of bony landmarks could be preferable due to increased precision [9] or reliability [10,18]. The present data do not confirm better precision of any particular WCmp, as assessed by the comparison of coefficients of variation obtained for each WCmp. Similar results have been reported elsewhere in the general population [35,36]. Additional research is warranted to support or reject the preferable use of bony landmarks, particularly for longitudinal assessments. Ease of measurement, meaning that WCmp should require less specific training and be less time consuming, has been proposed as an important criteria for the adoption of any measurement by general public and practitioners in routine clinical practice [9].

It has been suggested that WC measured at the superior border of the iliac crest would be more likely adopted by general public and practitioners as it requires only the palpation of one bony landmark [18] however this was not confirmed by practice nor research [9,10]. Bony landmarks have been subjectively reported to require more training and experience of observers, yet, in the present study only the lowest rib landmark proved demanding. Limited time availability has been proposed as one of the reasons for not using WC measurement in routine clinical practice [9]. Present data also objectively confirm a previous report [35] that subjectively pointed WC measurement at the midpoint between lowest rib and iliac crest as more time consuming than when using other studied WCmp.

There are several strengths and limitations to this study. The studied WCmp do not cover all protocols existent in the literature, yet the focus was set on the most commonly used and endorsed by prominent institutions for use in clinical setting [9,10,18]. Also the used BC assessment method (DXA), a gold standard instrument to assess BC in a three-compartment model, is unable to determine visceral adiposity independently from subcutaneous fat. However strong correlation between abdominal fat estimated from selected DXA ROI and visceral fat measured directly by magnetic resonance imaging (Park et al. 2002) and computed tomography [43,44] have been reported. Due to the cross-sectional approach used, the usefulness of the studied WCmp to assess longitudinal changes in the studied BF depots could not be established, based on the present results. Finally, the size of the sample was rather constrained due to difficulties in the recruitment of such a specific sub-population. 90 individuals were coveted to be included in the present sample in the initial research project. This would allow coefficients of correlation as low as 0.3, traditionally corresponding a moderate effect size, to be considered significant and unexposed to type I and II errors [29]. Unfortunately, despite all efforts on behalf of everyone involved in this research project, only 28 patients with NAFLD could be recruited, meeting all inclusion and exclusion criteria. Consequently, only associations equal or higher to r=0.50 could be considered to attain minimal statistical power of 80% and statistical significance of 5%, and could be considered fairly unexposed to type I and type II errors [29]. Nevertheess the aim of the present study was to find the preferable WCmp to use with patients with NAFLD, which should be found at the higher end of correlational range, therefore, the inability to find significant associations lower than r=0.5, though interesting are not the aim of the present study.

The present study confirms the strong association between WC and central BF, even after removing the effect of age, sex and BMI, regardless of the WCmp in use. Moreover, all tested WCmp could be considered useful and important low-cost assessment tools for clinical practice, though not interchangeable. This irrespective usefulness is bolstered by the similar precision found for all studied WCmp. On the other hand there could not be established one single preferable WCmp for the present sample of patients with NAFLD based on biological criteria. The use of bony landmarks showed no superiority, though research is needed to assess its' relevance in longitudinal assessments. WC measured at the superior border of the iliac crest may be a good choice if a bony landmark is valued together with time consuming.

Acknowledgements

The first author of this paper is supported by a research grant from the Foundation for Science and Technology (FCT), Ministry of Education and Science of Portugal (grant: SFRH/ BD/ 70515/ 2010).

The present study was funded by: the Centre for the Study of Human Performance, Portuguese Foundation for Science and Technology, Lisbon, Portugal;

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TABLES:

Table 1. Circumferences measurement protocols and references.

Measurement	Protocol	References
WC 1	Measured at the level of the narrowest site of the torso (minimal waist).	Lohman et al. (1988) [20]; ISAK (2006) [21]
WC 2	Measured right above the iliac crest.	NIH (1994) [22] CSEP (2010) [15]
WC 3	Measured at the mid-distance between the last rib and the top of the iliac crest.	WHO (1987, 2011) [23, 41]; Nishida et al. (2010) [8]
WC 4	Measured at the level of the umbilicus.	Targher et al. (2008) [25]; Rector et al. (2011) [24]

WC – waist circumference; ISAK – The International Society for the Advancement of Kinanthropometry; NIH – National Institute of Health; CSEP – Canadian Society of Exercise Physiology; WHO – World Health Organization.

Table 2. Descriptive data of the studied sample of patients with Non-Alcoholic Fatty Liver Disease.

	Patients with NAFLD (n=28)			
Variables	**Mean ± sd ***		**Min. – Max.**	
Age, yr (median, yr)	49.5 ± 12.8	(49)	25 – 68	
Sex, n female (% female)	9	(32,1)		
Anthropometry				
Weight, kg	87.6 ± 12.7		66.2 – 115.8	
Stature, cm	167.2 ± 9.2		149.5 – 183.7	
BMI, kg/m^2 (% obese)	29.1 ± 4.0	(32.1)	22.6 – 42.2	
WC 1, cm	100.7 ± 8.2		86.0 – 119.8	
WC 2, cm	104.8 ± 10.6		85.3 – 128.7	
WC 3, cm	103.7 ± 10.4		85.7 – 129.3	
WC 4, cm	106.3 ± 11.5		86.7 – 129.1	
Whole and Regional Body Composition as assessed by DXA				
BF, kg (%)	27.2 ± 9.3	(31.31 ± 8.20)	13.7 – 51.2	(18.84 – 46.28)
FFM, kg (%)	58.7 ± 9.1	(68.69 ± 8.20)	39.6 – 77.7	(53.72 – 81.16)
Trunk BF, kg (%)	15.2 ± 5.2	(33.15 ± 7.65)	7.4 – 25.0	(20.87 – 48.01)
Trunk FFM kg (%)	29.9 ± 3.9	(66.85 ± 7.65)	21.1 – 38.6	(51.99 –79.13)
Appendicular BF, kg (%)	10.8 ± 4.8	(30.42 ± 10.39)	5.2 – 25.7	(13.63 – 50.40)
Appendicular FFM, kg (%)	24.5 ± 5.1	(69.58 ± 10.39)	14.9 – 34.8	(49.60 – 86.37)
Abdominal BF, kg (%)	3.5 ± 1.2	(37.57 ± 6.59)	1.7 – 6.3	(26.09 – 49.40)
Central Abdominal BF, kg (%)	2.9 ± 0.8	(35.82 ± 5.70)	1.6 – 5.0	(24.28 – 44.64)

BMI – body mass index; WC1 – Waist circumference as measured by Lohman et al. [20] and The International Society for the Advancement of Kinanthropometry (ISAK) [21]; WC2 - Waist circumference as measured by the National Institute of Health (NIH) from the United States of America [22]; WC3 - Waist circumference as measured by the World Health Organization (WHO) [41]; WC4 - Waist circumference as measured by Ross et al., Masson et al., Targher et al. and others [7, 25, 36]; DXA – Dual-energy X-ray Absorptiometry; BF – body fat; FFM – fat free mass; * Results are presented as mean ± standard deviation, unless otherwise noted; Min. – lowest observed value; Máx. – highest observed value

Table 3. Mean differences and P values from paired samples T test used in the comparison between waist circumference results obtained with different measurement protocols, in 28 patients with NAFLD

Variables	WC 1, cm		WC 2, cm		WC 3, cm	
	Dif †	p	Dif †	p	Dif †	p
WC2	4,1	0.000	--	--	--	--
WC3	3,0	0.000	-1,1	0.000	--	--
WC4	5.6	0.000	1,5	0.012	2,6	0.001

NAFLD – Non-Alcoholic Fatty Liver Disease; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference measured just above iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; † - difference between results obtained using WC protocols in the Left column and the results from the WC protocols in the top line.

Table 4. Partial and semipartial correlations between waist circumferences, obtained using different measurement protocols, and the studied body fat depots, in 28 patients with NAFLD.

	WC 1, cm		WC 2, cm		WC 3, cm		WC 4, cm	
Variables	r †	r ‡	r †	r ‡	r †	r ‡	r †	r ‡
BF, kg	0.63*	0.34	0.68**	0.37	0.66*	0.36	0.77**	0.35
BF, %	0.46	0.23	0.45	0.22	0.46	0.22	0.54*	0.28
Trunk BF, kg	0.78**	0.52**	0.82**	0.54**	0.82**	0.54**	0.84**	0.51**
Trunk BF, %	0.56*	0.35	0.54*	0.34	0.56*	0.34	0.57*	0.30
Abdominal BF, kg	0.78**	0.68**	0.78**	0.69**	0.80**	0.70**	0.72**	0.66**
Abdominal BF, %	0.59*	0.49	0.57*	0.47	0.58*	0.48	0.47	0.44
Central Abd BF, kg	0.76**	0.73**	0.77**	0.74**	0.78**	0.75**	0.68**	0.70**
Central Abd BF, %	0.52*	0.48	0.53*	0.46	0.54*	0.47	0.48	0.44

NAFLD – Non-Alcoholic Fatty Liver Disease; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference measured just above iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; BF – body fat; Central Abd BF – Central abdominal body fat; † - partial correlations between studied circumferences and dependent variables, controlled for age, sex and body mass index; ‡ - semipartial correlations between studied circumferences and dependent variables, removing the effect of age, sex and body mass index; * - significant for p<0,01; ** - significant for p<0,001.

Table 5. Z statistic P values for the comparison between the coefficients of correlation found in partial and semipartial correlations between waist circumferences, obtained using different measurement protocols, and the absolute (lower-left half of table) and relative (upper-right half of the table) results for DXA assessed body fat depots in 28 patients with NAFLD.

Variables[a]		WC1 p†	WC1 p‡	WC2 p†	WC2 p‡	WC3 p†	WC3 p‡	WC4 p†	WC4 p‡		Variables[b]
				0.954	0.982	0.971	0.988	0.874	0.841	%BF	WC1
		Blank separator		0.923	0.962	0.980	0.990	0.715	0.853	Trunk %BF	
		Data do not apply		0.886	0.920	0.949	0.963	0.747	0.836	Abd %BF	
				0.909	0.928	0.952	0.964	0.836	0.865	C Abd %BF	
WC2	BF	0.744	0.907			0.982	0.994	0.920	0.824	%BF	WC2
	Trunk BF	0.754	0.918			0.944	0.971	0.788	0.828	Trunk %BF	
	Abd BF	0.950	0.968			0.937	0.957	0.899	0.915	Abd %BF	
	C Abd BF	0.912	0.921			0.957	0.964	0.927	0.936	C Abd %BF	
WC3	BF	0.828	0.939	0.913	0.968			0.902	0.830	%BF	WC3
	Trunk BF	0.746	0.918	0.992	1.000			0.734	0.862	Trunk %BF	
	Abd BF	0.799	0.876	0.848	0.908			0.837	0.872	Abd %BF	
	C Abd BF	0.842	0.864	0.929	0.943			0.884	0.901	C Abd %BF	
WC4	BF	0.881	0.958	0.859	0.948	0.946	0.981				
	Trunk BF	0.881	0.958	0.643	0.876	0.635	0.876				
	Abd BF	0.819	0.879	0.770	0.985	0.629	0.758				
	C Abd BF	0.849	0.862	0.763	0.784	0.697	0.730				

DXA – Dual-energy X-ray Absorptiometry; NAFLD – Non-Alcoholic Fatty Liver Disease; [a] – Variables in the left column apply to the lower-left half of the table: [b] – Variables in the right column apply to the upper-right half of the tale; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference measured just above iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; † - comparison between correlation coefficients obtained in partial correlations between waist circumferences and all dependent variables, controlled for age, sex and BMI; ‡ - comparison between correlation coefficients obtained in semipartial correlations between waist circumferences and all dependent variables, removing the effect of age, sex and BMI.

Table 6. Coefficient of variation and time length for measurement of each waist circumference protocol.

VARIABLES	WC 1	WC 2	WC 3	WC 4
COV	0.045	0.049	0.047	0.073
TLM, sec.	35±6*	44±4**	74±4**	34±5***

COV – mean coefficient of variation, TLM – mean±standard deviation of time length of measurements, in seconds; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference measured just above iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; * - different from WC2 and WC3 (p<0-001) but not WC4 (P=0.522); ** - different from all other WC TLM (p<0.001); *** - - different from WC2 and WC3 (p<0-001) but not WC1 (P=0.522).

FIGURE LEGENDS:

Figure 1 - Image of a DXA scan showing the abdominal region of interest defined as the area within the upper edge of the second lumbar vertebra and de lower edge of the fourth lumbar vertebra.

Figure 2 - Representation of waist circumference measuring sites over a DXA scan image. WC1 – waist circumference measured at minimal waist; WC2 - waist circumference measured just above iliac crest; WC3 – waist circumference measured at mid-distance between lowest rib and iliac crest; WC4 – waist circumference measured at the umbilicus.