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

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7 **Short title:** Waist circumference in Liver Disease

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28 **Abstract**

29 **Background**

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

34 **Material and methods**

35 Body fat (BF) was assessed through Dual Energy X-ray Absorptiometry (DXA) in 28 patients with NAFLD
36 (19 males, 51 + 13 yrs, and 9 females, 47 + 13 yrs). WC was measured using four different WC mp (WC1-
37 narrowest torso, WC2- just above iliac crest, WC3- mid-distance between iliac crest and last rib and
38 WC4- at the umbilicus).

39 **Results**

40 All WC measurements were highly correlated particularly with central BF depots, including trunk BF
41 ($r=0.78$; $r=0.82$; $r=0.82$; $r=0.84$; respectively for WC1, WC2, WC3 and WC4) abdominal BF ($r=0.78$;
42 $r=0.78$; $r=0.80$; $r=0.72$; respectively for WC1, WC2, WC3 and WC4) and central abdominal BF ($r=0.76$;
43 $r=0.77$; $r=0.78$; $r=0.68$; respectively for WC1, WC2, WC3 and WC4), controlling for age, sex and body
44 mass index. There were no differences between the correlation coefficients obtained between all
45 studied WC measurements and each whole and central analyzed BF variable.

46 **Conclusion**

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

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52 Introduction

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

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

74 and endorsed, which is at the superior border of the iliac crest^{9,10}. Nevertheless several other
75 measuring sites have been sparsely used¹⁰.

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

82 **Materials and methods**

83 **Subjects:**

84 This study was conducted at Exercise and Health Laboratory, from the Interdisciplinary
85 Centre for the Study of Human Performance (Faculty of Human Kinetics, Technical University of
86 Lisbon, Portugal). To be selected for the present study subjects had to be over 18 years of age,
87 diagnosed with NAFLD, without history of hepatotoxic substances intake (eg. steroids) and
88 tobacco consumption. Exclusion criteria included alcohol consumption over 20 gr/day; the
89 presence of other possible causes for fatty liver disease, including hepatitis, self-immune
90 disease and others; any physical and/or mental disabilities or any condition that constituted an
91 absolute restriction from exercise, or diseases, other than metabolic (insulin resistance,
92 hypertension or dyslipidemia), with mandatory specific pharmacologic therapy (eg. Asthma or
93 other). We studied 28 patients with NAFLD (19 males, 51 ± 13 yrs, and 9 females, 47 ± 13 yrs)
94 who were diagnosed through liver biopsy or ultrasound. Subjects were recruited from the
95 outpatient periodical medical consultations in Santa Maria Hospital and Curry Cabral Hospital;
96 59 consecutive patients were selected based on selection criteria; 37 of the selected subjects

97 accepted to participate and 28 were found eligible to enter the study after exclusion criteria
98 was considered. Subjects were taking one or more of the following medication: platelet
99 inhibitors, angiotensin-converting enzyme inhibitors, nitrates, statins, ezetimibe, nicotinic acid
100 and biguanides with similar use among both genders. All participants signed an informed
101 consent before being included in the present study and undergoing any study procedure. All
102 methods used in the present study complied with good ethics and Portuguese laws and were
103 approved by both the Portuguese Foundation for Science and Technology and Faculty of
104 Human Kinetics institutional review boards.

105 **Body composition:**

106 Body composition was assessed using Dual Energy X-ray Absorptiometry (DXA) (Explorer
107 W, Hologic; Waltham, MA, USA; Fan bean mode) whole body scans and anthropometric
108 measurements. Repeated measurements in 18 young adults showed a coefficient of variation
109 (CV) of 1.7% for total BF mass and 1.5% for total %BF. All scans were made in the morning after
110 an overnight 12-hour fast. Quality control with spine phantom was made every morning, and
111 with step phantom every week. By default DXA software (QDR for windows, version 12.4)
112 estimates the head, trunk, arms and legs, both left and right, regions fat content, according to a
113 three-compartment model (fat mass, lean tissue and bone mass). Whole BF, sometimes just
114 referred to as BF, includes the fat mass of the whole body, as opposed to central BF, which
115 refers to the trunk BF, abdominal BF and central abdominal BF variables, as analyzed by DXA
116 regions of interest. The trunk region of interest (ROI) (CV = 0.5%) includes chest, abdomen and
117 pelvis, to provide estimates of trunk BF and trunk %BF. All scans were submitted to additional
118 analysis by ROI to assess absolute a relative fat content of the abdominal (Abd BF and Abd %BF)
119 and central abdominal regions (CAbd BF and CAbd %BF) (Fig. 1) (CV = 1.0 %). The upper and
120 lower limits of the abdominal and central abdominal ROI were determined as the upper edge of

121 the second lumbar vertebra to the lower edge of the fourth lumbar vertebra, respectively¹⁹⁻²¹.
122 The sides' limits of the abdominal ROI were determined as to include all trunk length, but
123 exclude any upper limb scan area^{20,21}, whereas the vertical sides of central abdominal ROI were
124 the continuation of the lateral sides of the ribs cage, as to exclude the lateral subcutaneous fat
125 of the trunk, including the anterior and posterior subcutaneous abdominal fat, as well as the
126 intra-abdominal fat¹⁹, as seen in figure 1. Absolute and relative BF content results were
127 registered to the nearest 0.01kg and 0.1%, respectively.

128 Anthropometric measurements consisted of weight, height and body mass index (BMI)
129 as well as WC. Some standardization procedures were taken into account, as proposed by
130 Agarwal and colleagues²² to avoid any bias in the measurements, therefore all WC
131 measurements were made with subjects in a standing comfortable position, in their underwear,
132 in a 12-hour fasting state. All WC measurements were made by the same technician, who was a
133 trained level 2 technician, certified by the International Society for the Advancement of
134 Kinanthropometry, using an inelastic flexible metallic tape (Lufkin - W606PM, Vancouver,
135 Canada) parallel to the floor after a tidal expiration, to the nearest 0.1cm. The WC
136 measurement sites in the present study were the narrowest torso (WC1)^{23,24}, also called
137 minimal waist¹⁰, superior border of the iliac crest (WC2)^{18,25}, midpoint (WC3)²⁶ and umbilicus
138 (WC4)^{27,28}, as detailed in table 1, and represented in figure 2. These are the most commonly
139 used protocols endorsed by sound authorities in this field^{9,10}. Time length of each WC
140 measurement, including all procedures, from brief initial instruction results registering, was
141 recorded in seconds, to the nearest 1 second, with a standard watch chronometer (model
142 RS800, Polar, Oulu, Finland). Body weight was measured to the nearest 0.1kg, and height was
143 measured to the nearest 0.1 cm, on a scale with an attached stadiometer (model 770, Seca;
144 Hamburg, Deutschland), according to standard protocol²³. Both weight and height were used

145 to calculate the subjects' BMI, by dividing the weight, in kg, by the squared height, in meters
146 ($\text{BMI} = \text{weight [kg]} / \text{height [m]}^2$). All anthropometric measurements were repeated two times,
147 and if the second differed more than 1cm (for waist and height measurements) or 0,5kg (for
148 weight measurement) from the first measurement, a third measurement was carried out. We
149 always considered the result obtained in the second measurement unless a third measurement
150 was carried out. When a third measurement was taken we considered the mode or, if mode
151 was absent, the median value of all three measurements. By using this procedure we sought to
152 always use the most suitable value that was actually measured on the subjects (instead of
153 mean values).

154 **Statistical methods:**

155 Descriptive statistics are presented as mean \pm sd and range for all analyzed variables.
156 The Gaussian distribution of the data was assessed with the Shapiro-Wilk goodness-of-fit test.
157 Levene test was used for assessing sample variance homogeneity. Paired-samples T test was
158 used to compare WC results obtained with different WCmp. When homogeneity of variance
159 was not present, the corrected significance values of T test were used. The association between
160 whole and central BF and the results obtained with each WCmp was assessed using partial as
161 well as part, also called semipartial ²⁹, correlations, controlling for age and sex and BMI. Both
162 partial and semipartial correlation techniques allow controlling for confounders but, while
163 partial correlation remove from the analysis the confounders-related variation of both
164 dependent and independent variables, the semipartial technique only removes from the
165 correlation analysis the confounder-related variation of the independent variable ²⁹. In order to
166 accomplish a statistical power of 80% ($\beta = 0.20$) at a statistical significance level of 5% ($\alpha =$
167 0.05), only coefficients of correlation equal or superior to 0.5, corresponding to a large effect
168 size, were considered significant. This is in accordance with Cohen and Cohen ²⁹ to assure that

169 results are unexposed to type I and II errors, despite an rather modest sample size. Pairs of
170 correlation coefficients obtained between each WC with each dependent variable were
171 compared, using Z statistic, to find if any WC was more closely associated with whole and
172 central BF. To evaluate the precision of the studied WCmp we calculated coefficients of
173 variation for the repeated measurements, and compared them between WCmp using paired-
174 samples T test. To respond to the third criteria, comprised in the aim of the present study,
175 paired-samples T test was used to compare the time consumption of WC measurement
176 between different measurement protocols. Statistical calculations were performed using the
177 IBM SPSS Statistics version 19 (SPSS, inc, Chicago, IL), except for z statistic which was performed
178 using Medcalc version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium).

179 **Results**

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

188 Table 4 shows the results for partial and semipartial correlations between each WC and
189 each whole or central studied BF depot controlled for sex, age and BMI. All WC results were
190 somewhat correlated with the overall studied DXA assessed BF depots, controlling for age, sex
191 and BMI, often showing correlation coefficients above 0.5. WC4 was correlated with both

192 absolute and relative whole BF, while WC1, WC2 and WC3 were correlated only with the
193 absolute values of whole BF, but only in partial correlations. All studied WC were correlated
194 with absolute and relative trunk BF, in partial correlations, however, in semipartial correlations,
195 this was true only for the absolute values of trunk BF. WC 1, WC2 and WC3 were associated
196 with absolute a relative values of abdominal fat depots, except for WC4 which was only
197 correlated with Abd BF and Cabd BF, in partial correlations. Semipartial correlations only
198 confirmed the correlation with Abd BF and CAbd BF, for all WC.

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

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

210 Discussion

211 Even though WC has been a widely used measure in the sub-population of patients with
212 NAFLD^{12,30}, to our knowledge this is the first study to focus on the comparison of different
213 WCmp based on a scientific and practical rational. The prevalence of high levels of BMI,
214 including obese and morbidly obese patients, in the present sample was expected since

215 obesity, along with insulin resistance, have been identified among the strongest risk factors for
216 NAFLD, and therefore highly prevalent in this sub-population³¹⁻³⁴. WC results were also quite
217 high which was consistent with BMI levels. The magnitudes of WC mean values were different
218 according to the protocol in use, as has been previously reported in different populations³⁵⁻³⁷,
219 meaning they are not interchangeable. This has imperative implications advising the consistent
220 use of one single protocol to avoid misinterpretations either when monitoring longitudinal data
221 as when using cut-off for classification of WC values and subjects subject's risk level appraisal
222^{35,38,39}. Based on the small or absent differences reported, particularly in men, between
223 measurements of WC2 (at the superior border of the iliac crest) and WC3 (at the midpoint
224 between lowest rib and iliac crest)³⁵⁻³⁷, it was proposed that current WC thresholds,
225 generalized using WHO WCmp (WC3), could be applied to measurements using WC2¹⁰. Our
226 data do not support the mentioned generalization for patients with NAFLD, yet this analysis
227 was not in the aim of the present study, and additional research is needed to look into sex
228 differences and other possible influencing variables. These results reinforce the necessity of
229 searching for the most useful, consensual and standardized WCmp for use in clinical practice
230 with patients with NAFLD.

231 In the present sample of patients with NAFLD WC was highly associated with whole and
232 central BF, adjusted for age, sex and BMI. The association of WC with BC, particularly with
233 central BF has long been reported in diverse populations⁴⁰⁻⁴². Wang and colleagues (2003) had
234 already found stronger associations between WC and absolute central BF, as opposed to either
235 whole BF or any relatively expressed BF depot, assessed by DXA. In the present report, the
236 results obtained in partial correlation may suggest that WC1, WC2 and WC3 are more
237 consistently associated with central BF, and therefore seem better markers of a more
238 hazardous fat accumulation in the studied sample of patients with NAFLD, as opposed to WC4

239 which seems more consistently associated with Whole BF. When the results obtained in
240 semipartial correlations were taken into consideration, all studied WC were similarly only
241 correlated with central BF accumulation, namely trunk BF, Abd BF and CAbd BF, controlled for
242 age, sex and BMI, revealing a cognate association pattern among different WCmp. Central BF
243 accumulation has been found highly important in NAFLD etiology (Park et al. 2007) and is also a
244 risk factor for cardiovascular diseases incidence and outcomes^{7,8}, which is increased in NAFLD
245 (Targher et al. 2005). The present results support the use of WC measurement in patients with
246 NAFLD, as a cost-effective screening procedure, to assess hazardous body composition
247 phenotypes, namely central BF accumulation, in routine clinical appraisals, irrespective of the
248 WCmp in use.

249 Comparisons between pairs of competing WC correlations results with each dependent
250 variable showed no differences meaning all WC results are similarly associated with the
251 analyzed BF depots, irrespectively of the WCmp used, in the studied sample of patients with
252 NAFLD. Never the less, conflicting results can be found in general population^{36,37}. A report
253 focusing on different WC measuring sites, including at the superior border of the iliac crest
254 (WC2) and also at the midpoint between lowest rib and iliac crest (WC3), found differences
255 between correlation coefficients, in partial correlations, with abdominal adipose tissue
256 assessed with magnetic resonance imaging, in women but not in men³⁷. In agreement with the
257 present report, Wang and colleagues³⁶ found WC correlations to be stronger with trunk BF,
258 regardless of sex and WCmp. The present results showing a preferable association between WC
259 and preferably central BF, regardless of WCmp, together with the well-established recently
260 reported¹⁰ relationships between WC and morbidity of cardiovascular disease and diabetes
261 and with cardiovascular and all-cause mortality, also rather unaffected the use of different

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

264 In the absence of biological support for the use of one preferable WCmp, additional
265 criteria have been suggested in the attempt to substantiate the choice of one particular WCmp,
266 including the use of bony landmarks and ease of measurement^{9,10,18}. It was argued that the use
267 of bony landmarks could be preferable due to increased precision⁹ or reliability^{10,18}. The
268 present data do not confirm better precision of any particular WCmp, as assessed by the
269 comparison of coefficients of variation obtained for each WCmp. Similar results have been
270 reported elsewhere in the general population^{35,36}. Additional research is warranted to support
271 or reject the preferable use of bony landmarks, particularly for longitudinal assessments. Ease
272 of measurement, meaning that WCmp should require less specific training and be less time
273 consuming, has been proposed as an important criteria for the adoption of any measurement
274 by general public and practitioners in routine clinical practice⁹.

275 It has been suggested that WC measured at the superior border of the iliac crest would
276 be more likely adopted by general public and practitioners as it requires only the palpation of
277 one bony landmark¹⁸ however this was not confirmed by practice nor research^{9,10}. Bony
278 landmarks have been subjectively reported to require more training and experience of
279 observers, yet, in the present study only the lowest rib landmark proved demanding. Limited
280 time availability has been proposed as one of the reasons for not using WC measurement in
281 routine clinical practice⁹. Present data also objectively confirm a previous report³⁵ that
282 subjectively pointed WC measurement at the midpoint between lowest rib and iliac crest as
283 more time consuming than when using other studied WCmp.

284 There are several strengths and limitations to this study. The studied WCmp do not cover all
285 protocols existent in the literature, yet the focus was set on the most commonly used and

286 endorsed by prominent institutions for use in clinical setting^{9,10,18}. Also the used BC assessment
287 method (DXA), a gold standard instrument to assess BC in a three-compartment model, is
288 unable to determine visceral adiposity independently from subcutaneous fat. However strong
289 correlation between abdominal fat estimated from selected DXA ROI and visceral fat measured
290 directly by magnetic resonance imaging (Park et al. 2002) and computed tomography^{43,44} have
291 been reported. Due to the cross-sectional approach used, the usefulness of the studied WCmp
292 to assess longitudinal changes in the studied BF depots could not be established, based on the
293 present results. Finally, the size of the sample was rather constrained due to difficulties in the
294 recruitment of such a specific sub-population. 90 individuals were coveted to be included in the
295 present sample in the initial research project. This would allow coefficients of correlation as low
296 as 0.3, traditionally corresponding a moderate effect size, to be considered significant and
297 unexposed to type I and II errors²⁹. Unfortunately, despite all efforts on behalf of everyone
298 involved in this research project, only 28 patients with NAFLD could be recruited, meeting all
299 inclusion and exclusion criteria. Consequently, only associations equal or higher to $r=0.50$ could
300 be considered to attain minimal statistical power of 80% and statistical significance of 5%, and
301 could be considered fairly unexposed to type I and type II errors²⁹. Nevertheless the aim of the
302 present study was to find the preferable WCmp to use with patients with NAFLD, which should
303 be found at the higher end of correlational range, therefore, the inability to find significant
304 associations lower than $r=0.5$, though interesting are not the aim of the present study.

305 The present study confirms the strong association between WC and central BF, even
306 after removing the effect of age, sex and BMI, regardless of the WCmp in use. Moreover, all
307 tested WCmp could be considered useful and important low-cost assessment tools for clinical
308 practice, though not interchangeable. This irrespective usefulness is bolstered by the similar
309 precision found for all studied WCmp. On the other hand there could not be established one

310 single preferable WCmp for the present sample of patients with NAFLD based on biological
311 criteria. The use of bony landmarks showed no superiority, though research is needed to assess
312 its' relevance in longitudinal assessments. WC measured at the superior border of the iliac crest
313 may be a good choice if a bony landmark is valued together with time consuming.

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437

438

439 **TABLES:**440 **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]

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

443

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

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

445 BMI – body mass index; WC1 – Waist circumference as measured by Lohman et al. [20] and The International Society for the Advancement of Kinanthropometry (ISAK)
 446 [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
 447 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-**

448 energy X-ray Absorptiometry; BF – body fat; FFM – fat free mass; * Results are presented as mean ± standard deviation, unless otherwise noted; Min. – lowest observed
 449 value; Máx. – highest observed value

450

451

452 **Table 3.** Mean differences and P values from paired samples T test used in the
 453 comparison between waist circumference results obtained with different
 454 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

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

459

460

Table 4. Partial and semipartial correlations between waist circumferences, obtained using different

461

measurement protocols, and the studied body fat depots, in 28 patients with NAFLD.

Variables	WC 1, cm		WC 2, cm		WC 3, cm		WC 4, cm	
	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

462

NAFLD – Non-Alcoholic Fatty Liver Disease; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference measured just above

463

iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; BF – body fat; Central Abd

464

BF – Central abdominal body fat; † - partial correlations between studied circumferences and dependent variables, controlled for age, sex and body

465

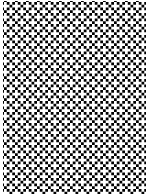
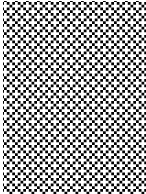
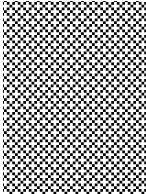
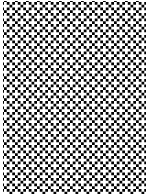
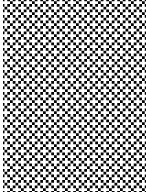
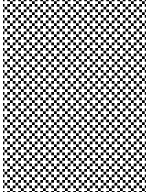
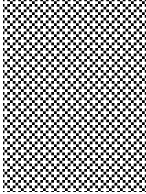
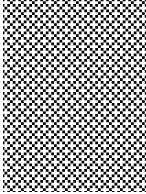
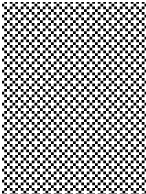
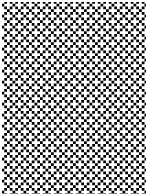
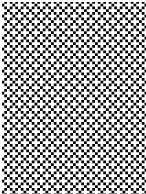
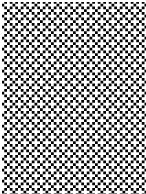
mass index; ‡ - semipartial correlations between studied circumferences and dependent variables, removing the effect of age, sex and body mass

466

index; * - significant for p<0,01; ** - significant for p<0,001.

467

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

Variables ^a		WC1		WC2		WC3		WC4		Variables ^b	
		p [†]	p [‡]	p [†]	p [‡]	p [†]	p [‡]	p [†]	p [‡]		
				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				

472 **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
 473 – Variables in the right column apply to the upper-right half of the table; WC1 – Waist circumference measured minimal waist; WC2 - Waist circumference
 474 measured just above iliac crest; WC3 - Waist circumference measured at mid-distance; WC4 - Waist circumference measured at the umbilicus; † - comparison
 475 between correlation coefficients obtained in partial correlations between waist circumferences and all dependent variables, controlled for age, sex and BMI; ‡ -
 476 comparison between correlation coefficients obtained in semipartial correlations between waist circumferences and all dependent variables, removing the
 477 effect of age, sex and BMI.

478

479

480 **Table 6.** Coefficient of variation and time length for
 481 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***

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

488

489 **FIGURE LEGENDS:**

490

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

494

495 **Figure 2** - Representation of waist circumference measuring sites over a DXA scan image.

496 WC1 – waist circumference measured at minimal waist; WC2 - waist circumference

497 measured just above iliac crest; WC3 – waist circumference measured at mid-distance

498 between lowest rib and iliac crest; WC4 – waist circumference measured at the umbilicus.

499