ACCEPTED version by the European Journal of Clinical Nutrition after peer-revision

Original Article

Body Composition and Body Fat Distribution are Related to Cardiac Autonomic Control in Non-Alcoholic Fatty Liver Disease Patients

Running Title: Body composition and Cardiac Autonomic Control

Nuno M. Pimenta, MS[1,2], Helena Santa-Clara, PhD[1], Helena Cortez-Pinto, MD, PhD[3], José Silva-Nunes, MD[4], Maria da Lapa Rosado, Bch[1], Pedro J. Sousa, MD[5], Rita Calé, MD[6], Xavier Melo, MS[1], Luís B. Sardinha, PhD[1], Bo Fernhall, PhD[7]

[1] Exercise and Health Laboratory, Interdisciplinary Centre for the Study of Human Performance, Faculty of Human Kinetics, Technical University of Lisbon, Cruz-Quebrada, Portugal; [2] Sport Sciences School of Rio Maior, Polytechnic Institute of Santarém, Portugal; [3] Unidade de Nutrição e Metabolismo, FML, IMM. Departamento de Gastrenterologia, Hospital Universitário de Santa Maria, Lisbon, Portugal; [4] Curry Cabral Hospital, Lisbon, Portugal; [5] Santa Cruz Hospital, Carnaxide, Portugal; [6] Garcia de Orta Hospital, Almada, Portugal; [7] College of Applied Health Sciences, University of Illinois, Chicago, USA.

Correspondence: Nuno Manuel Pimenta, MS. Sport Sciences School of Rio Maior – Polytechnic Institute of Santarém, Av. Dr. Mário Soares, 2040-413 Rio Maior - Portugal. Phone: +351-243 999 280, Fax: +351-243 999, E-mail: npimenta@esdrm.ipsantarem.pt

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

ABSTRACT

Background/Objectives: Heart rate recovery (HRR), a cardiac autonomic control marker, has been shown to be related to body composition (BC), yet this was not tested in Non-Alcoholic Fatty Liver Disease (NAFLD) patients. The aim of this study was to determine if, and to what extent, markers of BC and body fat (BF) distribution are related with cardiac autonomic control in NAFLD patients.

Subjects/Methods: BC was assessed with Dual Energy X-ray Absorptiometry in 28 NAFLD patients (19 males, 51 ± 13 yrs, and 9 females, 47 ± 13 yrs). BF depots ratios were calculated to assess BF distribution. Subjects' HRR was recorded 1 (HRR1) and 2 minutes (HRR2) immediately after a maximum graded exercise test.

Results: BC and BF distribution were related to HRR, particularly weight, trunk BF as well as trunk BF-to-appendicular BF ratio showed a negative relation with HRR1 (r=-0.613; r=-0.597 and r=-0.547; respectively, $p<0.01$) and HRR2 (r=-0.484; r=-0.446; $p<0.05$ and r=-0.590; $p<0.01$, respectively). Age seems to be somewhat related to both HRR1 and HRR2 except when controlled for BF distribution. The preferred model in multiple regression should include trunk BF-to-appendicular BF ratio and BF to predict HRR1 ($r^2=0.549$; $p<0.05$), and trunk BF-to-appendicular BF ratio alone to predict HRR2 ($r^2=0.430$; $p<0.001$).

Conclusions: BC and BF distribution were related to HRR in NAFLD patients. Trunk BF-to-appendicular BF ratio was the best independent predictor of HRR and therefore may be best related to cardiovascular increased risk, and possibly act as a mediator in age related cardiac autonomic control variation.

Keywords: Regional Body Fat; Dual Energy X-ray Absorptiometry; Hepatic Steatosis; Heart Rate Recovery; Parasympathetic Reactivation.

INTRODUCTION

Paragraph number 1 Non Alcoholic Fatty Liver Disease (NAFLD) is a condition present in up to 30% of developed countries, with a considerably higher prevalence in the obese populations, particularly in the presence of abdominal or morbid obesity (1-5). NAFLD was shown to result from hepatic fat metabolism imbalance and encompasses several stages, from the initial hepatocyte fat accumulation (hepatic steatosis), to hepatic inflammation (non-alcoholic steatohepatitis) along with a constellation of other disturbances, that ultimately can lead to advanced fibrosis, cirrhosis, liver failure and death (6). NAFLD patients have also been reported to have increased cardiovascular risk compared with the general population (7). Insulin resistance and obesity are major risk factors for NAFLD, yet BF accumulation, particularly that of the abdominal region, besides being strongly associated with NAFLD and found to precede presence of insulin resistance (8), may mimic the same metabolic abnormalities triggered by insulin resistance alone (9, 10) and is also associated with other metabolic disorders that can also increase the risk of NAFLD, therefore, BF may be a key factor in the etiology of NAFLD (6).

Paragraph number 2 Heart rate recovery (HRR) after exercise is a recognized cardiac autonomic control marker mostly reflective of parasympathetic reactivation (11, 12). Slow HRR is independently related to higher risk of mortality and other cardiovascular and metabolic outcomes (13-20). Autonomic nervous system (ANS) imbalance, including blunted HRR, has also been linked to obesity (21), higher body fat (BF) accumulation (22, 23). Kreier and colleagues (24) presented a neuroanatomical evidence for a reciprocal influence of BF, particularly intra-abdominal BF, and ANS, and suggested a pathway for ANS mediated imbalance in several other biological functions

including liver fat metabolism, meaning it may be somewhat involved in the etiology, progression, consequences and treatment of both obesity and NAFLD, however this has been largely overlooked, particularly in the population of NAFLD, and research is warranted in this field. Insulin resistance and obesity (main risk factors for hepatic fat accumulation) have been shown to precede the presence of slow HRR (20, 25). Thus, the BF accumulation and distribution has been suggested to be associated with ANS imbalance (22, 26, 27), but this has not yet been tested in NAFLD patients.

Paragraph number 3 Very few studies have focused on BF distribution and HRR associations and it is unknown if such a relationship exists in NAFLD patients. The purpose of the present study was to determine if, and to what extent, specific markers of BC and BF distribution, are related with reduced parasympathetic reactivation following maximal exercise, as assessed by heart rate recovery (HRR), in NAFLD patients.

SUBJECTS AND METHODS

Paragraph number 4 **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 without history of hepatotoxic substances intake (eg. steroids) and tobacco consumption. Exclusion criteria included alcohol consumption over 20 gr/day; the presence of other potential causes for fatty liver disease (viral hepatitis, auto-immune disease and others); any physical and/or mental disabilities or any condition that constituted an absolute restriction to exercise, or

other diagnosed diseases, with mandatory specific pharmacologic therapy. Not included in the exclusion criteria is the presence of metabolic and cardiovascular disease (insulin resistance, hypertension or dyslipidemia). We studied 25 NAFLD patients (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 medical departments 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 comply with ethics and Portuguese laws and were approved by Faculty of Human Kinetics institutional review board for human studies. The present investigation also complies with the principles outlined in the Declaration of Helsinki.

Paragraph number 5 **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 performed 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 the DXA software (QDR for windows, version 12.4) estimates the head, trunk,

arms and legs, both left and right, regional fat content, according to a three-compartment model (fat mass, lean tissue and bone mass). The trunk region of interest (ROI) (CV = 0.005%) includes chest, abdomen and pelvis. Appendicular ROI (CV = 0.004 %) includes both arms plus both legs. All scans were submitted to additional analysis by ROI to assess fat content of the abdominal and central abdominal regions (CV = 0.01 %). 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 (28-30). The lateral limits of the abdominal ROI were determined as to include all trunk length, but exclude any upper limb scan area (29, 30), 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 (28). Absolute and relative BF content results were registered to the nearest 0.01kg and 0.1%, respectively. All scans and analyses were made by the same observer.

Paragraph number 6 Anthropometric measurements consisted of weight, height and body mass index (BMI). 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 (31). Both weight and Height were used to calculate the subject's BMI, by dividing the weight, in kg, by the squared height, in meters (BMI = weight [kg] / height $[m]^2$).

Paragraph number 7 **Body fat distribution:**

BF distribution variables were calculated using ratios between BF content absolute values of different fat depots, obtained by DXA, as done elsewhere (30). The

trunk BF-to-appendicular BF ratio, also called trunk-to-extremity fat ratio (32) or central-to-peripheral fat mass ratio (33), was calculated as the trunk BF content divided by the sum of the BF content of the arms and legs, both left and right. The abdominal BF-to-trunk BF ratio was calculated as the fat content of the selected abdominal ROI divided by the trunk BF. The abdominal BF-to-total BF was calculated as the selected abdominal ROI fat content divided by the whole BF. Ratios were registered to the nearest 0,01.

Paragraph number 8 **Exercise testing:**

All subjects underwent a treadmill (Q-65, Quinton, Cardiac Science Corp; Bothell, WA, USA) graded exercise test (GXT) using Bruce standard protocol (34). All GXT were monitored using a 12 lead electrocardiogram PC-based acquisition module (Welch-Allyn PCE-210, Welch Allyn Inc.; Skaneateles Falls, NY, USA) and the data, including heart rate (HR), were monitored and recorded using Welch Allyn CardioPerfect software (Welch Allyn Inc.; Skaneateles Falls, NY, USA). Oxygen uptake was monitored during GXT using a MedGraphics CPX Ultima Cardio metabolic cart (Medical Graphics Corp; St Paul, MN, USA) and data was recorded using Breeze Suite software (version 6.4.1, Medical Graphics Corp; St Paul, MN). Subjects exercised until at least two of the following test termination criteria were reached (35): (1) subjects volitional fatigue; (2) respiratory exchange ratio reached 1.1 or higher; (3) subjects reached age predicted maximal HR (HRmax); (4) oxygen uptake did not increase in spite of increasing work load.

Paragraph number 9 **Heart Rate Recovery:**

When GXT termination criteria were reached patients started exercise recovery with a speed of 1.5mph and incline of 2.5% on the treadmill. Subjects remained

walking with the recovery treadmill mechanical load for 2 minutes. After 2 minutes of recovery the treadmill was stopped and subjects continued their recovery seated in an armless standard chair. HR was recorded beat-by-beat and was averaged at 15 seconds intervals for identifying HRmax. HR at the end of the first and second recovery minutes were recorded from beat-by-beat records (HR1 and HR2, respectively). HRR was calculated as the difference between observed HRmax and HR1 (HRR1 = HRmax – HR1) and HR2 (HRR2 = HRmax – HR2). Cut off value for identifying slow HRR was considered 12bpm for HRR1 (13-15, 19). The 22bpm cut off value for identifying slow HRR2 was developed using a supine recovery protocol (18, 36), however it has been used with diverse exercise recovery protocols, including seated (37) and walking (20) recovery protocols and therefore was adopted in the present study for descriptive purposes only.

Paragraph number 10 **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. Partial and part, also called semipartial (38), correlations were performed to assess the relations between dependent and independent variables controlling for age and sex. When age was an independent variable the correlation was controlled for sex and fat distribution. In order to accomplish a statistical power of 80% ($\beta = 0.20$) at a statistical significance level of 5% ($\alpha = 0.05$), as has been used as a convention (38), only coefficients of correlation equal or superior to 0.5, corresponding to a large effect size, were considered significant and unexposed to type I and II errors (38). Multiple linear regressions were conducted, using Enter method, between dependent variables and correlated independent variables to analyze r square change

when using two predictors in the model. Stepwise regressions were performed to find preferred models for the prediction of both dependent variables (HRR1 and HRR2). The level of significance was set at P<0.05 (two-tailed). Statistical calculations were performed using the IBM SPSS Statistics version 19 (SPSS, inc, Chicago, IL).

RESULTS

Paragraph number 11 Mean values for all studied variables are presented in Table 1. No clinical test interruption criteria, such as electrocardiogram signs of ischemia, new onset of arrhythmias, or excessive hypotensive/hypertensive response, were observed in any GXT. All subjects met termination criteria for ending the GXT. From among the 25 studied NAFLD patients slow HRR1 was present in 6 (22.2%, 2 were female) and slow HRR2 in 5 (18.5%, 2 were female) patients. Neither HRR1 nor HRR2 were different between men and women (p=0.754 and p=0.631 obtained in an independent samples t test comparison, respectively). Mean BMI of the studied sample was in the overweight category, with no differences between sexes (p=0.075 on independent samples t test). BMI was also not related with age (r= -0.218; p=0.285 on Pearson correlation).

Paragraph number 12 Table 2 shows the results for partial and semipartial correlations between each independent variable and each dependent variable (HRR1 and HRR2), controlled for sex and age (unless otherwise noted). Only the studied BF compartments, not fat free mass, were related to HRR. On a whole body analysis only weight was found negatively correlated with HRR1 (p=0.002), in partial correlations and semipartial correlations. The regional BC analysis showed that trunk BF (p=0.003) and central Abdominal BF (p=0.009) were negatively correlated with HRR1 but not with HRR2, both in partial and semipartial correlations, independently of sex and age.

The analysis of BF distribution indicated that the trunk BF divided by appendicular BF was the only studied BF distribution marker related to HRR1 (p=0.008) and the only studied independent variable to be related to HRR2 (p=0.003) in both partial and semipartial correlations, when controlled for sex and age. Age, when controlled for sex and BF distribution, was not related to neither HRR1 nor HRR2 (p=0.596 and p=0.483, respectively).

Paragraph number 13 All independent variables that showed significant relation with HRR in partial and semipartial correlations were included in multiple linear regression analysis shown in table 3. Regressions were performed using only trunk BF-to-appendicular BF ratio and age, which has been suggested to influence HRR in healthy adults (20), as predictors of either HRR1 or HRR2, and also between pairs of independent variables to predict HRR1. Because trunk BF-to-appendicular BF ratio was the only independent variable correlated with both dependent variables, it was chosen as a fixed independent variable in multiple linear regressions. The higher R square change in the prediction of HRR1 seems to be that obtained by adding weight to trunk BF-to-appendicular BF ratio in the prediction model. In the prediction of HRR2 Trunk BF-to-appendicular BF ratio alone was found to predict over 40% of the variation of HRR2, in this sample of NAFLD patients.

DISCUSSION

Paragraph number 14 To our knowledge this is the first study to focus on the association between HRR, and BC and/or BF distribution, in NAFLD patients. Most studies on HRR focus primarily on cardiovascular outcomes and have not included BC variables (12-16). Some previous population-based reports showed slower HRR in

patients with higher BMI (25, 39). Nilsson and colleagues found similar results in elders (27). In a recent report, BMI showed the highest odds ratio for slow HRR2 (OR=6.58) over a 20 yr period, after controlling for baseline HRR (20). In our sample BMI was not associated with either HRR1 or HRR2, after controlling for age and sex. Similar results had also been found in a sample of type 2 diabetes mellitus patients (19). These discrepancies may be explained by differences in studied samples as well as in research protocols, including different HRR record timing criterion as well as considerable exercise protocol differences either in the effort as in the recovery phase. Nevertheless the development of slow HRR seems more likely in those who have more BF accumulation (20, 25, 37).

Paragraph number 15 A recent report showed that the sum of skinfolds accounted for the greatest variance of both HRR1 and HRR2, as compared with BMI, waist circumference (WC) and maximal oxygen consumption (23). They used mainly skinfolds from the trunk region, including the abdominal skinfold, which can reinforce the importance of central BC for appropriate ANS function. In accordance to this, the present results showed trunk BF and CAbd BF to be significant correlated with HRR1, independent of age and sex. Few studies could be found using different BC markers, besides BMI, when focusing on HRR, nevertheless some investigations have used WC to assess central obesity or central as well as whole BF accumulation and found concordant results to ours (20). Mean WC has been shown to be higher in patients with slow HRR (20, 25). The association between slow HRR and WC has been shown to be stronger than with BMI (adjusted for age, race and sex) (25) as well as with all metabolic syndrome components (27). In the present study the results on central BF variables, particularly abdominal fat and central abdominal fat, also show a negative

correlation with HRR1, but not with HRR2. Kim and colleagues (22) found somewhat concordant results concerning the relation between visceral fat, particularly that around the myocardium, and both HRR1 and HRR2. The only study we found focusing on HRR and regional body composition analysis using DXA showed no differences in HRR between overweight young adults and lean control subjects, in a sample of overnight sleep apnea patients, even though overweight subjects were significantly heavier, and had higher BMI, %BF and central abdominal BF (40).

Paragraph number 17 In the present study Trunk BF: Appendicular BF ratio was the only BF distribution marker that was related to HRR, moreover this BF distribution marker was the only studied independent variable to show correlation magnitudes with both HRR1 and HRR2 that correspond to a large effect size, even after removing the effect of sex and age. Multiple regression also revealed that other BC variables added little predictive capacity to Trunk BF-to-Appendicular BF ratio. These results emphasize that BF distribution may be more important for ANS function than the absolute or relative amount of BF. Because HRR has been considered a powerful predictor of cardiovascular, as well as overall, mortality (13, 14, 17, 19, 41-44), the present results suggest that a central BF distribution, particularly Trunk BF-to-Appendicular BF ratio, can possibly relate more strongly to cardiovascular increased risk. The importance of a central distribution of BF was noticed before, using HRV to assess ANS function (26). In that study, abdominal-to-peripheral fat distribution, assess by dividing abdominal by thigh DXA estimated fat contents, was found to explain a significant variation of HRV (26). It is known that the ANS may influence adipocyte fat metabolism by an endocrine pathway and a neuronal pathway (45, 46), and adipocytes from different regions of the body respond differently to the intensity and duration of

the endocrine stimulation (47) and may also be controlled by different branches/neurons of the ANS (24). Therefore, the fact that BF distribution was the most consistent correlate with the studied autonomic markers, in the present study, gives strength to the theory that ANS may be somewhat involved, either as a cause or as a consequence, in BC and overall metabolic abnormalities associated with the central BF accumulation phenotype, though this is still speculative at this point. The potential implications of the ANS in the etiology, progression, consequences and treatment of both adverse body fat accumulation patterns and NAFLD should warrant further research.

Paragraph number 18 Carnethon et al. (20) showed an association of HRR with aging. In our cross-sectional study the relation of HRR1 and HRR2 with patient's age, was absent if controlled for BF distribution. Christou and colleagues (26) had long proposed that the changes in fat accumulation pattern that occurs with aging, resulting in BF distribution changes, may contribute to the ANS variation commonly attributed to aging. This is a matter that needs to be confirmed either in the general population as in specific sub-populations such as the NAFLD patients and other metabolic impaired sub-populations.

Paragraph number 19 The prevalence of slow HRR in the present study is in accordance with most of the published data, including that from the Cleveland Clinic Foundation (13-15) that focused on patients referred for symptom-limited exercise testing, as well as in patients with metabolic impairments (17, 19) or in even more heterogeneous populations (25), in accordance to the understanding that metabolic impairments are somewhat linked to abnormal ANS. Accordingly, when confronted with healthy cohort data, as shown recently by Carnethon and colleagues (20) the

311 prevalence of slow HRR in the present sample was fairly high. The prevalence of high
312 levels of BMI, including obese and morbidly obese patients, in the present sample was
313 expected since obesity, along with insulin resistance, have been identified as the
314 strongest risk factors for NAFLD, and therefore highly prevalent in this sub-population
315 (1-4).

316 ***Paragraph number 20*** There are several strengths and limitations to this study. In the
317 present report autonomic nervous system assessment was restricted to HRR. Previous
318 studies have validated the use of HRR as a marker of parasympathetic reactivation,
319 however HRR is not a direct measure of autonomic nervous system dysfunction but
320 rather is an estimate of parasympathetic response to a specific physiologic challenge
321 (i.e., exercise) (11, 12). Further studies with measures of different components of
322 autonomic nervous system function (e.g., sympathetic input), as well as
323 sympathetic/parasympathetic balance and resting cardiac autonomic control, are
324 warranted to confirm our observations. Also our BC assessment method (DXA) albeit
325 being a gold standard instrument to assess BC in a three compartment model, is
326 unable to determine visceral adiposity independently from subcutaneous fat.
327 Nevertheless, recent studies indicate strong correlation between abdominal fat
328 estimated from selected ROI and visceral fat assessed by magnetic resonance imaging
329 (29) and computed tomography (48, 49). Because a cross-sectional approach was used,
330 a causal relation between cardiac autonomic control variation and BC or BF
331 distribution could not be established, based on the present results. Finally, the size of
332 the sample was rather constrained due to difficulties in the recruitment of such a
333 specific sub-population. 90 individuals were coveted to be included in the present
334 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 (38). Unfortunately, despite all efforts on behalf of everyone involved in this research project, only 28 NAFLD could be recruited. This embodied acknowledged consequences in the statistical power of the present results. 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 1 and type 2 errors (38). However the aim of the present study was not compromised, neither it's importance. This study sought to find the best markers, which are found at the higher end of correlational range, so the inability to find significant associations lower than r=0.5, though interesting are not the aim of the present study. Moreover, the present results represent a relevant preliminary analysis to establish the importance of BC and BF distribution in the cardiac autonomic control of NAFLD patients.

Paragraph number 21 In the present study BF content and distribution were important contributors to HRR in NAFLD patients. Excess BF accumulated in the trunk or abdominal regions is associated with poor HRR. BF distribution appears to be more important than overall BF accumulation in explaining the variation of HRR and therefore can possibly be a better predictor of cardiovascular risk in NAFLD patients. Therefore, present results also highlight the importance of assessing BF distribution in NAFLD patients, rather than just markers of generalized BF.

ACKNOWLEDGMENTS

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

CONFLICT OF INTEREST

The authors have nothing to disclosure.

REFERENCES

1. Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med. 2000 Jan 18;132(2):112-7. PubMed PMID: 10644271.
2. Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Tani S, Goto M. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med. 1988 May;27(2):142-9. PubMed PMID: 3047469.
3. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004 Dec;40(6):1387-95. PubMed PMID: 15565570.
4. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. J Clin Gastroenterol. 2006 Mar;40 Suppl 1:S5-10. PubMed PMID: 16540768. Epub 2006/03/17. eng.
5. Cortez-Pinto H, Moura MC, Day CP. Non-alcoholic steatohepatitis: from cell biology to clinical practice. Journal of Hepatology. 2006;44:197-208. PubMed PMID: 10553793.
6. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002 Apr 18;346(16):1221-31. PubMed PMID: 11961152.
7. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Pichiri I, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol. 2010 Oct;53(4):713-8. PubMed PMID: 20619918.
8. Rector RS, Thyfault JP, Uptergrove GM, Morris EM, Naples SP, Borengasser SJ, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. J Hepatol. 2010 May;52(5):727-36. PubMed PMID: 20347174. Epub 2010/03/30. eng.
9. Cote M, Mauriege P, Bergeron J, Almeras N, Tremblay A, Lemieux I, et al. Adiponectinemia in visceral obesity: impact on glucose tolerance and plasma lipoprotein and lipid levels in men. J Clin Endocrinol Metab. 2005 Mar;90(3):1434-9. PubMed PMID: 15598678.

10. Gnacinska M, Malgorzewicz S, Stojek M, Lysiak-Szydlowska W, Sworczak K. Role of adipokines in complications related to obesity: a review. Advances in medical sciences. 2009;54(2):150-7. PubMed PMID: 19875356.
11. Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, et al. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. J Am Coll Cardiol. 1994 Nov 15;24(6):1529-35. PubMed PMID: 7930286. Epub 1994/11/15. eng.
12. Davrath LR, Akselrod S, Pinhas I, Toledo E, Beck A, Elian D, et al. Evaluation of autonomic function underlying slow postexercise heart rate recovery. Med Sci Sports Exerc. 2006 Dec;38(12):2095-101. PubMed PMID: 17146315. Epub 2006/12/06. eng.
13. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. N Engl J Med. 1999 Oct 28;341(18):1351-7. PubMed PMID: 10536127. Epub 1999/10/28. eng.
14. Vivekananthan DP, Blackstone EH, Pothier CE, Lauer MS. Heart rate recovery after exercise is a predictor of mortality, independent of the angiographic severity of coronary disease. J Am Coll Cardiol. 2003 Sep 3;42(5):831-8. PubMed PMID: 12957428. Epub 2003/09/06. eng.
15. Diaz LA, Brunken RC, Blackstone EH, Snader CE, Lauer MS. Independent contribution of myocardial perfusion defects to exercise capacity and heart rate recovery for prediction of all-cause mortality in patients with known or suspected coronary heart disease. J Am Coll Cardiol. 2001 May;37(6):1558-64. PubMed PMID: 11345365. Epub 2001/05/10. eng.
16. Arena R, Guazzi M, Myers J, Peberdy MA. Prognostic value of heart rate recovery in patients with heart failure. Am Heart J. 2006 Apr;151(4):851 e7-13. PubMed PMID: 16569547. Epub 2006/03/30. eng.
17. Cheng YJ, Lauer MS, Earnest CP, Church TS, Kampert JB, Gibbons LW, et al. Heart rate recovery following maximal exercise testing as a predictor of cardiovascular disease and all-cause mortality in men with diabetes. Diabetes Care. 2003 Jul;26(7):2052-7. PubMed PMID: 12832312. Epub 2003/07/02. eng.
18. Lipinski MJ, Vetrovec GW, Froelicher VF. Importance of the first two minutes of heart rate recovery after exercise treadmill testing in predicting mortality and the presence of coronary artery disease in men. Am J Cardiol. 2004 Feb 15;93(4):445-9. PubMed PMID: 14969619. Epub 2004/02/19. eng.
19. Chacko KM, Bauer TA, Dale RA, Dixon JA, Schrier RW, Estacio RO. Heart rate recovery predicts mortality and cardiovascular events in patients with type 2 diabetes. Med Sci Sports Exerc. 2008 Feb;40(2):288-95. PubMed PMID: 18202573. Epub 2008/01/19. eng.
20. Carnethon MR, Sternfeld B, Liu K, Jacobs DR, Jr., Schreiner PJ, Williams OD, et al. Correlates of heart rate recovery over 20 years in a healthy population sample. Med Sci Sports Exerc. 2012 Feb;44(2):273-9. PubMed PMID: 21796053. Epub 2011/07/29. eng.
21. Bray GA. Obesity, a disorder of nutrient partitioning: the MONA LISA hypothesis. J Nutr. 1991 Aug;121(8):1146-62. PubMed PMID: 1861165. Epub 1991/08/01. eng.
22. Kim MK, Tanaka K, Kim MJ, Matsuo T, Tomita T, Ohkubo H, et al. Epicardial fat tissue: relationship with cardiorespiratory fitness in men. Med Sci Sports Exerc. 2010 Mar;42(3):463-9. PubMed PMID: 19952810. Epub 2009/12/03. eng.

23. Esco MR, Williford HN, Olson MS. Skinfold thickness is related to cardiovascular autonomic control as assessed by heart rate variability and heart rate recovery. J Strength Cond Res. 2011 Aug;25(8):2304-10. PubMed PMID: 21691230. Epub 2011/06/22. eng.
24. Kreier F, Kap YS, Mettenleiter TC, van Heijningen C, van der Vliet J, Kalsbeek A, et al. Tracing from fat tissue, liver, and pancreas: a neuroanatomical framework for the role of the brain in type 2 diabetes. Endocrinology. 2006 Mar;147(3):1140-7. PubMed PMID: 16339209. Epub 2005/12/13. eng.
25. Kizilbash MA, Carnethon MR, Chan C, Jacobs DR, Sidney S, Liu K. The temporal relationship between heart rate recovery immediately after exercise and the metabolic syndrome: the CARDIA study. Eur Heart J. 2006 Jul;27(13):1592-6. PubMed PMID: 16728422. Epub 2006/05/27. eng.
26. Christou DD, Jones PP, Pimentel AE, Seals DR. Increased abdominal-to-peripheral fat distribution contributes to altered autonomic-circulatory control with human aging. Am J Physiol Heart Circ Physiol. 2004 Oct;287(4):H1530-7. PubMed PMID: 15178547. Epub 2004/06/05. eng.
27. Nilsson G, Hedberg P, Jonason T, Lonnberg I, Ohrvik J. Heart rate recovery is more strongly associated with the metabolic syndrome, waist circumference, and insulin sensitivity in women than in men among the elderly in the general population. Am Heart J. 2007 Sep;154(3):460 e1-7. PubMed PMID: 17719290. Epub 2007/08/28. eng.
28. Kamel EG, McNeill G, Van Wijk MC. Usefulness of anthropometry and DXA in predicting intra-abdominal fat in obese men and women. Obes Res. 2000 Jan;8(1):36-42. PubMed PMID: 10678257.
29. Park YW, Heymsfield SB, Gallagher D. Are dual-energy X-ray absorptiometry regional estimates associated with visceral adipose tissue mass? Int J Obes Relat Metab Disord. 2002 Jul;26(7):978-83. PubMed PMID: 12080453.
30. Pimenta NM, Santa-Clara H, Sardinha LB, Fernhall B. Body Fat Responses to a 1-Year Combined Exercise Training Program in Male Coronary Artery Disease Patients. Obesity. 2013 Apr/2013;21(4):723-30. PubMed PMID: 22810978. Epub 2012/07/20. Eng.
31. Lohman TG, Roche AF, Martorell R. Anthropometric Standardization Reference Manual. Champaign: Human Kinetics; 1988.
32. Savgan-Gurol E, Bredella M, Russell M, Mendes N, Klibanski A, Misra M. Waist to hip ratio and trunk to extremity fat (DXA) are better surrogates for IMCL and for visceral fat respectively than for subcutaneous fat in adolescent girls. Nutr Metab (Lond). 2010;7:86. PubMed PMID: 21143876. Epub 2010/12/15. eng.
33. Ketel IJ, Volman MN, Seidell JC, Stehouwer CD, Twisk JW, Lambalk CB. Superiority of skinfold measurements and waist over waist-to-hip ratio for determination of body fat distribution in a population-based cohort of Caucasian Dutch adults. Eur J Endocrinol. 2007 Jun;156(6):655-61. PubMed PMID: 17535865. Epub 2007/05/31. eng.
34. Bruce RA. Exercise testing of patients with coronary heart disease. Principles and normal standards for evaluation. Ann Clin Res. 1971 Dec;3(6):323-32. PubMed PMID: 5156892. Epub 1971/12/01. eng.

35. Howley ET, Bassett DR, Jr., Welch HG. Criteria for maximal oxygen uptake: review and commentary. Med Sci Sports Exerc. 1995 Sep;27(9):1292-301. PubMed PMID: 8531628. Epub 1995/09/01. eng.
36. Shetler K, Marcus R, Froelicher VF, Vora S, Kalisetti D, Prakash M, et al. Heart rate recovery: validation and methodologic issues. J Am Coll Cardiol. 2001 Dec;38(7):1980-7. PubMed PMID: 11738304. Epub 2001/12/12. eng.
37. Carnethon MR, Jacobs DR, Jr., Sidney S, Sternfeld B, Gidding SS, Shoushtari C, et al. A longitudinal study of physical activity and heart rate recovery: CARDIA, 1987-1993. Med Sci Sports Exerc. 2005 Apr;37(4):606-12. PubMed PMID: 15809559. Epub 2005/04/06. eng.
38. Cohen J, Cohen P. Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. 2nd ed. Hillsdale, NJ: Lawrence Erkbaum Associates, Inc.; 1983. 545 p.
39. Panzer C, Lauer MS, Brieke A, Blackstone E, Hoogwerf B. Association of fasting plasma glucose with heart rate recovery in healthy adults: a population-based study. Diabetes. 2002 Mar;51(3):803-7. PubMed PMID: 11872683. Epub 2002/03/02. eng.
40. Hargens TA, Guill SG, Zedalis D, Gregg JM, Nickols-Richardson SM, Herbert WG. Attenuated heart rate recovery following exercise testing in overweight young men with untreated obstructive sleep apnea. Sleep. 2008 Jan;31(1):104-10. PubMed PMID: 18220083. Epub 2008/01/29. eng.
41. Chen MS, Blackstone EH, Pothier CE, Lauer MS. Heart rate recovery and impact of myocardial revascularization on long-term mortality. Circulation. 2004 Nov 2;110(18):2851-7. PubMed PMID: 15505081. Epub 2004/10/27. eng.
42. Hadley DM, Dewey FE, Freeman JV, Myers JN, Froelicher VF. Prediction of cardiovascular death using a novel heart rate recovery parameter. Med Sci Sports Exerc. 2008 Jun;40(6):1072-9. PubMed PMID: 18460999. Epub 2008/05/08. eng.
43. Myers J, Tan SY, Abella J, Aleti V, Froelicher VF. Comparison of the chronotropic response to exercise and heart rate recovery in predicting cardiovascular mortality. Eur J Cardiovasc Prev Rehabil. 2007 Apr;14(2):215-21. PubMed PMID: 17446799. Epub 2007/04/21. eng.
44. Nishime EO, Cole CR, Blackstone EH, Pashkow FJ, Lauer MS. Heart rate recovery and treadmill exercise score as predictors of mortality in patients referred for exercise ECG. Jama. 2000 Sep 20;284(11):1392-8. PubMed PMID: 10989401. Epub 2000/09/16. eng.
45. Horowitz JF. Adipose Tissue Lipid Metabolism During Exercise. In: Hargreaves M, Spriet L, editors. Exercise Metabolism. Champaign: Human Kinetics; 2006. p. 89-104.
46. Bartness TJ, Song CK. Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. Journal of lipid research. 2007 Aug;48(8):1655-72. PubMed PMID: 17460327.
47. Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. Journal of lipid research. 1993 Jul;34(7):1057-91. PubMed PMID: 8371057.
48. Bertin E, Marcus C, Ruiz JC, Eschard JP, Leutenegger M. Measurement of visceral adipose tissue by DXA combined with anthropometry in obese humans. Int J Obes

Relat Metab Disord. 2000 Mar;24(3):263-70. PubMed PMID: 10757618. Epub 2000/04/11. eng.

49. Snijder MB, Visser M, Dekker JM, Seidell JC, Fuerst T, Tylavsky F, et al. The prediction of visceral fat by dual-energy X-ray absorptiometry in the elderly: a comparison with computed tomography and anthropometry. Int J Obes Relat Metab Disord. 2002 Jul;26(7):984-93. PubMed PMID: 12080454. Epub 2002/06/25. eng.

546 **TABLES:**

547

548 Table 1. Descriptive data of the studied sample.

	NAFLD Patients (n=25)			
Variables	**Mean ± sd ***		**Min. – Max.**	
Age, yr (median, yr)	48.6 ± 12.8	(49)	25 – 68	
Sex, n female (% female)	8	(30.8)		
VO_2max, ml/kg/min	24.9 ± 6.4		13.8 – 38.0	
Type 2 Diabetes Mellitus, n (%)	8	(28.6)		
Insulin resistance, n (%)	12	(42.9)		
HRR1, bpm	19.4 ± 10.1		-4.0 – 37.0	
HRR2, bpm	35.9 ± 16.7		-8.0 – 67.0	
Whole Body Analisys				
Weight, kg	88.0 ± 12.8		66.2 – 115.8	
Stature, cm	167.3 ± 9.4		149.5 – 183.7	
BMI, kg/m^2 (% obese)	29.1 ± 4.1	(34.6)	22.6 – 42.2	
BF, kg (%)	27.5 ± 9.4	(31.52 ± 8.29)	13.7 – 51.2	(18.84 – 46.28)
FFM, kg (%)	58.8 ± 9.2	(68.48 ± 8.29)	39.6 – 77.7	(53.72 – 81.16)
Regional Body Analisys				
Trunk BF, kg (%)	15.4 ± 5.2	(33.37 ± 7.71)	7.4 – 25.0	(20.87 – 48.01)
Trunk FFM kg (%)	29.9 ± 4.0	(66.63 ± 7.31)	21.1 – 38.6	(51.99 – 79.13)
Appendicular BF, kg (%)	11.0 ± 4.8	(30.63 ± 10.54)	5.2 – 25.7	(13.63 – 50.40)
Appendicular FFM, kg (%)	28.5 ± 5.1	(80.40 ± 6.56)	19.2 – 36.7	(68.64 – 90.66)
Abdominal BF, kg (%)	3.5 ± 1.2	(37.99 ± 6.67)	1.7 – 6.3	(26.09 – 49.40)
Central Abdominal BF, kg (%)	2.9 ± 0.8	(35.94 ± 5.78)	1.6 – 5.0	(24.28 – 44.64)
Body Fat Distribution (Ratios)				
Trunk BF-to-Appendicular BF ratio	1.478 ± 0.378		0.958 – 2.547	
Abdominal BF-to-Total BF ratio	0.130 ± 0.026		0.045 – 0.185	
Abdominal BF-to-Trunk BF ratio	0.233 ± 0.040		0.095 – 0.299	

549 * results are presented as mean ± standard deviation, unless otherwise noted; VO_2max – maximal oxygen
550 consumption; BF – body fat; BMI – body mass index; FFM – fat free mass; HRR1 – heart rate recovery at 1 min.;
551 HRR2 – heart rate recovery at 2 min.; Máx. – highest observed value; Min. – lowest observed value.

552

553

554

555 Table 2. Partial and semipartial correlations between dependent and
556 independent variables.

	HRR 1		HRR 2	
Variables	**r** †	**r** ‡	**r** †	**r** ‡
Age	- 0.120 §	- 0.093 ¶	- 0.154 §	- 0.115 ¶
Whole Body Analisys				
Weight, kg	- 0.613 **	- 0.565 **	- 0.484 *	- 0.440 *
Stature, cm	- 0.176	- 0.162	- 0.161	- 0.147
BMI, kg/m^2	- 0.325	- 0.299	- 0.164	- 0.149
BF, kg	- 0.493 *	- 0.453	- 0.313	- 0.285
BF, %	- 0.241	- 0.222	- 0.068	- 0.062
FFM, kg	- 0.190	- 0.172	- 0.144	- 0.129
FFM, %	0.235	0.213	0.192	0.172
Regional Body Analisys				
Trunk BF, kg	- 0.597 **	- 0.550 **	- 0.446 *	- 0.406 *
Trunk BF, %	- 0.356	- 0.327	- 0.232	- 0.211
Trunk FFM, kg	- 0.211	- 0.192	- 0.151	- 0.135
Trunk FFM, %	0.288	0.262	0.259	0.232
Appendicular BF, kg	- 0.273	- 0.251	- 0.096	- 0.088
Appendicular BF, %	- 0.020	- 0.018	0.186	0.170
Appendicular FFM, kg	- 0.179	- 0.163	- 0.140	- 0.125
Appendicular FFM, %	0.171	0.156	0.144	0.129
Abdominal BF, kg	- 0.491 *	- 0.451 *	- 0.265	- 0.241
Abdominal BF, %	- 0.296	- 0.272	- 0.093	- 0.085
Central Abdominal BF, kg	- 0.553 **	- 0.508 **	- 0.335	- 0.304
Central Abdominal BF, %	- 0.376	- 0.345	- 0.170	- 0.154
Body Fat Distribution (Ratios)				
Trunk BF-to-Appendicular BF ratio	- 0.547 **	- 0.503 **	- 0.590 **	- 0.537 **
Abdominal BF-to-Total BF ratio	- 0.150	- 0.138	- 0.042	- 0.038
Abdominal BF-to-Trunk BF ratio	0.086	- 0.079	0.260	0.236

557 BF – body fat; BMI – body mass index; FFM – fat free mass; HRR1 – heart rate recovery at 1
558 min.; HRR2 – heart rate recovery at 2 min.; † – partial correlations controlling for age and sex
559 (except when age is a variable); ‡ – semipartial correlations removing the effect of age and
560 sex (except when age is a variable); § – partial correlation controlling for trunk BF/ Limb BF
561 ratio and sex; ¶ – semipartial correlation removing the effect of trunk BF/ Limb BF ratio and
562 sex. * - significant for $p<0.05$; ** - significant for $p<0.01$; *** - significant for $p<0.001$.
563
564

565 Table 3. Linear regressions with R square change analisys (Enter method) between dependent and
566 related independent variables.

Variables	Model †	R	R square	R square change	P
HRR 1 ‡					
Trunk BF-to-Appendicular BF ratio		0.617	0.380	--	0.001 **
	Weight, kg	0.739	0.546	0.166	0.012 *
	BF, kg †	0.741	0.549	0.169	0.011 *
	Trunk BF, kg	0.724	0.524	0.144	0.020 *
	Abdominal BF, kg	0.657	0.432	0.052	0.167
	Central Abdominal BF, kg	0.664	0.441	0.061	0.138
	Age, yr	0.625	0.391	0.011	0.346
HRR 2 ‡					
Trunk BF-to-Appendicular BF ratio †		0.655	0.430	--	0.000 ***
	Weight, kg	0.709	0.502	0.072	0.087
	Trunk BF, kg	0.698	0.487	0.057	0.131
	Age, yr	0.666	0.444	0.014	0.467

567 BF – body fat; HRR1 – heart rate recovery at 1 min.; HRR2 – heart rate recovery at 2 min.; † – Regressions were
568 conducted using pairs of independent variables, which include always Trunk BF/Appendicular BF ratio plus one
569 of the listed variables; ‡ – Dependent variable in the following regressions. * – significant for $p<0.05$; ** –
570 significant for $p<0.01$; *** – significant for $p<0.001$.
571

572