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# NON-ALCOHOLIC FATTY LIVER DISEASE SEVERITY, CENTRAL FAT MASS AND ADINOPECTIN: A CLOSE RELATIONSHIP

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***Abstract***

***Aim.*** *Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the general population. Overweight is a common condition in patients with NAFLD, and body composition (BC) assessment is useful to evaluate nutritional status and the efficacy of nutritional strategies. A valid tool for assessing BC is dual-energy X-ray absorptiometry (DXA). Adiponectin has been shown to be relevant to the pathogenesis of NAFLD. The aim of this observational study is to define the relationship between the severity of NAFLD, the central fat mass evaluated by DXA, and the circulating levels of adiponectin.*

***Methods.*** *The study was carried out in 31 overweight patients. The degree of liver steatosis was evaluated by ultrasound (US) examination. Anthropometric parameters were measured according to standard methods. Fasting glucose and insulin level were used also to calculate insulin resistance (IR), according to the homeostasis model assessment-insulin resistance (HOMA-IR). The enzyme-linked immunosorbent assay technique was performed to dose fasting serum levels of adiponectin.*

***Results****. NAFLD progression was significantly associated with increased central fat ($p<0.05$). Using DXA, we quantified the regional distribution of adipose tissue and found the expected association between central fat and the US severity of NAFLD. Serum levels of adiponectin, were inversely related to NAFLD progression ($p<0.05$).*

***Conclusion.*** *BC evaluated by anthropometry and DXA, may be used as indicator of NAFLD severity in overweight patients. The evaluation of BC in clinical practice, can improve the nutritional strategies and follow-up. In the clinical setting adiponectin may represent a potential marker for the staging of NAFLD.*

**Keywords:** non-alcoholic fatty liver disease, body composition, central obesity, insulin resistance, adiponectin

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is a relevant issue in public health [1,2]. It represents the most common chronic liver disease in the general population and is expected to increase in the future, as a result of an ageing population, obesity and diabetes [2]. NAFLD is the hepatic manifestation of metabolic syndrome and includes a spectrum of disease ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis. NAFLD occurs in 60-95% of

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patients with obesity and in 28-55% of patients with type 2 diabetes mellitus (T2DM). Insulin resistance (IR), with compensatory hyperinsulinemia, plays a pathogenetic key role in NAFLD progression [3].

Given its low cost, repeatability, safety and availability, ultrasound (US) is routinely the first-line imaging technique to detect liver steatosis [4]. US is used for the diagnosis of steatosis on conventional grayscale mode. Central obesity, defined as a presence of excess fat in the abdominal area, is frequently associated with NAFLD and their coexistence in the same subjects increases the likelihood of having more advanced forms of liver disease [5,6]. Liver steatosis progression is associated not only with the body mass index (BMI), but also with visceral adiposity [6]. However, fat mass and fat distribution can be different in subjects with the same BMI [7]. For these reasons, the body composition (BC) evaluation is necessary for assess the nutritional status and the efficacy of nutritional strategies.

A valid and precise tool to measuring BC, is the dual-energy X-ray absorptiometry (DXA), an important method for studying not only osteoporosis but also the soft-tissue composition changes [8]. The accuracy of DXA in measuring the fat mass is 98.8%, and combined with other standard anthropometric parameters, it is a reliable tool for the estimation of central fat [9].

The adipose tissue, has been considered an energy storage organ, but over the last decade several findings have encouraged researches on its the endocrine role. Furthermore, data highlighted that central adipose tissue is a metabolic and inflammatory organ that modulates the action and the metabolism of brain, liver, muscle and cardiovascular system [10]. Recently, the important role of adipokines, peptides synthesized from adipocytes, in the pathogenesis of IR and NAFLD has been discussed [11]. In particular, adiponectin is considered an anti-inflammatory adipokine, able to reduce body fat, to improve hepatic and peripheral insulin sensitivity, and inversely associated with BMI, IR and hepatic fat accumulation [12].

The aim of this observational study was to investigate, in overweight patients, the possible relationship between central fat mass, liver steatosis and the circulating levels of adiponectin.

## Materials and methods

The study was carried out in overweight patients, referred to the Division of Clinical Nutrition and Nutrigenomics, University of Rome "Tor Vergata" of Rome (Italy), for a nutritional evaluation, in the period January - July 2013. The study was performed in accordance with Good Clinical Practice and complying with the principles laid down of Declaration of Helsinki. The study was approved by the appropriate Ethical Committee of the Centre, and all patients gave their written informed consent before the recruitment.

We excluded patients with heart diseases, renal failure even if newly discovered. Other exclusion criteria were: insulin therapy, smoking habits, alcohol intake (>20 g/day), hepatic virus infection, auto-immune diseases, and use of drugs known to induce liver steatosis or NASH. After the initial evaluation of 52 patients, 21 (40.4%) were excluded: 5 for heart diseases, 4 for insulin therapy, 2 for hepatitis B virus infection, 6 for excessive daily alcohol intake and 4 for treatment with drugs-inducing steatosis or NASH.

***Anthropometric and laboratory evaluation***

Body weight, height, BMI, and waist circumference were measured according to standard methods [13]. All subjects underwent a biochemical examination of fasting glucose, fasting insulin and alanine aminotransferase (ALT). Fasting glucose and insulin level were used also to calculate IR, according to the homeostasis homeostasis model assessment (HOMA-IR) [14]. The enzyme-linked immunosorbent assay technique was used to measure fasting serum levels of adiponectin in all patients (R&D Systems Inc., USA).

***Ultrasound assessment of NAFLD***

The patients underwent US liver by a Toshiba Eccocee, with a convex transducer of 3.7 Mhz. The results were interpreted by an investigator (LA) with experience in the field. The liver images were considered normal if the texture was homogenous, exhibited fine level echoes and isoechoic compared to the renal cortex and there was adequate visualization of the hepatic vessels and diaphragm (grade 0). Criteria for determining the stage of liver steatosis, in according to the Hamaguchi score, included: presence of bright echoes or increased hepato-renal contrast indicative of mild steatosis (grade 1); presence of both bright echoes and increased hepato-renal contrast as well as vessel blurring indicative of moderate steatosis (grade 2); severe steatosis was considered when in addition to the criteria for moderate steatosis, there was evidence of posterior beam attenuation and non-visualization of the diaphragm (grade 3) [4].

***Body composition analysis***

BC was assessed by DXA (Lunar DPX-IQ; GE Medical Systems, Milwaukee, WI), according to the standardized described procedure [15]. Standard DXA quality control and calibration measures, were performed prior to each testing session. The subjects, were instructed to avoid exercise within 24 hours from the test. The test takes about twenty minutes, and the subjects remain in a supine position during the scanning. The results, were transmitted to a connected computer for further analysis, according to the manufacturer orientation.

***Statistical analysis***

In order to show the relation between DXA parameters and US stage of NAFLD, we displayed graphically the evolution of these features among classes, and we assessed the statistical significance of clinical and

laboratory features. All study variables, were treated as continuous variables. Summary outcome measures were reported as mean±standard deviation. Statistical differences of laboratory and clinical variables between the four groups were evaluated by the ANOVA test. A p-value of $\leq 0.05$ was considered statistically significant. Data analysis was performed using the statistical package Primer for Windows.

## Results

Clinical and laboratory characteristics of the patients, stratified on the basis of the US stage of NAFLD, are reported in table I. Of the 31 patients included in the study, 13 were male (41.9%). The median value of anthropometric parameters assessed were: BMI 31.4±5.9 and waist circumference 97.2±16.2 cm, with 16 patients (7 males) that presented central obesity, according to the definition of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) [16].

US evaluation of the liver showed that: in 8 patients (25.8%) steatosis was absent, in 9 (29%) mild (grade 1), in 6 (19.4%) moderate (grade 2), and in 8 (25.8%) severe (grade 3). The median value of ALT was 33.6±27.3 Ul-1, with four patients that reported high ALT value, one without, two with mild and one with severe US liver steatosis.

Of all DXA parameters analyzed, those that present a statistical significant difference between the US stages of NAFLD were (Figure 1): abdominal fat (AF, %), mean value 42.6±11.9 ($p<0.05$); abdominal tissue mass (ATM: fat plus lean mass, kg), (mean value 7±2.5, $p<0.05$); abdominal fat mass (AFM, kg), (mean value 3.9±1, $p<0.05$). Considering the only distribution of L2-L5 fat tissue (kg), (mean value 5.2±1.2), we observed that the value was higher, but reached no statistical significance, in patients with moderate and severe steatosis, compared to those with the mild type. The assessment of IR by HOMA-IR, showed a statistically significant increase of insulin level, related to steatosis progression ($p<0.05$). Finally, the concentration of adiponectin (7.78±2.2 µg/mL), was significantly lower in the advanced US stages of NAFLD ($p<0.05$).

**Table I**. Clinical and laboratory characteristics of the patients on the basis of US severity of NAFLD.

| Variable | Overall | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|---|---|
| Age (years) | 49.2 ± 12.1 | 44.4±10.9 | 50.9±10.3 | 55.7±17.0 | 47.1±11 |
| Weight (kg) | 87.2±20.3 | 75.3±13.3 | 81.6±21.5 | 84.51±18.5 | 106.86±23.7 |
| Male | 13 (41.9%) | 3 (9.6%) | 3 (9.6%) | 2 (6.4%) | 5 (16.1%) |
| BMI (Kg/m $^2$) | 31.4±5.9 | 28.0±4.4 | 30.6±2.7 | 31.5±7.6 | 35.3±5.4 |
| Waist circumference (cm) | 97.2±16.2 | 87.1±11.7 | 95±15.2 | 101.3±4.8 | 108.2±20.5 |
| ALT (n.v. <32 Ul $^{-1}$) | 33.6±27.3 | 19.5±11.1 | 30.4.±20.6 | 40.1±26.1 | 49.3±42.1 |
| Fasting glucose (n.v. 70 -100 mg/100 ml) | 105.8±13.8* | 92 ±5.85 | 101.3±1.4 | 105±6.5 | 124.75±9.24 |
| Fasting Insulin (n.v. <25 mU ml $^{-1}$) | 25.1±4.3* | 21.8±2.8 | 22.9±1.8 | 24.7±1.46 | 30.9±2.6 |
| HOMA-IR Index (n.v. <1.5) | 1.2±0.3* | 0.9±0.1 | 1.0±0.1 | 1.2±0,1 | 1.7±0.2 |
| Adiponectin (n.v. 10µg/mL) | 7.8±2.2* | 11.2±0.7 | 7.1±0.8 | 6.1±0.3 | 5.9±0.3 |
| AF (%) | 42.6±11.9* | 33.6±15 | 43.2±11 | 47.15±6.7 | 48.5±7.5 |
| ATM (kg) | 7±2.5* | 5.2±1.5 | 6.8±1.9 | 7.3±1.1 | 9.22±3.1 |
| AFM (kg) | 3.9±1* | 4±0.8 | 4±1 | 4.3±0.7 | 4.6±1.2 |
| Distribution of L2 -L5 (g) | 5.2±1.2 | 4.6±1.1 | 4.9±1.5 | 5.5±1.2 | 5.9±1 |

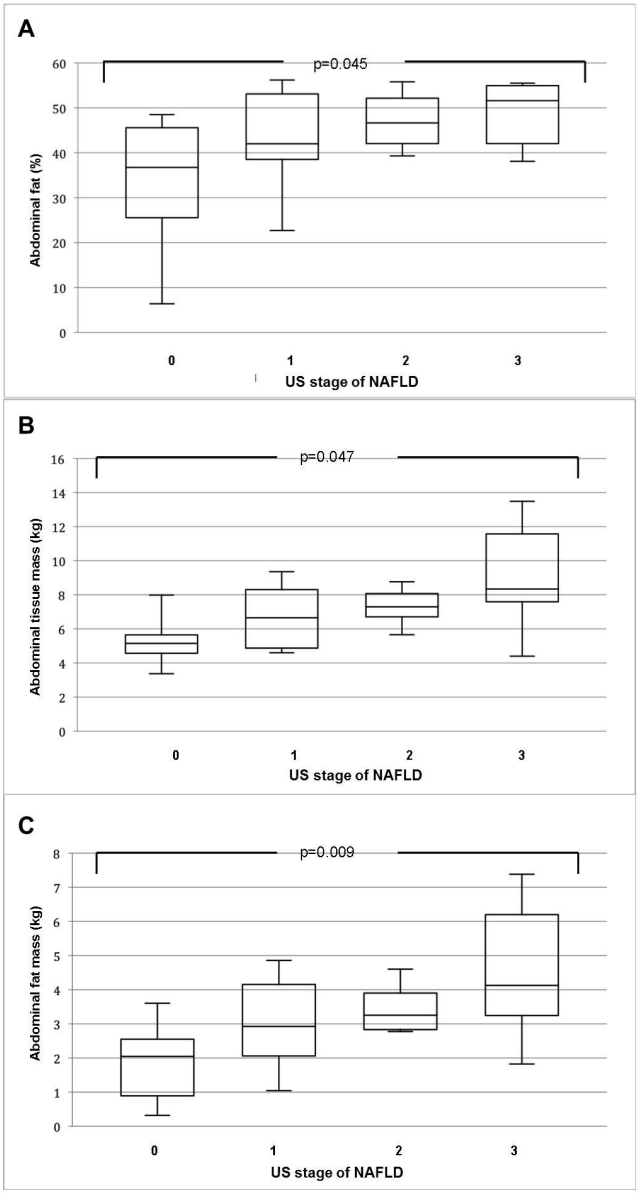


**Figure 1.** Distribution of abdominal fat (box A), abdominal tissue mass (box B) and abdominal fat mass (box C) in different US stages of NAFLD.

## Discussion

Literature reported the association between central obesity and liver steatosis [16]. Obesity, which is often associated with IR, represents a chronic low-grade inflammatory state, characterized by elevated circulating levels of cytokines and activation of pro-inflammatory signaling pathways [17]. In particular, IR plays a key role in the pathogenesis of NAFLD, causing alterations in the uptake, degradation or secretion of lipid molecules, with consequent accumulation of lipid in the hepatocytes [17,18]. NAFLD is usually prevalent in obese subjects, and several studies have reported that regional fat distribution associated with IR is an important factor for development and progression of liver steatosis [4,11]. In our study, abdominal fat accumulation and anthropometric parameters, increased with US severity of liver steatosis and IR. In this context, DXA seems to be an useful tool to evaluate overweight patients. The first observation is that by using DXA, we quantified the regional distribution of adipose tissue and we found the association between increased central fat mass, and liver steatosis severity. This observation is in agreement with the progression of the values of BMI and circumference observed in our series. Concerning the relationship between BC and NAFLD, this study has shown that central fat accumulation constitutes an important determinant of liver steatosis in overweight patients, independently to BMI.

Recent data document that the abdominal adipose tissue is an active endocrine organ capable to secrete a multitude of hormones, cytokines, chemokines, and enzymes, collectively known as adipokines [5]. In particular adiponectin, leptin, resistin, visfatin and pro-inflammatory cytokines, such as tumor necrosis factor-α, and interleukins, have been shown to be involved in pathogenesis and progression of NAFLD [11,12]. The increased number of abdominal adipocytes produces a disequilibrium in adipokines secretion, which promotes liver fat accumulation, and subsequently the development of NAFLD. In particular, adiponectin improves muscular and hepatic insulin sensitivity, through its anti-inflammatory and anti-atherogenic activity, and its ability to decrease triglyceride synthesis and stimulate β-oxidation [3,11]. In fact adiponectin protects hepatocytes from triglycerides accumulation by increasing β-oxidation of free fatty acid and/or decreasing *de novo* free fatty acid production in hepatocytes [19]. Adiponectin levels are reduced in obese patients, and T2DM, and the plasma concentrations are inversely related to body weight, especially to visceral adiposity [20]. Moreover, adiponectin is inversely associated with other traditional cardiovascular risk factors, such as blood pressure, low-density lipoprotein cholesterol and triglyceride levels, and is positively related to high-density lipoprotein cholesterol levels [21]. In our cohort, US liver steatosis progression is characterized by IR and low adiponectin serum levels, pathogenetic factors that can increase the concentrations of intra-cellular fatty acids, and may enhance oxidative stress that is the second stage in the pathogenesis of NASH.

## Conclusion

BC assessed by anthropometry and DXA, may be used as indicator of NAFLD severity in overweight patients. In particular we report the direct relationship between central fat mass and NAFLD progression. However, further studies are required to better understand not only this correlation, but also to define the pathogenetic role of central fat distribution and the changes in adipokine levels in the progression of NAFLD.

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