Influence of lifestyle habits, nutritional status and insulin resistance in NAFLD

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1. ABSTRACT

Non alcoholic fatty liver disease (NAFLD) is associated with obesity, diabetes and insulin resistance (IR). The aim of our study was to assess the relationship between IR, anthropometry, lifestyle habits, resting energy expenditure (REE) and degree of fatty liver at ultrasound in 48 overweight patients with NAFLD as compared to 24 controls without fatty liver, matched for age. Nutritional status, alcohol intake and physical activity were assessed by skinfold thickness measurements, a 7-day diary, and SenseWear armband (SWA). REE was assessed by both SWA (REE-SWA) and a Vmax metabolic cart (REE-Vmax). Fatty liver was measured by US and the Doppler Power Index was calculated. IR was assessed using the HOMA index. There was significant correlation between waist circumference, HOMA, Doppler power index and fatty liver grade at US. Multivariate analysis showed that alteration of waist circumference, Doppler power index, and HOMA were the major significant predictors of fatty liver. Our data demonstrated a significant association between NAFLD and central adiposity and IR.

2. INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) has been found to be the most prevalent chronic liver disease in Western countries (1-3), it is closely associated with the presence of insulin resistance (IR), and is now considered the early hepatic manifestation of metabolic syndrome (3, 4). NAFLD encompasses a histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis (5), its histological features resembles those of alcoholic steatohepatitis, however it occurs in patients who consume less than 20 g of alcohol per day (6). Liver biopsy still remains the gold-standard and most cost-effective test in patients with NAFLD for the diagnosis of NASH, but not for diagnosis of NAFLD itself (7), which is usually formulated, particularly in general population studies, with liver ultrasound. The benefits of changes in lifestyle habits in NAFLD, meaning gradual weight loss, increase in physical activity, and behaviour therapy, are fairly well established as the first choice treatment for NAFLD (8-11). However systematic and complete analysis of the possible

differences in eating habits, physical activity, anthropometric measurements, body composition, energy expenditure, and IR between overweight patients with and without NAFLD are lacking.

The aim of this study was to evaluate the possible correlation between the presence of fatty liver, diagnosed by the non-invasive method of liver ultrasound, with diet, energy expenditure, body composition, physical activity and IR, as detected by the homeostasis model assessment (HOMA) index, in overweight adult patients with NAFLD as compared to controls.

3. MATERIAL AND METHODS

3.1. Subjects

A convenience sample of 72 subjects: 48 patients with NAFLD (29 males and 19 females), and 24 apparently healthy controls without NAFLD (14 males and 10 females), matched for age, but not for body mass index (BMI), since this could be one of the predictors of NAFLD, were enrolled in the study.

Subjects were asked to follow their usual diet during the week preceding the study. Each subject underwent a general history and physical examination, an anthropometric assessment, a dietary and daily physical activity evaluation, a clinical and laboratory evaluation, and a liver ultrasound. The diagnosis of FL was performed by ultrasound, using standardised criteria (12) , and the amount of fat in the liver was graded from 1 to 4 on the basis of the presence of 1 or more of the following signs: a) Poor visibility of intrahepatic venous vessels and no expansion of the hepatic and portal veins diameters at deep breath; b) Diffuse hyperechoic echotexture (“bright liver”); c) Deep echo attenuation; d) Increased liver echotexture compared with the kidney. Liver ultrasound was performed in all subjects by the same operator, who was unaware of the clinical and laboratory data. NAFLD was operationally defined as the presence of fatty liver in a subject drinking no more than 20 g/day of ethanol, in the absence of both HBsAg and HCV-RNA positivity (13). Any patients with other medical illnesses or other causes of chronic or acute liver disease were excluded from the study. Clinical examination included a detailed interview aimed at excluding the use, in the last 6 months, of drugs able to induce fatty liver (*e.g.,* amiodarone, anti-oestrogen receptors), surgical procedures able to induce fatty liver (*e.g.,* bilio-pancreatic diversion) or the presence of other significant steatosis-inducing condition, such as hypothyroidism or polycystic ovary syndrome. Systolic and diastolic blood pressure was measured in triplicate on the same day, and the mean value of the three measurements was used for analysis(14).

The study was conducted according to the guidelines established by the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the local ethics committee. Written informed consent was obtained from all subjects.

3.2. Laboratory tests

Laboratory evaluation included HBsAg and anti-HCV antibodies, measured as described elsewhere (15). Alanine transaminase (ALT), gamma-glutamyl transferase (GGT), fasting glucose, triglycerides, cholesterol and high-density lipoprotein (HDL) cholesterol, were performed using standard laboratory methods. Insulin was measured by radioimmunoassay (ADVIA Insulin Ready Pack 100, Bayer Diagnostics, Milan, Italy), with intra- and inter-assay coefficients of variation 5%, and insulin sensitivity was estimated using the HOMA method ([glucose (mmol/L) / insulin (mU/L)]/22.5)(16).

3.3. Anthropometry

All anthropometric measurements were performed by the same operator according to the Anthropometric Standardization Reference Manual (17). Skinfold thicknesses (biceps, triceps, sub-scapular, supra-iliac, calf and mid-thigh) and circumferences (arm, waist, hip, calf and mid-thigh) were measured to the nearest millimetre using callipers on the right-hand side of the body and an anthropometric tape, respectively (Holtain, Crymich, UK) (17). All skinfold and circumference measurements were repeated three times and the three values were averaged. Weight (Wt) was measured to the nearest 100 g and height (Ht) to the nearest 0.1 cm using an electronic scale with a built-in stadiometer (Tanita, Tokyo, Japan). Body mass index (BMI) was calculated as Wt/Ht2 (kg/m2).

3.4. Resting energy expenditure (REE) measurement.

REE was measured in the morning (08:00e10:00am), using both Sense Wear Armband (SWA - SenseWear Pro2 Armband, BodyMedia Inc, Pittsburgh, PA, USA) and) and a Sensor Medics Vmax metabolic cart with a ventilated canopy (SM-29N Metabolic Cart, Yorbe Linda CA, USA), after a 12 h fast and at least 24 h free of structured PA on different days of the same week (18). The SWA is a portable device that monitors various physiological parameters (heat flux, skin temperature, galvanic skin response and near-body temperature), demographic characteristics (gender, age, height, weight), introduced into proprietary algorithms to estimate energy expenditure, and movement (accelerometer). SWA has already undergone validation studies in different fields (19, 20) and has been shown to be not completely reliable for the assessment of REE (20), but very useful for the assessment of physical movement and lifestyles (21, 22).

The SWA was positioned in all patients over the triceps muscle on the upper right arm, halfway between the acromion and olecranon processes, 10 min before data collection (23). SWA data were collected for at least 3 work days. REE- SWA was estimated by the Innerview Research Software version 6.0.

All measurements with the ventilated canopy and the SM-29N Metabolic Cart were made in a thermoneutral environment and in the absence of external stimuli. All the subjects were asked to rest quietly in a supine position for approximately 30 or 40 min in an isolated room, with a temperature of between 20 and 25°C, after which REE measurements were performed with subjects remaining in the supine position. The data collected during the first 5-10 min were discarded to allow the subjects to adapt to the

canopy and instrument noise (24). The criterion for a valid REE was 15 min of steady state, determined as <5% variation in respiratory quotient (RQ)/minute and oxygen consumption/minute. At least 30 min of respiratory gas exchange data were collected. Oxygen consumption and carbon dioxide production were used to calculate REE in accordance with the Weir formula (25).

3.5. Dietary assessment

A simple 7-day diary of food intake exploring time, type and quantity of all the food and beverage (enclosed water) eaten and drunk was explained and administered to the subjects by a trained dietician, who discussed it with the subject when he or she handed it in one week later. To avoid the possible confounding effect of seasonality on food intake, the 7-day diary was administered to a similar number of cases and controls each month. Mean daily ethanol intake was calculated as the mean value of ethanol intake as assessed by the 7-day diary, which is the recognized gold standard for the assessment of food intake (16) and is more accurate than conventional methods for the evaluation of alcohol intake (26).

3.6. Physical activity evaluation

Physical activity was measured by using both a standardized physical exercise diary (27), and the SWA mentioned above. Both the diary and SWA measurements were expressed in metabolic equivalent. One metabolic equivalent is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml O_2 per kg body weight x min. The metabolic equivalent concept represents a simple, practical, and easily understood procedure for expressing the energy cost of physical activity as a multiple of the resting metabolic rate. The energy cost of an activity can be determined by dividing the relative oxygen cost of the activity (ml O2/kg/min) x by 3.5 (28).

3.7. Power Doppler measurements

Doppler Perfusion Index (DPI) was measured as suggested by Kakkos SK and coworkers (29). Briefly, for performing liver and Doppler ultrasound we used a Colour Doppler Ultrasound machine and a 3-MHz Convex array transducer (Esaote, Italy). The instrument was used with the proper software for direct and automatic calculation of haemodynamic parameters based on the spectral Doppler waveform.

The following haemodynamic indices were measured at the portal vein and the common hepatic artery:

1. Time averaged mean velocity (mean TAV);
2. Portal vein and hepatic artery flow volume (PFV, HFV) [Vessel Cross Section x mean TAV];
3. Doppler perfusion index (DPI) [HFV/PFV + HFV]

Patients were fasting and had not taken any medication for at least 12 h prior to the examination. Portal vein measurements were taken in a supine position right at the origin of the vessel from the splenic and superior mesenteric veins. Hepatic artery measurements were also taken right at the origin of the vessel from the coeliac trunk. All patients were asked to hold their breath briefly. We recorded the Doppler waveforms for at least four cardiac cycles. The size of the volume sample was equal to the vessel diameter in all cases. The angle between the beam and the vessel was always less than 60° and the vessel diameter was always measured at the sampling point.

All portal vein and common hepatic artery measurements for each individual were obtained at least three times and the average values were entered in the calculation of the DPI. All the measurements were taken by the same expert operator (L.M.). The intra-observer variability in the obese patients was similar to that with normal controls.

3.8. Statistical analysis

Continuous variables are given as medians and interquartile ranges on account of skewed distributions. Comparisons of continuous variables between subjects with and without NAFLD were performed with the Mann-Whitney *U* test and those of nominal variables with Fisher's exact test. The analysis of risk factors for NAFLD was performed in the pooled sample by comparing subjects with NAFLD with those with normal liver (controls). Comparisons of continuous variables between subjects with NAFLD, and normal liver were performed with the Kruskal-Wallis *H* test and those of nominal variables with Pearson's chi-square test.

Pearson and Spearman correlations were used to evaluate the relationship between REE-SWA and REE-Vmax, and between fat mass percentage and fat-free mass percentage with different body composition methods. Bland and Altman's method was also used to calculate the limits of agreement between REE-SWA and REE-Vmax (30). Bias was defined as the difference between REE-SWA and REE-Vmax . Pitman's test was used to evaluate proportional bias (30). Lin's concordance correlation coefficient was calculated as a further measure of agreement (31).

Predictors or risk factors for the presence of NAFDL were evaluated with a multinomial logistic regression model, and the Cochran Mantel-Haenszel likelihood ratio test was used to assess overall significance (32). Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated. Significant predictors of the presence of NAFLD at univariate analysis were evaluated at multivariate analysis. The cut-offs for all the variables used were based on the upper limit of the median value found in the control group and reported in Table 1, and the Wald test statistic was used to assess overall significance. Statistical significance for all the tests was set as p values less than 0.05. Statistical analysis was performed using SPSS for windows version 14.0 (SPSS Inc., Chicago, Illinois, USA).

4. RESULTS

4.1. Characteristics of subjects with and without NAFLD

As reported in Table 1, the only parameters found to be significantly different in both sexes in patients with

Table 1. Baseline characteristic of the population studied with and without NAFLD

	Controls (n=24)		NAFLD (n=48)		P (NAFLD vs Controls)	
	M (14)	F(10)	M (29)	F(19)	M	F
Age (years)	60(21)	59(18)	51(21)	58(19)	N.S.	N.S.
Weight (kg)	74.25(13.8)	65.0(15)	82.5(19.5)	77(19.5)	0.016	0.007
Stature (cm)	174(9)	160(14)	171 (12)	157(8)	N.S.	N.S.
BMI	24.9(4.8)	25.5(6.1)	29.4(4.4)	32.8(7.1)	0.001	0.004
Sistolic BP (mmHg)	130(10)	130(10)	130(15)	135(15)	N.S.	N.S.
Diastolic BP (mmHg)	80(10)	85(5)	80(5)	85(10)	N.S.	N.S.
Glicemia (mg/dl)	106(9)	93(6)	103(16)	97(20)	N.S.	N.S.
ALT (UI/l)	23(10)	19(7)	41(24)	22(13)	0.008	N.S.
Gamma-GT (UI/l)	21(14)	15(18)	32(15)	22(13)	0.008	N.S.
Insulinemia (ng/ml)	4.0(4)	5.3(3.4)	8.3(5.6)	8.5(3.8)	0.010	0.008
HOMA	0.9(1.1)	1.2(0.8)	2.15(1.4)	2.13(1.7)	0.008	0.002
Tryglicerides (mg/dl)	72(39)	87(44)	132(73)	110(103)	0.011	N.S.
HDL-Cholesterol (mg/dl)	49(18)	54(26)	42(19)	51(12)	N.S.	N.S.
Doppler Power Index (DPI)	0.13(0.05)	0.18(0.04)	0.20(0.08)	0.20(0.08)	0.001	0.020
Phys. Act (MET-SW ArmBand)	1.4(0.3)	1.4(0.7)	1.4(0.4)	1.2(0.5)	N.S.	N.S.
Physical Activity (MET Diary)	1.2(0.6)	1.0(0,3)	1.3(0.4)	1.0(0.3)	N.S.	N.S.
Waist Circumference (WC)	91(8)	80(17)	95(11)	95(11)	0.020	0.001
Hip Circumference (HC)	98(10)	100(13)	104(6)	114(19)	N.S.	0.004
W/Hip Ratio	0.91(0.08)	0.79(0.09)	0.92(0.07)	0.84(0.06)	N.S.	N.S.
Trycipt fold (mm)	9(4)	19(12)	11(7)	26(13)	N.S.	N.S.
Bycipt fold (mm)	5(1)	10(5)	6(4.5)	14(8)	N.S.	0.020
Under-scapula fold (mm)	15(6)	15(10)	17(6)	23(9)	N.S.	0.005
Upper-iliac fold (mm)	16(5)	15(11)	16(5)	30(10)	N.S.	0.001
Fat (kg)	17(4)	22(9)	20(8)	30(10)	N.S.	0.004
FFM (kg)	59(11)	45(5)	63(14)	47(10)	N.S.	N.S.
Food Diary (KCal consumed)	2069(464)	1652(443)	1667(706)	1423(484)	N.S.	N.S.
REE (kcal – Indir. Calorimetry)	1526(283)	1434(224)	1697(514)	1501(527)	N.S.	0.033
REE (kcal - SW Armband)	1653(366)	1362(246)	1771(461)	1553(259)	N.S.	N.S.
Proteins (g)	70(24)	61(12)	61(32)	57(16)	N.S.	N.S.
Carbohydrate (simple) (g)	61(70)	74(46)	72(53)	54(21)	N.S.	N.S.
Carbohydrate (complex) (g)	173(84)	166(71)	149(53)	109(49)	N.S.	N.S.
Fat (g)	66(25)	63(18)	58(24)	54(13)	N.S.	N.S.
Cholesterol (Total) (mg)	252(97)	224(95)	223(118)	172(82)	N.S.	N.S.
Saturated Fatty Acid (/100 g food g)	20(12)	21(7)	20(13)	18(6)	N.S.	N.S.
MUFA (/100 g food g)	30(10)	30(9)	25(9)	26(5)	N.S.	N.S.
PUFA (/100 g food g)	8(3)	9(3)	8(5)	7(1)	N.S.	N.S.
Fiber (g)	6(3)	7(2)	5(2)	6(2)	N.S.	N.S

Values are expressed as median and interquartile range (IQR)

NAFLD vs C were weight, BMI, waist circumference, DPI, insulin serum levels, and consequently HOMA index. Median serum triglyceride, ALT and GGT levels were significantly higher in patients with NAFLD vs C in males only. On the contrary, hip circumference and skinfold thickness were found to be significantly higher in females with NAFLD, but not in males with NAFLD, which simply confirms the "physiological" differences in fat distribution between males and females. No differences were found between NAFLD patients and controls in either energy expenditure or physical activity, calorie intake and diet composition.

4.2. Validation of REE and PA measurements

The agreement between the 3-day physical diary and the SWA measurements, expressed in metabolic equivalent, of both NAFLD and controls patients pooled together (43 men and 29 women) was highly significant ($p < 0.0001$) as calculated using both Pearson's correlation coefficient ($r = 0.765$; C.I. 0.601-0.867) and Lin's Concordance Correlation Coefficient (data not shown). On the contrary, as shown in Figure 1, the level of agreement (LOA) between REE-SWA with REE-Vmax, expressed in kcal/day, even when significant, was less high in both men and women with wide confidential intervals ($r = 0.639$ with confidence interval 0.304-0.679 in males and $r = 0.676$ with confidence interval 0.273-0.673 in females). Figure 2 shows Lin's Concordance Correlation Coefficient (CCC) plots of REE-SWA and REE-Vmax . The fixed bias of REE-SWA was 132.42 in males and 85.63 kcal/day in females, with high standard deviations, causing wide limit of agreement (350 to 521 kcal/day in females and 292 to 557 kcal/day in males). The Lin's CCC was similarly low in both women and men ($p<0.0001$)

4.3. Univariate and multivariate analysis of predictors for NAFLD

Tables 2 and 3 give the results of the univariable and multivariable analysis of risk factors for NAFLD in both sexes. Surprisingly any significant difference was found in eating habits, physical activity and REE between NAFLD and C. At univariate analysis, increased IR, as expressed by both insulinaemia (Figure 3) and the HOMA index (Figure 4), increased BMI and waist circumference (Figure 5), especially in females, were significant predictors of the presence of NAFLD. Interestingly, DPI was able to distinguish FL from normal liver, especially in males (odd ratio = 29; C.I. 3-295, when DPI > 0.13), and it was significantly correlated with the grade of FL in both men (Spearman correlation coefficient = 0.594, $p<0.0002$)

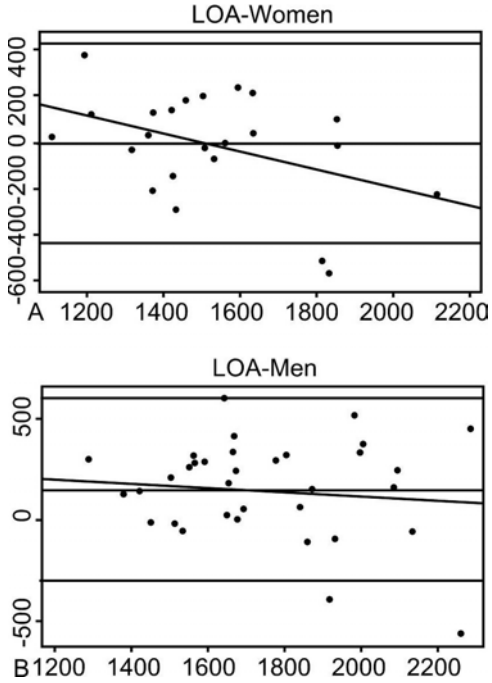

Figure 1. Levels of agreement (LOA) between Resting Energy Expenditure as measured with either a SenseWear armband (REE-SWA, in ordinate) or a Sensor Medics Vmax metabolic cart with a ventilated canopy (REE-Vmax, in abscissa) in women and men.

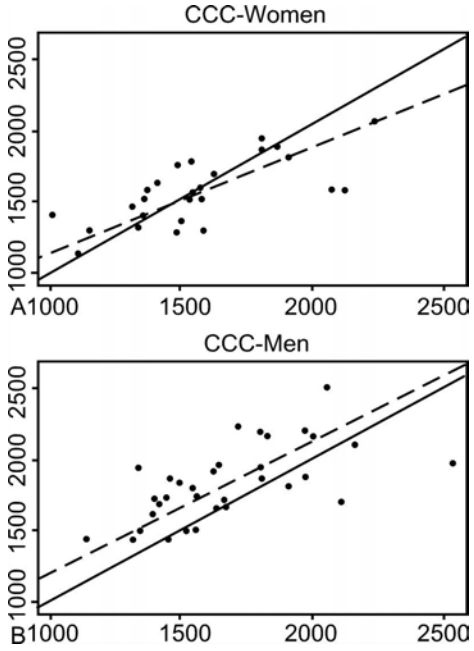

Figure 2. Lin's Concordance Correlation Coefficient (CCC) plots between Resting Energy Expenditure as measured with either a SenseWear armband (REE-SWA, in ordinate) or a Sensor Medics Vmax metabolic cart with a ventilated canopy (REE-Vmax, in abscissa) in women and men.

and women (Spearman correlation coefficient = 0.501 $p<0.01$), as shown in Figures 6. When all these variables significant for the presence of NAFLD at univariate analysis were put together and multivariate analysis was performed (see Table 3), the only 3 parameters that remained statistically significant predictors for the presence of NAFLD, as detected by both the likelihood and Wald test, were DPI greater than 0.13 in males and 0.18 in females ($p=0.025$), waist circumference (WC) greater than 91 cm in males and 80 cm in females, and a HOMA index greater than 0.9 in males and 1.2 in females. When the multivariate analysis was applied to each gender, interestingly, an altered WC was able to predict the presence of FL in females (p Wald <0.030) alone, whereas the HOMA index was able to predict FL in males (p Wald < 0.004, Table 3) alone. Similarly, when Spearman correlation coefficients were calculated, the highest correlation coefficients with the grade of FL were waist circumference in females ($r=0.759$, $p< 0.000001$) and insulinaemia in males ($r=0.651$, $p< 0.0001$) (data not shown).

5. DISCUSSION

Studies exploring the potential differences present in energy expenditure, dietary and lifestyle habits, or the correlation of different tools in measuring physical activity, and the reliability of the SWA in estimating REE as compared with simultaneous indirect calorimetry, in patients with NAFLD are lacking (19-23). The present study, the first to measure all variables simultaneously, ry to cover this lack, in order to design a comprehensive picture of habits and conditions associated with NAFLD. We evaluated the relationship between IR, anthropometry, lifestyle habits, REE and degree of FL at ultrasound in NAFLD vs C patients. We found that there were no significant differences in eating habits, PA and REE between NAFLD and C, although a significant correlation was found between waist circumference, HOMA-IR index, DPI and grade FL at ultrasound.

The agreement between the 3-day physical diary and the SWA measurements, expressed in metabolic equivalent, in both NAFLD and controls patients, was significant, suggesting that SWA is a reliable instrument for measuring lifestyle habits, particularly physical activity. On the contrary, the agreement between REE-SWA and REE-Vmax, even if significant, was not so high in both men and women with wide confidential intervals, thus confirming the data of Bertoli *et al.* (20), and suggesting that SWA is not an accurate tool for evaluating REE. Although the SWA method does not appear to have the same accuracy as indirect calorimetry, it could certainly have some practical advantages over traditional metabolic carts, which are primarily restricted to research laboratories and hospitals, because it can be easily used by physicians and dieticians, and in many other healthcare settings, both in normal subjects, and in patients with NAFLD.

Although weight and BMI were significantly different in the 2 groups studied, NAFLD was not associated with a different pattern of dietary intake and

Table 2. Unvaried analysis for the presence of NAFLD

	TOTAL	MALES	FEMALES									
	Controls	NAFLD	OR (95%C.I.)	p	Controls	NAFLD	OR (95%C.I.)	p	Controls	NAFLD	OR (95%C.I.)	p
BMI (>24.9M and >25.5F)	9/22 (41)	38/46 (83)	7 (2-21)	0,001	4/12 (33)	22/29 (76)	6 (1-27)	0,014	5/10 (50)	16/17 (94)	16 (1.5-171,2)	0,022
WC (cm) (>91M and > 80F)	9/21 (43)	35/44 (80)	5 (2-16)	0,005	5/11 (45)	19/27 (70)	3 (0.7-12)	0,266	4/10 (40%)	16/17 (94)	24 (2,2-260,3)	0,009
Insulin (ng/ml) (>4M and 5.3F)	10/21 (48)	31/37 (84)	6 (2-19)	0,006	5/11 (45)	17/21 (81)	5 (1-25)	0,056	5/10 (50%)	14/16 (87,5)	7 (1-48,3)	0,050
HOMA (>0.9M and 1.2F)	8/21 (38)	33/36 (92)	18 (4-78)	0,0001	4/11 (36)	18/20 (90)	16 (2-106)	0,003	4/10 (40)	15/16 (93,8)	22,5 (2,1-244,8)	0,011
DPI (>0.13M and 0.18F)	8/19 (42)	37/42 (88)	10 (3-38)	0,0001	5/11 (45)	24/25 (96)	29 (3-295)	0,010	3/8 (37,5)	13/17 (76,5)	5,4 (0,88-33,4)	0,050

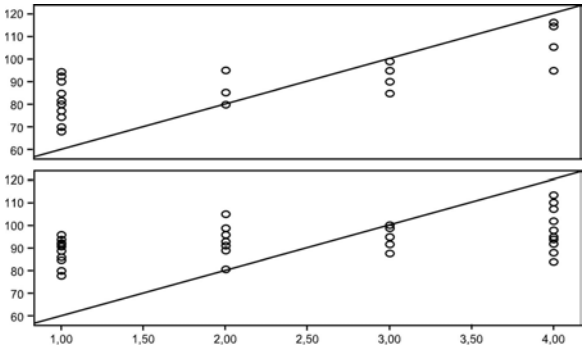

Figure 3. Correlation between Insulinemia (expressed in mU/L in ordinate) and the grade of fatty liver as scored with ultrasonography in both men and women. (The values of Spearman correlation coefficient and statistical significances are reported within the table).

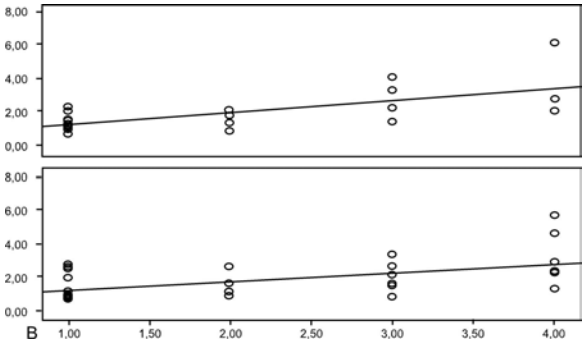

Figure 4. Correlation between HOMA index and the grade of fatty liver as scored with ultrasonography in both men and women. (The values of Spearman correlation coefficient and statistical significances are reported within the table).

energy expenditure. This is certainly surprising, since weight was remarkably different, however this is not the first such finding in literature (33). Very few pilot and non-controlled studies have demonstrated a close relationship between diet and the presence of fatty liver (3, 34-37). The role of either calorie intake or diet composition in modulating the presence of fat in the liver is still unclear. Some authors have shown that NASH patients report a diet that is richer in fat, particularly monounsaturated fat, and poorer in carbohydrates (34), others (35) that patients with NASH had an higher consumption of saturated fat. Recent works suggest that patients with NAFLD have a deficiency of n- 3 polyunsaturated fatty acids (n-3 PUFA), and an increase in n-6: n-3 fatty acid ratio, which is probably related to IR (36), and others authors (37) have demonstrated that supplementation with n-3 PUFA in patients with NAFLD improves the biochemical, ultrasonographic and haemodynamic features of liver steatosis. Unlike these preliminary results published by other authors (34) (35-37), it hasn't been found any significant differences in eating habits and calorie intake between NAFLD and controls, however, this could simply suggest that the tests routinely used, such as the 7-day diary or the semi-quantitative illustrated food questionnaire, are too insensitive to measure small changes in dietary intake.

Furthermore, physical activity and REE seem to be similar, thus suggesting that the only evident effect is metabolic. NAFLD is considered the hepatic component of metabolic syndrome, and lifestyle modifications, including increased physical activity, is definitely useful, and possibly more useful than pharmacological interventions to avoid progression of this disease(3). Only recently, Zelber-Sagi *et al.*, in a larger cohort of patients, demonstrated that habitual leisure-time physical activity, especially anaerobic, may play a protective role in reducing abdominal obesity in NAFLD (4). However, the difference found in previous studies was minimal and their cohort was larger than the cohort of this study. The few studies available thus far do not allow causal inference between reduced physical activity and the presence of fatty liver, since it is also possible that NAFLD patients tend to be less physically active because of their obesity-associated disorders.

In this study, the only parameters found to be significant predictors of NAFLD at univariate analysis in both sexes, were all inter-related: weight, BMI, waist circumference, DPI, and IR. Interestingly, DPI was significantly correlated with the grade of fatty liver in both sexes. This confirms that, when correctly assessed, DPI could be used as a reliable indirect measurement to quantify the amount of hepatic fat content (29, 37). At

Table 3. Multivariate analysis of risk factors for the presence of NAFLD

	TOTAL Controls vs NAFLD (p- wald)	**TOTAL Controls vs NAFLD (p- LR)**	**MALES Controls vs NAFLD (p- wald)**	**MALES Controls vs NAFLD (p- LR)**	**FEMALES Controls vs NAFLD (p- wald)**	**FEMALES Controls vs NAFLD (p- LR)**
BMI (24.9M and 25.5F)	0.589*	0.525*	0.300*	0.300*	0.302*	0.386*
WC (cm) (>91M and > 80F)	**0.021**	**0.003**	0.185*	0.185*	**0.030**	**0.001**
Insulin (ng/ml) (>4M and 5.3F)	0.171*	0.089*	0.384*	0.384*	0.854*	0.096*
HOMA (>0.9M and 1.2F)	**0.008**	**0.001**	**0.050**	**0.004**	0.087*	**0.006**
DPI (>0.13M and 0.18F)	**0.025**	**0.025**	0.269*	0.269*	0.120*	0.090*

*in the stepwise analysis they were taken out of the model

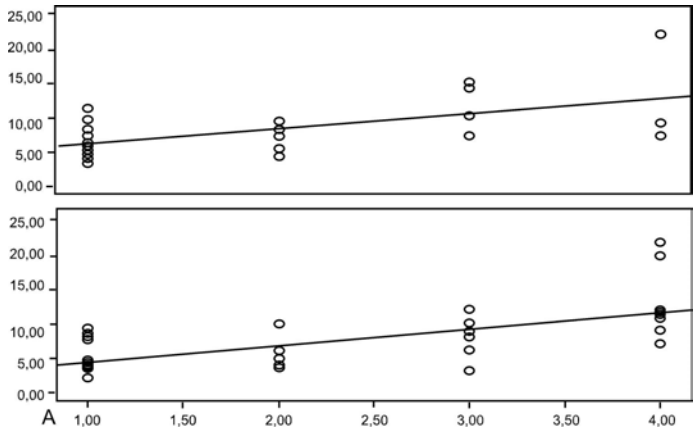

Figure 5. Correlation between WC (expressed in cm in ordinate) and the grade of fatty liver as scored with ultrasonography in both men and women. (The values of Spearman correlation coefficient and statistical significances are reported within the table).

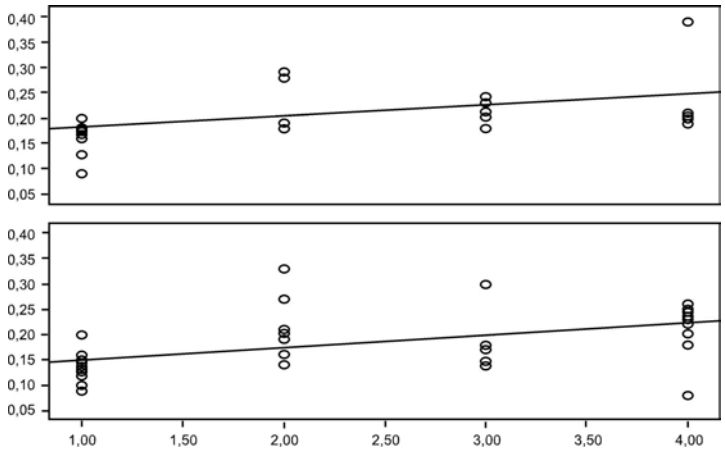

Figure 6. Correlation between Doppler Power Index (DPI) and the grade of fatty liver as scored with ultrasonography in both men and women. (The values of Spearman correlation coefficient and statistical significances are reported within the table).

multivariate analysis, the only 3 parameters that remained significant predictors for the presence of NAFLD, were DPI in both sexes (> 0.13 in men and > 0.18 in women), WC in females (> 80 cm), and the HOMA index in males (> 0.9), suggesting that the presence of IR is better predicted by measuring waist circumference in females, and the HOMA index in males.

We may conclude that, in a short series of consecutive asymptomatic overweight patients referred to our Liver Centre outpatient facility, SWA could be used as a simple, valid and practical instrument to measure lifestyle habits in patients with NAFLD. This study also suggest that there is strong evidence of a significant association between the presence of NAFLD, central adiposity and IR, as

measured by waist circumference and the HOMA index, and that by calculating DPI during hepatic ultrasound, it is possible to obtain a sensitive measurement of the amount of fat present within the liver. However more and, larger studies are needed to validate these results.

6. ACKNOWLEDGEEMNTS

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F2-2009-241762 for the project FLIP, and from Banca Popolare dell'Emilia-Romagna (Modena)".

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Key Words: NAFLD, HOMA, Resting Energy Expenditure, REE, SenseWear armband, Doppler Power Index. DPI

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