

Impaired Fat Oxidation and Reduced Resting Energy Expenditure after a Fat Load in Individuals with Liver Steatosis

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Abstract

To date, the factors involved in the progression from simple liver steatosis to liver fibrosis have not been clarified. The postprandial phase after a high fat meal is pro-inflammatory and chronic low-grade inflammation is associated with the development of non-alcoholic fatty liver disease. The purpose of this study was to evaluate the effect of an oral fat load on fat oxidation in individuals with vs. without liver steatosis. Twelve adults without liver steatosis and seventeen with liver steatosis underwent an oral tolerance tests with 190 grams of a fresh cream (343 kcal/100 g, calories from fats 84%). Respiratory quotient and resting energy expenditure were evaluated using indirect calorimetry at 0, 90 and 120 minutes after load ingestion. In the subjects without liver steatosis, the fat load induced a reduction in the respiratory quotient (basal value=0.88 ± 0.07 vs. 90 min value=0.85 ± 0.06, p=0.020; and basal value=0.88 ± 0.07 120 min value=0.84 ± 0.06, p=0.001) with no change in resting energy expenditure. The liver steatosis led to a delayed switch to fat oxidation after the fat load (90 min value 0.88 ± 0.07 vs. 120 min value = 0.85 ± 0.06; p=0.032) with a significant reduction of the resting energy expenditure after 120 min (p=0.011). An impaired fat oxidation, suggesting a reduced metabolic flexibility, and reduced resting energy expenditure is demonstrated after a fat load in individuals with liver steatosis. These mechanisms may be involved in the progression from liver steatosis to fibrosis.

Keywords: Fat oxidation; Liver steatosis; Fat load; Resting energy expenditure; Metabolic flexibility

Abbreviations

NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; HF: High-Fat; T1DM: Type 1 Diabetes; T2DM: Type 2 Diabetes; CV: Cardiovascular; CAP: Controlled Attenuation Parameter; TE: Liver Transient Elastography; CIMT: Carotid Intima-Media Thickness; BMI: Body Mass Index; WC: Waist Circumference; HC: Hip Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate; TBW: Total Body Water; FFM: Fat-Free Mass; MM: Muscle Mass; FM: Fat Mass; REE: Resting Energy Expenditure; RQ: Respiratory Quotient; VO²: Volume of Oxygen; VCO²: Volume of Carbon Dioxide; IQR: Interquartile Range; HDL-Cholesterol: High Density Lipoprotein-Cholesterol

Introduction

Non-alcoholic fatty liver disease (NAFLD) is becoming a major problem in global public health [1]. Indeed, with a prevalence of 20% to 40%, it constitutes a common liver disease in Western countries [1]. At present, a number of factors are considered to be involved in the progression from simple liver steatosis to non-alcoholic steatohepatitis (NASH) and/or cirrhosis [2-4], but the leading cause remains unclear.

Although a number of studies have examined the relationship between whole-body substrate oxidation and NAFLD, the findings have been conflicting [5,6]. Furthermore, the question of whether individuals with hepatic steatosis can also be classified as “low-fat

oxidizers” in response to an acute fat load has not yet been explored. This issue is important since it has already been demonstrated that, under fasting conditions, “low-fat oxidizers” individuals exhibit a higher risk of gaining weight and of exhibiting comorbidities compared with “high-fat (HF) oxidizer” individuals [7-10]. Moreover, it is well known that the postprandial phase that follows a HF meal is generally considered to be pro-inflammatory [11] and chronic low-grade inflammation is associated with the development of NAFLD or non-alcoholic steatohepatitis (NASH) [11]. Individuals with hepatic fat accumulation may have an abnormal regulation of fat oxidation after a HF meal, which may contribute to disease progression in the liver [12].

Thus, in the present investigation, we set out to evaluate whether an oral fat load can induce different responses, in terms of fat oxidation, in individuals with liver steatosis vs. healthy controls.

Material and Methods

Design

This is a sub-study of the single centre intervention study entitled “Development of new functional food”, whose protocol was approved by the local ethics committee at the “Mater Domini” University Hospital in Catanzaro, Italy (projects codes 117/2015). Written informed consent was obtained from all participants. The investigation conforms to the principles outlined in the Declaration of Helsinki [13].

Population

The participants were white volunteers of both gender, aged 20-45 years, regular consumers of milk and its derivatives, free of metabolic disease, who were invited to participate in the study by newspapers advertisements.

We excluded from the study those who, during the medical interview and examination, had clinical and laboratory signs of chronic hepatitis B and/or C virus infection, past and current alcohol abuse (>20 g of alcohol per day; 350 mL (12 oz) of beer, 120 mL (4 oz) of wine, and 45 mL (1.5 oz) of hard liquor each contain 10 g of alcohol), current use or history of drug treatment causing hepatic steatosis (e.g., corticosteroids, high-dose estrogen, methotrexate, or amiodarone), impaired liver function, presence of autoimmune or cholestatic liver disease, type 1 and 2 diabetes mellitus (T1DM and T2DM), hyperlipidemia, hypertension and those to whom it was not possible measure the Controlled Attenuation Parameter (CAP) to diagnose liver steatosis for technical reasons. Exclusion criteria included: milk allergies, inflammatory disease or cancer, a restrictive diet during the 3 months prior to study, and recent weight change of >5 kg in the prior 3 months, intense physical activity.

The following criteria were used to define the classical CV risk factors of the participants:

diabetes: fasting blood glucose ≥ 126 mg/dL or antidiabetic treatment [14]; hyperlipidemia: total cholesterol >200 mg/dl and/or triglycerides >200 mg/dL or lipid lowering drugs use; hypertension: systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mm Hg or antihypertensive treatment; overweight: $25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$; obesity; body mass index (BMI) $\geq 30 \text{ kg/m}^2$, respectively; smoking: current smoker: who has smoked more than 100 cigarettes in their lifetime and smoke cigarettes every day or some days; sedentary individuals were those having <3 hours/week of low-to-moderate exercise [15,16].

All participants underwent a Liver Transient Elastography (TE) to assess the presence of liver steatosis. Furthermore, to confirm that participants were free from cardiovascular risk factors, an ultrasonographical assessment of the carotid intima-media thickness (CIMT) was performed. Thus, a total of 12 individuals with liver steatosis and 17 without liver steatosis were consecutively enrolled.

Oral fat loading test

The participants were asked to eat according to their usual dietary habits the day before the study, but to avoid drinking alcohol. The fat load consisted of 190 grams of a commercially available fresh cream (343 kcal/100 g; calories from fats, carbohydrates and proteins were 84% [17], 13% and 3% respectively; 70% saturated fatty acid, 0% cholesterol). The amount of the fresh cream (a dose equivalent to three full glasses) was chosen on the base of results of a previous investigation [3], taking into account the compliance of the participants to consume this food, assessed before the start of the study (190 g providing 652 kcal and containing 61 g of fats). Participants consumed the oral fat load within 5 min and remained seated during the test.

Vital function assessment

The measurement of the systemic blood pressure (systolic blood pressure -SBP and diastolic blood pressure -DBP) of both arms was obtained by auscultatory blood pressure technique with aneroid

sphygmomanometer at baseline and 120 min after loading. Clinic BP was obtained in supine patients, after 5 min of quiet rest. A minimum of three BP readings were taken using an appropriate BP cuff size (the inflatable part of the BP cuff covered about 80 percent of the circumference of upper arm) as previously described [18].

The Heart rate (HR), as beats per minutes (bpm), was measured via a Polar HR transmitter (Quark CPET Cosmed, Rome, Italy).

Biochemical evaluation

Venous blood was collected after fasting overnight into vacutainer tubes (Becton & Dickinson, Plymouth, England) and centrifuged within 4 h. Serum glucose, total cholesterol, and triglycerides were measured with Enzymatic colorimetric test [18]. Quality control was assessed daily for all determinations. Furthermore, a capillary blood glucose measurement was performed before each test.

Dietary intake assessment

Dietary intake data were assessed by a 24-hour recall and a seven-day diet record and calculated using nutritional software MetaDieta 3.0.1 (Metedasrl, San Benedetto del Tronto, Italy). The 24-hour recall was collected via an interview by a dietitian who used images associated with a comprehensive food list in the program. All participants were also given a food diary, measuring sheet with life-size images of a spoon, cup and bottle sizes for food diaries. The INRAN (National Institute of Food Research) 2000 and IEO (European Institute of Oncology) 2008 database serves as the source of food composition information in the program [8]. The data were entered by dietitians into the program. All foods are assigned a unique code which allows categorisation of foods into food groups. The resulting database was exported into SPSS for analysis.

Anthropometric measurements

All tests were performed after a 12 h overnight fast. Before tests, participants had no caffeinated beverages between their evening meal and the conclusion of the tests on the examination's morning. Body weight was measured before breakfast with the subjects lightly dressed, subtracting the weight of clothes. Body weight was measured with a calibrated scale and height measured with a wall-mounted stadiometer. BMI was calculated with the following equation: weight (kg)/height (m)². Waist circumferences and hip circumferences (WC and HC) were measured with a nonstretchable tape over the unclotted abdomen at the narrowest point between costal margin and iliac crest and over light clothing at the level of the widest diameter around the buttocks, respectively, as described in the past [8]. Bioelectrical impedance analysis (BIA) (BIA-101, Akernsrl, Florence, Italy) was performed to estimate the percentage of Total Body Water (TBW), Fat Mass (FM, also in kg), Muscle Mass (MM, also in kg), total Fat-Free Mass (FFM) [8,19].

RQ assessment - indirect calorimetry

Fasting RQ and the Resting Energy Expenditure (REE) were measured with the participants in their post-absorptive state (baseline) and 90 min and 120 min after loading. The Indirect Calorimetry instrument (Quark CPET Cosmed, Rome, Italy) was used for all measurements. Respiratory gas exchange was measured in a sedentary position, using the open circuit technique between hours of 7 a.m. and 8:30 a.m., after 48 h abstention from exercise. The participant rested

quietly for 30 min in an isolated room with temperature controlled (21-24°C) environment. Quark CPET is equipped with a canopy hood for spontaneously breathing subjects. The subject must breathe in the canopy hood for at least 30 min, until steady state was achieved. With this instrument, the flow rate is directly measured with a digital bidirectional turbine flowmeter. Accuracy of the flowmeter is 2%. Software uses the Weir equation to assess REE at rest. Criteria for a valid measurement was a minimum of 30 minutes of steady state, with steady state determined as less than 10% fluctuation in minute ventilation and oxygen consumption and less than 5% fluctuation in RQ. RQ was calculated as CO₂ production/O₂ consumption [8,20,21].

Liver transient elastography

All patients were evaluated using the 3.5 MHz standard M probe on the right lobe of the liver through intercostal spaces with the patient lying supine and placing the right arm behind the head to facilitate access to the right upper quadrant of the abdomen. The tip of the probe transducer was placed on the skin between the rib bones at the level of the right lobe of the liver. TE can quantify liver steatosis by CAP assessment and measure liver stiffness [22]. (Fibroscan; Echosense SASU, made in France, 75013, Paris). Both stiffness and CAP were obtained simultaneously and in the same volume of liver parenchyma. All scans were performed by the same investigator. Liver stiffness was expressed by the median value (in kPa) of ten measurements performed between 25 and 65 mm depth. Only results with 10 valid shots and interquartile range (IQR)/median liver stiffness ratio <30% were included. The cut-off value for defining the presence of fibrosis was liver stiffness >7 kPa. We assessed CAP using only the M probe because the CAP algorithm is specific to this device. Ten successful measurements were performed on each patient, and only cases with ten successful acquisitions were taken into account for this study. The success rate was calculated as the number of successful measurements divided by the total number of measurements. The ratio of the IQR of liver stiffness to the median (IQR/MLSM) was calculated as an indicator of variability. The final CAP value (ranged from 100 to 400 decibels per meter (dBm⁻¹), was the median of individual measurements. The ratio of IQR in CAP values to the median (IQR/M CAP) was used as an indicator of variability for the final CAP. The presence of liver steatosis was defined by the presence of CAP values ≥ 216 dBm⁻¹ [22].

Carotid arteries assessment

The subjects underwent B-mode ultrasonography of the extracranial carotid arteries by use of a high-resolution ultrasound instrument (Toshiba Medical Systems Corporation, model TUS-A500, 1385, Shimoishigami, Otawara-Shi, Tochigi 324-8550, Japan) with a 5- to 12-MHz linear array multifrequency transducer. All the examinations were performed by the same ultrasonographer blinded to clinical information. All patients rested in the supine position for at least 10 min before the study and were kept in this position during the procedure. ECG leads were attached to the ultrasound recorder for on-line continuous heart rate monitoring. The right and left common (CCA) and internal carotid arteries (including bifurcations) were valuated with the head of the subjects turned away from the sonographer and the neck extended with mild rotation. The CIMT, defined as the distance between intimal-luminal interface and medial-adventitial interface, was measured as previously described [8,9]. Briefly, in posterior approach and with the sound beam set perpendicular to the arterial surface, 1 cm from the bifurcation, three

longitudinal measurements of CIMT were completed on the right and left common carotid arteries far-wall, at sites free of any discrete plaques. The mean of the three right and left longitudinal measurements was then calculated. Then, we calculated and used for statistical analysis the mean CIMT between right and left CCA. The coefficient variation of the methods was 3.3%. To evaluate artery diameters, images were magnified, whereas depth and gain settings were set to optimize the image of the vessel wall, in particular, the media-adventitia interface ("m" line). The end-diastolic diameter of the vessel, defined as the distance between near-wall and far-wall junctions of the media and adventitia, was measured over four cardiac cycles with the use of digital callipers and the average was then calculated.

Statistical analysis

Data are reported as mean ± SD. To find a significant reduction in RQ from baseline to 90 minutes of 0.05, with an estimated effect/standard deviation (E/S) equal to 1 and 80% power on a two-sided level of significance of 0.05, 16 subjects for group are required.

Significant changes in RQ and REE from baseline to 90 and 120 min were evaluated using paired Student's t-test (two-tailed). The unpaired t-test was used to compare the averages between individual with and without liver steatosis. A chi square test was performed to compare the prevalence between these two groups.

The Pearson's correlation was used to identify the variables correlated with basal, 90 min and 120 min RQ and basal, 90 min and 120 min REE, given that the continuous variables were normally distributed. We analyzed the correlation with the following variables: age, BMI, FFM, WC, glucose, LDL, HDL, triglycerides, calories, carbohydrates, lipids, proteins, fats, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids.

Furthermore, the area under the receiver operating characteristic (ROC) curve was used to analyse the capacity of CAP to predict the reduction of REE of at least 50 kcal after the oral fat load (after 90 min). Significant differences were assumed to be present at p<0.05 (two-tailed). All comparisons were performed using SPSS 20.0 for Windows (IBM Corporation, New York, NY, United States).

Results

Tables 1 and 2 show the characteristics of the population, according to the presence or not of liver steatosis. Nevertheless, we excluded the subjects with CV risk factors, there was, as expected, a significant difference in the total cholesterol and triglycerides average among the groups. Furthermore, the participants with liver steatosis had a lower value of FFM (%) compared to those without steatosis (p=0.001). In this population, the habitual diet provided more than 30% of the total calories from fat (Table 2).

Among those with liver steatosis, 9 had obesity, 1 was overweight and 2 were normal-weight. At Pearson's correlation, basal, 90 min and 120 min REE were significantly correlated only with FFM, CAP and calories from diet. Thus, we adjusted these values for FFM. RQ was not correlated to FFM.

Figures 1 and 2 show the response, in term of change in RQ and REE (adjusted for FFM), after a fat load. An oral fat load induced a significant early increase in fat oxidation (reduced RQ) already after 90 min (paired t-test, basal RQ=0.88 ± 0.07 vs. 90min RQ=0.85 ± 0.06, p=0.020) as well as at 120 min (basal RQ=0.88 ± 0.07 vs. 120 min RQ=0.84 ± 0.06, p=0.001) in the subjects without liver steatosis, while

this phenomenon was delayed in those with liver steatosis (basal RQ=0.88 ± 0.07 vs. 90 min RQ=0.88 ± 0.07, p=0.82; basal RQ=0.88 ± 0.07 vs. 120 min RQ=0.85 ± 0.06, p=0.13; 90 min RQ=0.88 ± 0.07 vs. 120 min RQ= 0.85 ± 0.06; p=0.032).

Variables	Without steatosis (N=17)	With steatosis (N=12)	p-values
CAP (dB/m)	181 ± 29	281 ± 42	
Age (years)	26 ± 4	30 ± 5	0.049
Males (%)	29.4	41.7	0.38
Smokers (%)	47.1	33	0.36
Sedentary (%)	24	58	0.065
BMI (Kg/m ²)	24 ± 4	32 ± 5	0.001
WC (cm)	79 ± 9	104 ± 16	<0.001
HC (cm)	101 ± 9	112 ± 8	0.002
Skinfold thickness (mm)	1.5 ± 0.6	2.0 ± 0.5	0.026
SBP (mmHg)	114 ± 10	120 ± 13	0.24
DBP (mmHg)	72 ± 6	76 ± 10	0.27
HR baseline (bpm)	72 ± 10	72 ± 11	0.88
HR 120 min (bpm)	69 ± 7	68 ± 6	0.73
TBW (%)	54.7 ± 6	46.3 ± 6	0.001
FFM (Kg)	49.9 ± 8	60.9 ± 11	0.012
FFM (%)	74.9 ± 8	63.3 ± 8	0.001
MM (Kg)	34.0 ± 6	42.7 ± 10	0.023
MM (%)	50.9 ± 6	44.2 ± 7	0.018
FM (Kg)	17.5 ± 9	35.2 ± 9	<0.001
FM (%)	25.1 ± 8	36.7 ± 8	0.001
REE (kcal)	1581 ± 301	1881 ± 367	0.029
REE* (kcal)	1700 ± 57	1788 ± 72	0.39
RQ	0.89 ± 0.06	0.89 ± 0.07	0.99
VO ² (ml/min)	226 ± 45	269 ± 53	0.035
VCO ² (ml/min)	199 ± 32	238 ± 48	0.026
Liver Stiffness (kPa)	4.8 ± 1.0	5.5 ± 0.5	0.019

Values expressed as mean ± SD

*Adjusted for age and FFM.

Note: CAP = Controlled Attenuation Parameter; BMI = Body Mass Index; WC = Waist Circumference; HC = Hip Circumference; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; HR = Heart Rate; TBW = Total Body Water; FFM = Fat-Free Mass; MM = Muscle Mass; FM = Fat Mass; REE = Resting Energy Expenditure; RQ = Respiratory Quotient; VO² = Volume of Oxygen; VCO² = Volume of Carbon Dioxide; IQR = Interquartile Range.

Table 1: Demographic, clinical and instrumental characteristics of the population.

Furthermore, the oral fat load induced a significant reduction in REE after 120 min in the subjects with liver steatosis (basal REE=1766 ± 256 kcal vs. 120 min REE=1686 ± 214 kcal; p=0.002; 90 min REE=1724 ± 235 kcal vs. 120 min REE=1686 ± 214 kcal; p=0.011), while REE didn't change in those without liver disease (basal REE=1715 ± 154 kcal vs. 90 min REE=1689 ± 136 kcal, p=0.43; basal REE=1715 ± 154 kcal vs. 120 min REE=1651 ± 109, p=0.052).

Variables	Without steatosis (N=17)	With steatosis (N=12)	p-values
CAP (dB/m)	181 ± 29	281 ± 42	
Capillary glycemia (mg/dl)	90 ± 10	94 ± 9	0.37
Glucose (mg/dl)	90 ± 6	90 ± 8	0.98
Total-cholesterol (mg/dl)	162 ± 18	182 ± 19	0.009
HDL-cholesterol (mg/dl)	43 ± 3	45 ± 2	0.11
Triglycerides (mg/dl)	83 ± 17	108 ± 21	0.003
Calories Intake (kcal)	1928 ± 682	1955 ± 589	0.91
Carbohydrates (%)	48 ± 5	47 ± 4	0.68
Carbohydrates (g)	230 ± 97	243 ± 80	0.69
Proteins (%)	16 ± 3	18 ± 2	0.060
Proteins (g)	75 ± 22	84 ± 19	0.25
Fats (%)	36 ± 6	35 ± 3	0.48
Fats (g)	78 ± 36	74 ± 21	0.37
Saturated fatty acids (g)	22 ± 12	22 ± 8	0.95
Monounsaturated fatty acids (g)	35 ± 16	37 ± 9	0.58
Polyunsaturated fatty acids (g)	8 ± 4	8 ± 1	0.43

Values expressed as mean ± SD

Note: CAP = Controlled Attenuation Cholesterol Parameter; HDL-Cholesterol = High Density Lipoprotein- Cholesterol

Table 2: Laboratory parameters and nutrient intake assessment of the population.

			95% C.I.	
Area	SE	p-values	Lower Limit	Higher Limit
0.740	0.095	0.028	0.555	0.926

Note: SE= Standard Error; C.I. = Confidence Interval

Table 3: The area under the receiver operating characteristic (ROC) curve analysis: Capacity of CAP to predict a REE reduction after 90 min.

The area under the ROC curve for CAP to predict a reduction in 90 min REE of at least 50 kcal was 0,74 (SE=0.095; p=0.028; Table 3). The CAP equal to 216 achieved satisfactory sensitivity (61%) and specificity (74%) to predict the reduction of 90 min REE (Figure 3).

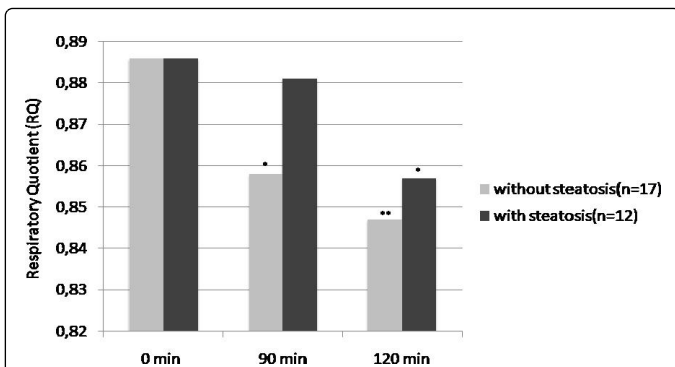


Figure 1: Change in respiratory quotient after a fat load. **Note:** Paired student's t-test analysis. Significance: ** $p < 0.005$ (basal vs. 120'), * $p < 0.05$ (basal vs. 90' in those without steatosis; 90' vs. 120' in those with steatosis).

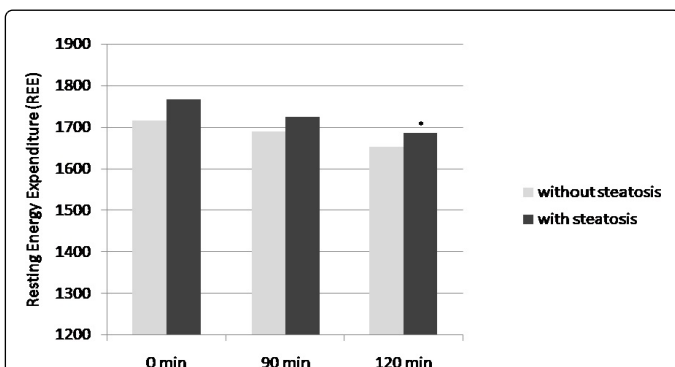


Figure 2: Change in resting energy expenditure^s after a fat load. **Note:** Paired student's t-test analysis. Significance: * $p < 0.05$ (baseline vs. 120' and 90' vs. 120'). ^sREE adjusted for FFM.

individuals are better able to match fat oxidation to fat intake, with less positive fat balance, than obese individuals [26].

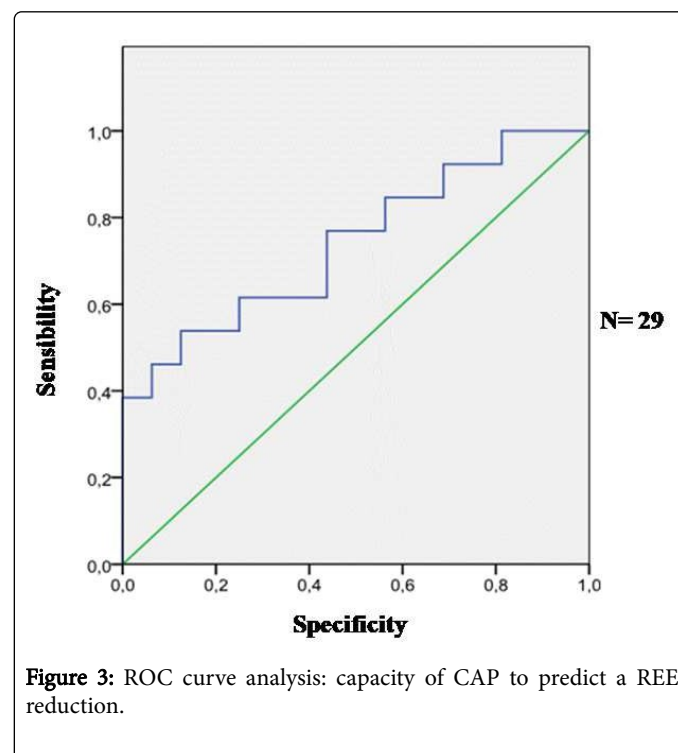


Figure 3: ROC curve analysis: capacity of CAP to predict a REE reduction.

Moderate overeating leads to the suppression of fat oxidation in the obesity-prone population [27]. A 3-week HF diet leads to intrahepatic lipid accumulation and decreased metabolic flexibility in healthy overweight individuals [12]. Our study contributes to the literature by promoting the understanding of the pathophysiological mechanisms of fat utilisation after an acute fat load in individuals with hepatic fat accumulation; our results also suggest which factors may be implicated in the progression from liver steatosis to NASH and /or cirrhosis.

Previous studies, performed in healthy individuals, have shown opposite results for an acute fat load with regard to its effect on REE and fat oxidation [11,17,28]. In line with other investigations [6,29], no significant difference in the basal fat oxidation was demonstrated between individuals with vs. without hepatic fat accumulation. However, our observed reduction in REE is plausible since it has been suggested that some individuals may be prone to diet-induced obesity as a consequence of a lower REE [30]. In addition, it has been demonstrated that REE, total EE and fat oxidation are acutely up-regulated in lipodystrophic subjects, who have a limited fat storage capability, in contrast with normal subjects [31]. Thus, we can hypothesize that individuals with an excessive adiposity down-regulate REE in response to a fat load.

In the above-cited study [31], the change in REE was largely attributable to a parallel change in fat oxidation. However, the fact that the change in EE was evident during REE measurement and sleep suggests that non-exercise-mediated thermogenesis may be involved [31]. Thermogenesis was not specifically measured in our study; thus, its contribution can only be postulated. Another explanation for the REE reduction may be related to hormonal influences (but no hormone measurements were taken in our investigation). Indeed, it has been demonstrated that Leptin administration can reduce EE [32]. Moreover, peripheral administration of adiponectin reduces visceral

Discussion

In the present study, we demonstrate that an oral fat load, given to individuals exposed to a HF diet with liver steatosis (but not presenting any CV risk factors), impairs the fat oxidation and induces a significant reduction in REE after 120 min (Figure 1).

On the contrary, the acute fat load induces an early increase in fat oxidation (already observed after 90 min), with no significant change in REE, in individuals without liver steatosis (Figure 2).

The hepatic fat accumulation led to a delayed switch to fat oxidation after the fat load, indicating reduced metabolic flexibility (RQ was significantly reduced only between 90 and 120 min).

It has been already demonstrated that a diet with a HF content, especially saturated fatty acids, can promote the development of both obesity and NAFLD [12,23-25]. However, investigations addressing changes in fat oxidation and REE after the consumption of a HF meal are lacking in individuals with hepatic steatosis.

Our study would suggest that less fat may be oxidized and more fat stored after a HF meal in subjects with liver steatosis (who were also prevalently obese). In this regard, it has been demonstrated that lean

fat mass and increases EE [33] and a low adiponectin level is an independent risk factor for NAFLD [34]. Adiponectin deficient mice show increased lipid accumulation due to impaired mitochondrial function [34]. In contrast, a reduction in REE may simply reflect the relatively lower FFM in individuals with an excess of adiposity compared with those without an excess (Table 1) [31].

Indeed, when REE was adjusted for FFM, comparable values were obtained for obese vs. non-obese individuals (Table 1) [35,36]. For these reasons, we only used the REE value adjusted for FFM in our statistical analyses, revealing a significant difference between basal REE and that at 120 minutes post fat load in individuals with liver steatosis. A CAP value of 216 dBm⁻¹, suggesting the presence of liver steatosis, is highly sensitive and specific in predicting a reduction in REE after 90 min (Figure 3). We hypothesize that the mechanisms of energy-balance regulation and fat utilization only function properly in individuals that do not have hepatic fat liver accumulation. The results of our study suggest that alterations in REE and fat utilization may be involved in the progression from simple liver steatosis to NASH and fibrosis. In this regard, the postprandial phase after a HF meal is generally considered to be pro-inflammatory [11]. Postprandial inflammation is a normal metabolic response of the human body to regulate EE after a meal [11], but which becomes abnormal in individuals with hepatic fat accumulation. Chronic low-grade inflammation is associated with the development of chronic diseases. We hypothesize that the regular consumption of HF meals by individuals with liver steatosis contributes to disease progression in the liver.

In this study some limitations must be pointed out. Our study design does not allow causal relationships to be ascertained. The molecular mechanisms underlying the differential responses to a fat load in individuals with vs. without liver steatosis are still unknown and our study was not designed to shed light on these mechanisms. Nevertheless, our results will be useful for generating future research hypotheses.

Due to the small sample size, we did not perform statistical analyses to investigate differences according to steatosis grade. In this study, the participants had a HF diet, thus, these results are applicable only to similar population. Finally, this study did not foresee the repeated assessment of triglyceride levels following the HF load. In normotriglyceridemic individuals, the magnitude of triglyceridemia that follows the ingestion of a fat meal is well known: it is directly proportional to the fat content of the meal [37]. Our aim was only to assess the response to a fat load in terms of fat oxidation, thus only basal triglyceride levels were assessed.

Conclusion

The health risks and health care costs associated with obesity and its complications, like hepatic fat accumulation or liver fibrosis, are considerable. The progression from liver steatosis to fibrosis involves complex interactions between hormones, genes and environmental factors. More in-depth knowledge about the mechanisms of energy balance in response to such dietary factors may be required, in order to identify strategies that can help reduce hepatic fat accumulation. Our study suggests that less fat may be oxidized and more fat stored after a HF meal in subjects with liver steatosis. This phenomenon is associated with a parallel decrease in REE. These mechanisms may be involved in the progression of the simple liver steatosis to liver fibrosis.

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Specific author contributions: SM performed the research and collected data; DD, EM, performed liver assessment; FP, CR designed the research study, YF analysed the data and wrote the paper. All authors approved the final version of the manuscript.

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