Abstract

Background. Non-Alcoholic Fatty Liver Disease occurs mainly in severely obese patients and its relationship to Metabolic Syndrome is increasingly recognized. The aim of this study was to determine energy production-utilization by measuring the Basal Metabolic Rate in severely obese patients, characterized by NAFLD, with or without Metabolic Syndrome. Then, the role of systemic inflammation was assessed.

Patients and methods. Twenty severely obese men with Metabolic Syndrome were compared with a well-matched cohort of patients without Metabolic Syndrome. All showed hepatic steatosis at UltraSonography. Basal Metabolic Rate was measured by indirect calorimetry using a canopy system and single-frequency bio-impedance analysis. Serum Interleukin-6 and fibrinogen levels were measured as markers of inflammation.

Results. Basal Metabolic Rate was higher in severely obese patients with Metabolic Syndrome than in those without it: 2,496±358 kcal/d vs 2,126±253 kcal/d, P = 0.001. Laboratory findings of concurrent chronic inflammation were also higher in these patients, i.e., Il6 4.35±1.34 pg/ml vs 6.23±2.1 pg/ml, P = 0.034; fibrinogenemia 285±40 mg/dL vs 376±91 mg/dL, P = 0.020; these of of cytochrome oxidase, i.e., AlainaminoTransferase, equally behaved 32.3±7.9 UI vs 65.7±28.2 UI, P < 0.001. Visceral adiposity and arterial hypertension were more frequently detected in patients with Metabolic Syndrome.

Conclusion. Increased energy expenditure, observed in morbidly obese patients as a consequence of a systemic, low-grade, inflammatory process, may explain progression from obesity to Metabolic Syndrome, independent of the presence of NAFLD. In this context, increased Basal Metabolic Rate may be a clue of Metabolic Syndrome.

Abbreviations

NonAlcoholic Fatty Liver Disease NAFLD
NonAlcoholic SteatoHepatitis NASH
Fatty Liver FL
Metabolic Syndrome MS
Basal Metabolic Rate BMR
Liver Biopsy LB
Bio Impedance Analysis BIA
AlAlanin aminoTransferase ALT
AST
ASpartate aminoTrasferase TEE
Total Energy Expenditure fibr
Oxygen Radical Species ROS
Interleukin 6 IL6
t
Evidence is accumulating that NonAlcoholic Fatty Liver Disease (NAFLD), ranging from Fatty Liver (FL) to NonAlcoholic SteatoHepatitis (NASH) and cirrhosis, is present mainly in severely obese patients.
The diagnosis of NAFLD is based on liver biopsy (LB), that has some limitations, i.e., patient acceptability and sampling error. When ethical and/or technical reasons make the performance of LB difficult, the presence of NAFLD may be inferred, with good specificity, by hepatic hyperechogenity with Ultrasound (US) in the absence of other liver disease. LB confirms US findings of steatosis without differentiating benign FL from the more severe NASH and does not generally influence the therapeutic approach. Most patients with normal US findings have normal or near-normal biopsies.

Currently, the importance of NAFLD and its relationship to Metabolic Syndrome (MS) is increasingly recognized. The evidence suggests that NAFLD is likely to be associated with increased cardiovascular risk, and raises the possibility that NAFLD may not only be a marker but also an early mediator of atherosclerosis. On the other hand, some authorities consider that serum hypertransaminasemia is a sign of NASH.

Because ATP is critical for maintaining cellular integrity, abnormal production may predispose to hepatocellular injury and mitochondrial dysfunction could be the key-mechanism. There are few studies based on estimates of energy metabolism, mainly BMR, in patients with liver disease. This involves measurement of subjects at rest, under thermo neutral temperatures (i.e. no thermogenic stress), in a post-absorptive (not digesting food) and inactive state. The underlying machinery that fuels BMR is identical to that which fuels all the other sources of energy utilisation, i.e., oxidative phosphorylation. ATP is generated in mitochondria, and is subsequently hydrolysed to ADP and phosphate to release energy for useful work. This process of electron transport during oxidative phosphorylation is the primary source of Oxygen Radical Species (ROS). Total energy expenditure (TEE) is commonly predicted on the basis of patient weight, activity level, and degree of metabolic stress (metabolic demands). BMR accounts for about 70% of TEE; the remainder is provided by energy dissipated by metabolism of food (10% of TEE), and energy expended during physical activity (20% of TEE). Conditions that increase metabolic stress, such as infection, critical illness, or trauma, having inflammation in common, can increase BMR. Elevated levels of pro-inflammatory cytokines, especially interleukin-6 (IL6), have been associated with increased cardiovascular morbidity and mortality amongst general populations. Fibrinogen, with its active participation in endothelial function, thrombosis and inflammation is an independent variable to cardiovascular risk. The two substances are strictly linked. All cells involved in the atherogenetic process are able to produce cytokines, mainly IL6, which induce an acute phase reaction that increases fibrinogen levels in plasma.

The aim of this study was to determine energy production-utilization, due to mitochondrial derangement. First, energy expenditure was evaluated in severely obese patients, characterized by NAFLD presence, with or without full-fledged MS. Then, the inflammation role was assessed by measuring IL6 and fibrinogen, as markers of systemic involvement.

Methods
The protocol of the study was approved by local Ethical Committee. All patients gave informed consent for participation.

Patients
Forty male, morbidly obese, patients showing similar increased liver echogenicity at US were included in this case-control study. Twenty patients with MS (OB-MS) were compared with 20 patients without MS (Ob). Patients were well matched for age, weight, height and Body Mass Index (BMI). Any liver disease of viral, toxic, genetic or immunological origin was excluded. None of the patients had significant daily alcohol consumption (> 20 g alcohol/day). All patients were defined as sedentary according to WHO criteria.
The presence of MS was diagnosed according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III, accepting its presence when at least three criteria were repeatedly (at least in four occasions, during a follow-up period of twelve months) satisfied.12

All subjects underwent the same measurements according to the sequence: indirect calorimetry - bio impedance analysis (BIA) - anthropometry. All tests were carried out in the first part of the morning, after a 12-14 h fast and at a room temperature of 22-24 °C.

**Indirect calorimetry**

BMR was measured by indirect calorimetry using a canopy system (MMC Horizon, Sensor Medics, Anaheim, U.S.A.) in a quiet environment and with patients in the supine position for 30 min before measurement. After an adaptation period of 15-20 min, oxygen consumption and carbon dioxide production were determined for 45 min. Energy expenditure was derived from CO2 production and O2 consumption, with the appropriate Weir’s formula, neglecting protein oxidation (Weir 1949). The apparatus was calibrated with gas mixtures of known composition before each test, and regularly checked by burning ethanol. The inter-day coefficient of variation of such measurements (determined in 6 patients on subsequent days) was always < 3%, without any sequence effect.

**Biochemistry**

Patients underwent a laboratory examination including: alanine aminotransferase, aspartate aminotrasferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, pseudo-cholinesterase, total and HDL-cholesterol, triglyceride, haemoglobin, total blood red cell count, glucose, insulin, serum ferritin, transferrin, fibrinogen, and erythrocyte sedimentation rate. Laboratory data were detected by using current haemato-chemical kits. IL6 was estimated by Pelikine Compact Human IL6 ELISA Kit with sensitivity 0.2 - 0.4 pg/ml. The index of insulin resistance was calculated on the basis of fasting values of plasma glucose and insulin, according to the Homeostasis Model Assessment (HOMA) method.

**Ultrasound Features**

The degree of fatty infiltration of the liver was graded according to ultrasonic appearance of liver echotexture, liver-diaphragm differentiation in echo amplitude, hepatic echo penetration and clarity of hepatic blood vessels and the features were quantified according to the following scale: 1, mild; 2, moderate; 3, severe.13

**Single-frequency-BIA analysis**

Single-frequency-Bioelectrical Impedance Analysis (BIA), a fast and convenient field technique for the estimation of total body Fat-Free Mass (FFM), was carried out by the same operator using a BIA 101-S plethysmograph (injection of an alternating current, at 800 µA and 50 kHz) (RJL/Akern System, Florence, Italy). Measurements were performed on the non-dominant side of the body, at an ambient temperature of 22-24 °C, after voiding and after being in the supine position for 20 min. A standard tetra polar technique was used, with the measuring electrodes placed on the anterior surface of the wrist and ankle, and the injecting electrodes placed on the dorsal surface of the hand and the foot, respectively. The instrument was routinely checked with resistors and capacitors of known values. The BIA variables considered were resistance, reactance, and phase angle. The bio impedance index was calculated as the ratio height²/resistance (cm²/ohm).14

**Anthropometry**

The same observer, according to standard procedures, performed anthropometric measurements. Height was measured to the nearest 0.1 cm with a stadiometer and body weight to the nearest 0.1 kg on a balance beam.
scale with the subject barefoot and wearing only light undergarment.

Statistical analysis

A paired t test was performed, for the majority of variables that showed normal distribution. The US steatosis scores were managed with the Wilcoxon test. \( P < 0.05 \) was considered statistically significant.

Results

The two study groups OB-MS and OB showed overlapping values of the main variables such as age, weight, BMI, US score of steatosis, expressed as median plus range, e.g., 2 (1-3) versus 2 (1-3), and FFM, calculated by bio impedance according to Lukaski\textsuperscript{14}, i.e., \( 76.0 \pm 11.5 \text{ kg} \) vs \( 72.1 \pm 9.3 \text{ kg} \), \( P = \text{NS} \). BMR was higher in the OB-MS (2.496±358 kcal/d) than in the OB cohort (2.126±253 kcal/d), \( P = 0.001 \). BMR, after correction for FFM (BMR-FFM = BMR/FFM kcal/kg), remained higher in the OB-MS (33.1±3.8 kcal/kg) than in the OB (29.6±2.6 kcal/kg) group, \( P = 0.003 \).

Insulin resistance was not significantly higher in the OB-MS than in the OB cohort (Insulin 25.0±12.8 vs 18.8±7.5 \( \mu \text{U/ml} \); HOMA 5.76±3.36 vs 4.23±1.72); triglycerides showed similar behaviour (133±45 in the OB-MS vs 97.0±36.5 \( \mu \text{g/dL} \) in the OB group). Fibrinogen and IL6 were higher in the OB-MS group in respect to the OB group, \( P = 0.020 \) and 0.034, respectively; ALT and AST values were increased in the OB-MS group (table). Visceral adiposity, waist circumference, and arterial hypertension were more frequently detected in OB-MS patients.

Discussion

An increase in BMR was found in morbidly obese NAFLD patients with MS compared with patients without MS but similar BMI. The same patients showed high serum IL6 and fibrinogen values. Furthermore, laboratory data expressing hepato-cellular damage (elevated transaminases) were not related to the NAFLD existence, but, they were to SM co-existence. Finally, the most represented criteria of MS were visceral adiposity and arterial hypertension. We infer that the increased BMR that we observed in obese NAFLD patients with stigmata of MS is due to low-grade, chronic inflammation. When body composition and metabolic changes were studied following bariatric surgery in obese patients, there was a significant kcalories decrease in BMR\textsuperscript{15} a finding that support our results.

The present data are not consistent with the observation that the severity of systemic adipocytokine-driven inflammatory response is related to the absolute body fat excess\textsuperscript{16} independent of its distribution. Cytokine production by adipocytes, has been suggested to enhance the production of ROS, which can induce subsequent liver damage via the intermediary step in which certain components of the mitochondrial respiratory chain are inhibited or altered.\textsuperscript{17} Such mitochondrial derangement would cause excessive heat production, accounting for the appearance of hyper metabolism and, potentially, a local energy deficit, which can lead to cellular degradation. A similar patho-physiologic mechanism has also been suspected in the development of Parkinson’s disease.\textsuperscript{18} The discovery of increased AST/ALT activity in OB-MS patients could be explained in this way. Another possible mechanism is based on individual susceptibility to the inflammatory response.

It is possible that there may be a continuum in energy metabolism alteration, likely time-dependent. As a final consideration, we cannot exclude the possibility that morbidly obese patients with MS, increased serum IL6 and fibrinogen\textsuperscript{19}, and hypertransaminasemia may suffer from a more severe form of NAFLD. This could have been ascertained by histology.

In conclusion, this analysis confirms that habitual energy expenditure is substantially raised in obese patients\textsuperscript{20} and expands our knowledge of the additional role of MS in increasing energy expenditure. The data
TABLE. Demographic characteristics.

<table>
<thead>
<tr>
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<th>OB Patients (n.20)</th>
<th>OB-MS Patients (n. 20)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>78.5</td>
<td>± 6.2</td>
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MS = Metabolic Syndrome
OB Patients = Obese Patients without MS
OB-MS Patients = Obese Patients with MS
ALT = ALanine aminOtransferase
AST = ASpartate aminOtransferase
GGT = Gamma-Glutamyltransferase
CHE = pseudo-Choline Esterase
AP = Alkaline Phosphatase
t-Bil = total Bilirubin
HDL-Chol = HDL-Cholesterol
TG = Triglyceride
Fibr = Fibrinogen
IL6 = Interleukin 6
PLTs = Platelets
Lymph = Lymphocytes
ESR = Erythro Sedimentation Rate
HOMA = Homeostasis Model Assessment method
BMI = Body Mass Index
warrant further investigation, to generate a working hypothesis.

References


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