Liver breath tests non-invasively predict higher stages of non-alcoholic steatohepatitis

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ABSTRACT

Effectively assessing subtle hepatic metabolic functions by novel non-invasive tests might be of clinical utility in scoring NAFLD (non-alcoholic fatty liver disease) and in identifying altered metabolic pathways. The present study was conducted on 39 (20 lean and 19 obese) hypertransaminasemic patients with histologically proven NAFLD (ranging from simple steatosis to severe steatohepatitis [NASH (non-alcoholic steatohepatitis)] and fibrosis) and 28 (20 lean and eight overweight) healthy controls, who underwent stable isotope breath testing ([13C]methacetin and [13C]ketoisocaproate) for microsomal and mitochondrial liver function in relation to histology, serum hyaluronate, as a marker of liver fibrosis, and body size. Compared with healthy subjects and patients with simple steatosis, NASH patients had enhanced methacetin demethylation (P = 0.001), but decreased (P = 0.001) and delayed (P = 0.006) ketoisocaproate decarboxylation, which was inversely related (P = 0.001) to the degree of histological fibrosis (r = −0.701), serum hyaluronate (r = −0.644) and body size (r = −0.485). Ketoisocaproate decarboxylation was impaired further in obese patients with NASH, but not in patients with simple steatosis and in overweight controls. NASH and insulin resistance were independently associated with an abnormal ketoisocaproate breath test (P = 0.001). The cut-off value of 9.6 % cumulative expired 13CO2 for ketoisocaproate at 60 min was associated with the highest prediction (positive predictive value, 0.90; negative predictive value, 0.73) for NASH, yielding an overall sensitivity of 68 % and specificity of 94 %. In conclusion, both microsomal and mitochondrial functions are disturbed in NASH. Therefore stable isotope breath tests may usefully contribute to a better and non-invasive characterization of patients with NAFLD.

INTRODUCTION

Chronic liver disorders are conditions with potential severe sequelae. A valid assessment of liver function is essential to establish prognosis [1]. ‘Dynamic’ LFTs (liver function tests), investigating time-dependent metabolic processes, have been found to be useful in predicting histological severity and survival, playing an adjunctive role to the most commonly used prognostic models especially in patients with cirrhosis awaiting liver transplantation [2,3]. Until now, however, the complexity of liver metabolism has not allowed the identification of a single ‘ideal’ LFT for exploring the function of the whole organ [4], although some tests may be helpful as indices of residual liver mass [5,6].

NASH (non-alcoholic steatohepatitis), the inflammatory form of NAFLD (non-alcoholic fatty liver disease), is currently recognized as a frequent condition...
and is often associated with central obesity, insulin resistance and other features of the so-called ‘metabolic syndrome’. The main problem in NASH resides in the accumulation of triacylglycerols (triglycerides) within hepatocytes with a concomitant inflammatory reaction and fibrosis [7,8] and a potential evolution towards end-stage liver disease [9]. Pathophysiological mechanisms include microsomal hypertrophy [10,11], mitochondrial dysfunction [12,13] and the activation of the peroxisomal metabolism of long-chain fatty acids [10,14,15].

The diagnosis of NASH requires histological confirmation by liver biopsy, an invasive procedure not always accepted by the patient [16]. Although regarded as the best tool for staging fibrosis, it has a number of potential biases, including sampling error and intra- and inter-observer discrepancies [17,18]. Therefore the potential usefulness of non-invasive tests able to identify patients at risk of progression of liver damage or with advanced disease is actively investigated.

\[ ^{13}C \text{-Labelled substrates undergoing metabolism in liver can be administered orally [19], and measurement of } ^{13}\text{CO}_2, \text{ resulting from their metabolism, in the expired air reflects specific hepatic function. Thus } ^{13}\text{C}]\text{methacetin [20] and } ^{13}\text{C}]\text{KICA (ketoisocaproate) [21] allow liver microsomal cytochrome P450 1A2 [22] and mitochondrial branched-chain amino acid decarboxylation [23] function respectively, to be explored reliably. However, there is a paucity of data on LFTs specifically in NAFLD.} \]

Therefore the present study investigates the clinical utility of two different breath tests \[^{13}\text{C}-\text{MBT (methacetin breath test) and } ^{13}\text{C}-\text{KBT (KICA breath test)}\] in exploring liver function in patients with NAFLD and their potential role in the better screening and scoring of these patients.

Part of this work was presented at the 40th Annual Meeting of the European Association for the Study of the Liver, held in Paris, on 13–17 April 2005, and at the Annual Meeting of the American Gastroenterological Association, held in Chicago on 14–19 May 2005, and subsequently published in abstract form [23a,23b].

**MATERIALS AND METHODS**

**Patients**

The study was conducted on 39 (20 lean and 19 obese) consecutive hypertransaminemic patients with histologically proven NAFLD ranging from simple steatosis to severe steatohepatitis (NASH) and fibrosis [24], all referred to the Department of Internal Medicine at the University of Bari. Inclusion criteria were elevated ALT (alanine aminotransferase) levels >1.5 times above the upper limits of the normal range on more than one occasion, absence of major viral hepatitis infection [HBV (hepatitis B virus) and HCV (hepatitis C virus)] and of autoimmune, inherited and cholestatic liver disorders, no history of recent intake of hepatotoxic drugs and evidence of a ‘bright liver’ by abdominal ultrasound (Table 1).

Twenty-eight healthy subjects (20 lean and eight overweight) served as controls. Lean subjects had a BMI (body mass index) between 20.0 and 24.9 kg/m². Overweight

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**Table 1  Characteristics of NAFLD patients and healthy controls**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal levels</th>
<th>NAFLD patients</th>
<th>Healthy controls</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Values are means ± S.D.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>38 ± 7</td>
<td>37 ± 7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Males/females (n)</td>
<td>21/18</td>
<td>14/14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>102 ± 12</td>
<td>74 ± 9</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31 ± 3</td>
<td>23 ± 3</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Overweight (%)</td>
<td></td>
<td>22.4</td>
<td>25.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Obese (%)†</td>
<td></td>
<td>48.7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Midarm muscle circumference (cm)</td>
<td>47.1 ± 1.6</td>
<td>35.1 ± 1.9</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>10–37</td>
<td>54 ± 6</td>
<td>26 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>12–37</td>
<td>136 ± 27</td>
<td>32 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>γ-GT (IU/l)</td>
<td>5–55</td>
<td>71 ± 11</td>
<td>41 ± 4</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerols (mg/dl)</td>
<td>&lt; 200</td>
<td>249 ± 18</td>
<td>139 ± 17</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterolis (mg/dl)</td>
<td>&lt; 110</td>
<td>113 ± 4.1</td>
<td>89 ± 3.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Serum insulin (µ-IU/ml)</td>
<td>4.3–19.9</td>
<td>27 ± 0.6</td>
<td>15.1 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>HOMA</td>
<td>&lt; 1.64</td>
<td>9.5 ± 3.1</td>
<td>1.5 ± 0.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* BMI > 24.9 kg/m² < 29.9 kg/m².
† BMI > 30.0 kg/m².
controls (large and tall athletic individuals) had a BMI between 25.0 and 29.9 kg/m², which had remained stable for at least 2 years. Controls had normal fasting glucose levels (<110 mg/dl), blood pressure, and serum total cholesterol and triacylglycerols (Table 1). The healthy subjects also had normal livers as determined by ultrasonography and repeatedly normal serum transaminases. Alcohol ingestion was either absent or <10 g/day in all subjects, and no subjects smoked.

As four patients with NASH had already developed liver cirrhosis as determined by histology, they were also compared with an additional group of HCV-related cirrhotic patients (n = 8; six females and two males; age, 56 ± 8 years; Child–Pugh A score; BMI, 26 ± 3 kg/m²).

All subjects were enrolled into the study by giving their written informed consent, and the protocol was approved by the Ethical Committee of the University Medical School of Bari.

**Blood analysis**

Blood tests of lipid, glucose and liver enzymes were performed using routine methods after an overnight fast. Serum hyaluronate levels were measured by using an ELISA (Corgenix). Insulin resistance was calculated by the HOMA (homeostatic model assessment) formula, i.e. basal insulin (m-international unit/l) × fasting glucose (mmol/l)/22.5.

**Histology**

Histological examination was performed on liver specimens obtained at ultrasound-guided liver biopsy, and the biopsies were scored according to the system devised by Brunt [24].

**Breath tests**

$^{13}$C-MBT and $^{13}$C-KBT were performed in subjects after an overnight fast at least 3 days apart. Subjects abstained from the ingestion of any alcoholic beverages or drugs interfering with liver function [25] for at least 5 days before breath testing. A dose of 100 mg of $[^{13}]$Cmethacetin (isotopic purity of 99%; Isotec) diluted in 200 ml of water was administered orally. The $^{13}$C-KBT was performed by giving orally 1 mg of $[^{13}]$CKICA (isotopic purity of 99%; Isotec) diluted in 200 ml of water per kg of body weight to which 1 g of unlabelled L-leucine (Sigma) was added. Air samples were collected in Exetainer® test tubes (Labco) before administration of the substrate and every 15 min for a total of 120 min. In order to standardize CO² production and minimize extrahepatic metabolism of the labelled substrates, patients rested for 30 min before and during the whole test. In order to minimize interference by sex hormones, fertile women performed the test in the pre-ovulatory phase of the menstrual cycle [26].

All the breath test measurements were performed in the Center for Liver, Intestinal and Metabolic Diseases, University Hospital Groningen, Groningen, The Netherlands by gas isotope ratio MS (Heliview; Mediconis). The rate of exhalation of $^{13}$CO² at each time point was calculated from the measured increment in the isotopic abundance of $^{13}$CO² ($δ^{13}$CO₂), the known purity of the labelled compound and an assumed constant endogenous production of CO² (300 mmol m⁻² h⁻¹), and is expressed as the percentage of the administered dose exhaled/h [27]. The cumulative recovery of $^{13}$CO² in breath was calculated as the AUC (area under the curve) of the $^{13}$CO² exhalation rate compared with the time curve determined by linear interpolation using the trapezoidal rule.

**Statistical analysis**

Values are means ± S.E.M., unless otherwise stated. Statistical significances were determined using Student’s t test for unpaired data or the rank sum test and two-way ANOVA for repeated measures, followed by post-hoc pairwise comparisons. To address the relationship between two parameters, linear regression analysis was performed. The effects of morphological anthropometric and metabolic parameters (NASH, overweight/obesity, insulin resistance, transaminases and serum lipids) on microsomal and mitochondrial function, determined in all the subjects included in the study (n = 67), were calculated as coefficients in a retrospective model of multiple logistic regression with subject groups (absent compared with present) as independent factors. The sensitivity of single and coupled breath tests, and positive and negative predictive values, were calculated to differentiate patients with more advanced NASH from those with simple steatosis and healthy controls. Calculations were performed with NCSS 2004 statistical software.

**RESULTS**

All NAFLD patients had significantly higher serum levels of transaminases, cholesterol, triacylglycerols and basal insulin than control subjects (Table 1). Although no patient was diabetic, the majority of them (36 out of 39) had a HOMA index higher than the reference value. Liver histology confirmed the presence of NAFLD in all cases. Histological classification of the patients is depicted in Table 2.

As $^{13}$C-MBT is affected by hepatic blood flow and liver cirrhosis [28], patients with advanced fibrosis (i.e. stage IV; n = 4) were excluded from the calculations when NAFLD patients were compared with the healthy subjects. These patients were included, however, in the $^{13}$C-KBT, which is not affected by hepatic blood flow [29].

**MBT for liver microsomal function**

The percentage cumulative recovery of $^{13}$CO² in breath in healthy controls after ingestion of methacetin is shown...
Table 2  Liver histology of patients with NAFLD according to the system devised by Brunt [24] and the sex distribution over the different disease stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9 (5)</td>
</tr>
<tr>
<td>I</td>
<td>6 (4)</td>
</tr>
<tr>
<td>II</td>
<td>12 (5)</td>
</tr>
<tr>
<td>III</td>
<td>8 (6)</td>
</tr>
<tr>
<td>IV</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11 (5)</td>
</tr>
<tr>
<td>2</td>
<td>20 (12)</td>
</tr>
<tr>
<td>3</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Steatosis</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>21 (11)</td>
</tr>
<tr>
<td>Severe</td>
<td>7 (4)</td>
</tr>
</tbody>
</table>

The maximal exhalation rate (0.31 ± 0.02 % of the administered dose exhaled/min) was recorded at 30 min (median value) (Figure 2).

Compared with controls, patients with NASH both at stages 0–I and at stages II–III had a significantly increased maximal exhalation rate (0.41 ± 0.04 % and 0.40 ± 0.02 % of the administered dose exhaled/min; Figure 2). The cumulative recovery of $^{13}$CO$_2$ in breath (percentage of the administered dose exhaled at 30, 60 and 120 min) was also significantly higher in NASH patients compared with controls (Figure 1).

**KBT for liver mitochondrial function**

The percentage cumulative recovery of $^{13}$CO$_2$ in breath in healthy controls after $^{13}$C-KICA is shown in Figure 1. The maximal rate of exhalation (0.32 ± 0.01 % of the administered dose exhaled/min) was observed at 30 min (median value) (Figure 2).

Overall, patients with stages II–IV of NASH had a significantly lower mitochondrial decarboxylation capacity compared with healthy subjects. This was confirmed by a significantly lower ($P = 0.001$) and delayed ($P = 0.006$) maximal rate of exhalation and a significantly lower cumulative recovery of $^{13}$CO$_2$ in breath (Figure 1). Major differences between NASH patients and healthy controls were maintained when the groups were divided by sex, which were distributed comparably over the histological stage classes (Table 2).

**Correlations between breath tests and metabolic markers and histology**

No correlation was found between $^{13}$C-MBT or $^{13}$C-KBT values and most of the hepatic and metabolic parameters considered, including HOMA. An inverse correlation was observed between $^{13}$CO$_2$ cumulative recovery values following $^{13}$C-KICA and the serum hyaluronate levels, an indirect marker of liver fibrosis (Figure 3). The hyaluronate levels were comparable between healthy subjects and patients with stages 0–II of NASH. From stage III, all NASH subjects had significantly higher hyaluronate levels. An inverse correlation was also found between

![Figure 1](image1.png)

**Figure 1**  Cumulative recovery of $^{13}$CO$_2$ with $[^{13}$C]methacetin and $[^{13}$C]KICA in breath of healthy controls and NAFLD patients with stages 0–I and II–IV of NASH

Healthy controls, $n = 28$; NAFLD patients, $n = 39$. For methacetin, patients with stage IV of NASH ($n = 4$) were not included (see text for explanation). Results are means ± S.E.M. * $P = 0.03$ and ** $P = 0.001$ compared with healthy controls.
Figure 2  Time course of $^{13}$CO$_2$ exhalation rate with $^{13}$C-methacetin and $^{13}$C-KICA in breath of healthy controls and NAFLD patients with stages 0–I and II–IV of NASH
Healthy controls, $n = 28$; NAFLD patients, $n = 39$. For methacetin, patients with stage IV of NASH ($n = 4$) were not included (see text for explanation). Results are means $\pm$ S.E.M. at each time point. $^* P = 0.006$ compared with controls; and $^{**} P = 0.001$ compared with controls and stages 0–I of NASH (as determined by ANOVA).

Figure 3  Correlation between $^{13}$C-KICA metabolism and serum hyaluronate levels in healthy controls and NAFLD patients according to histological stage of NASH
Healthy controls, $n = 28$; NAFLD patients, $n = 39$. +, Controls; $\triangle$, stage 0; $\checkmark$, stage I; $\bigcirc$, stage II; $\square$, stage III; $\bigstar$, stage IV. A negative and statistically significant correlation is best depicted by a third-order distribution curve.

Figure 4  Inverse correlation between $^{13}$C-KICA metabolism in NAFLD patients and histological stage (0–IV) of NASH
NAFLD patients, $n = 39$. Note that some of the points are partially overlapping.

$^{13}$CO$_2$ cumulative recovery values following $^{13}$C-KICA and the histological stage in NAFLD patients (Figure 4).

Considering an $\alpha$ value of 0.05 and a sample size of 67 subjects, KBT was able to significantly ($P = 0.001$; power ANOVA = 0.814) discriminate within NAFLD patients according to fibrosis stage, but not for inflammatory grade and extent of steatosis. In particular, patients with greater than stage II of NASH were significantly different from healthy controls and patients with simple steatosis or stage I of NASH. Regression analysis showed the presence of NASH ($r^2 = 0.324, P = 0.001$) and insulin resistance ($r^2 = 0.279, P = 0.001$) as highly significant and
Effect of obesity on mitochondrial function as assessed by $^{13}$C-KBT

Whereas obese individuals with simple fatty liver and without steatohepatitis had a faster KICA decarboxylation than lean and overweight healthy controls (peak rate of exhalation of 22 min compared with 30 min respectively), resulting in a significantly ($P = 0.05$) higher decarboxylation capacity at 30 min ($6.1 \pm 0.5$ compared with $5.5 \pm 0.4$ % of the administered dose respectively), obese NASH patients had significantly ($P = 0.001$) lower KICA decarboxylation than lean NASH patients (Figure 5). A significant inverse correlation was also found between the cumulative recovery of $^{13}$CO$_2$ after $^{13}$C-KICA and BMI in NASH patients (Figure 5). No such relationship was evident in obese individuals with simple fatty liver or in overweight controls.

**Effect of liver cirrhosis on hepatic function as assessed by breath tests**

The cumulative recovery of $^{13}$CO$_2$ after ingestion of $^{13}$C-methacetin, expressed as a percentage of the administered dose, was $2.8 \pm 0.4$ and $3.5 \pm 0.7$ % at 30 min, $8.6 \pm 1.6$ and $8.4 \pm 1.5$ % at 60 min, and $16.4 \pm 2.4$ and $15.5 \pm 2.3$ % at 120 min in patients with histological stage IV of NASH and HCV-related cirrhotic patients respectively. Irrespective of the aetiology, the presence of liver cirrhosis significantly ($P = 0.001$) decreased the hepatic methacetin demethylation capacity. By contrast, KICA decarboxylation was significantly impaired in patients with histological stage IV of NASH (1.4 $\pm$ 0.4, 5.7 $\pm$ 1.1 and 14.1 $\pm$ 1.4 % of the administered dose at 30, 60 and 120 min; $P = 0.001$ compared with stages I–III of NASH, for comparison see above and Figure 1), but remained approximately normal in HCV-related cirrhotic patients (4.0 $\pm$ 0.7, 11.1 $\pm$ 1.4 and 20.4 $\pm$ 2.2 % of the administered dose at 30, 60 and 120 min respectively).

**DISCUSSION**

By using non-invasive stable isotope breath tests, the present study has shown that both microsomal and mitochondrial functions are disturbed in patients with NASH, but not in those with simple fatty liver. In particular, our findings suggest that, by discriminating controls and patients with simple fatty liver from NASH patients, the use of $^{13}$C-MBT and $^{13}$C-KBT has clinical utility in characterizing NASH.

The extent of liver fibrosis is difficult to identify non-invasively [30]. Recently, liver fibrosis was predicted by using a complex algorithm of nine surrogate serum markers and, in patients with NASH, serum hyaluronic levels were reliably predictive only in patients with extensive fibrosis [31].
Ultrasonography is the easiest mode of imaging the liver; however, it has high sensitivity for steatosis, but a low specificity for fibrosis [32]. Therefore the ultimate diagnosis of NASH requires liver biopsy with histology, currently regarded as the gold standard diagnostic tool [16,33]. Several patients with NASH, however, show asymptomatic elevation of transaminases [34], and liver biopsy becomes difficult to advise. As age appears to be a major predictor of fibrosis in NASH patients [35], a recent study suggests a liver biopsy is suitable if the patient’s age is over 40 years, especially in obese patients [36].

No ‘ideal’ predictive test, however, has been identified in patients younger than 40 years of age. Thus non-invasive techniques are awaited to predict advanced or progressive forms of liver diseases, and for better selection of patients in whom a liver biopsy might be indicated in an area where basal levels are often normal [37]. In this regard, stable isotope breath tests have shown promising results in predicting fibrosis and response to therapy in patients with viral liver diseases [38]. In this respect, the role of breath tests were investigated in the present study.

Breath tests employing $^{13}$C as the stable isotope within have been used to explore specific liver functions in patients with liver steatosis. Mion et al. [39] found that KBT may discriminate between patients with alcoholic fatty liver disease and those with NAFLD. However, in that study [39], NAFLD patients had normal transaminase levels and no anthropometric data were available; thus the possibility that the patients were affected by ‘simple’ steatosis without NASH cannot be excluded. Another test of mitochondrial function employing radiolabelled methionine as a substrate has potential to be able to differentiate between liver disease patients, including steatosis [40]. By means of breath analysis, it is also possible to measure the microsomal mass which is increased in NASH [10]. Among several substrates exploring microsomal function in vivo, MBT is gaining acceptance as it is easy to perform and not burdened by side effects [6,41].

By showing little inter-individual variability within the group of healthy subjects in the present study, both MBT and KBT confirm their reliability in estimating microsomal and mitochondrial functions in vivo.

The present study shows that methacetin demethylation occurs to a greater extent in patients with stages 0–III of NASH compared with healthy subjects. This finding appears to reflect an increased metabolic activity of the cytochrome P450 system, independent of the use of known inducers of microsomal activity (i.e. chronic ethanol consumption or medication). Conversely, the same patients had a decreased decarboxylation of KICA, which indicates an impaired metabolic pathway for branched-chain amino acids at the mitochondrial level. These findings suggest a common pathophysiological mechanism underlying both of these dysfunctions in patients with NASH. Other chronic liver disease conditions exhibit different patterns of functional alterations. In fact, patients with chronic non-cirrhotic viral hepatitis have a methacetin demethylation capacity lower or even comparable with healthy subjects [42], whereas, in our present study, those with virus-related liver cirrhosis differed from NASH patients by their significantly decreased methacetin metabolism.

A previous report [6] indicates that the MBT is able to differentiate among Child–Pugh classes and provides an early prediction of the recovery after transplantation. Consistent with such observations, our present results support this breath test as a reliable index of microsomal metabolism in relation to the extent of liver injury.

Ultrastructural changes in mitochondria, decreased mitochondrial respiration and impaired ATP generation have been described in patients with NASH [43]. These findings correlate with serum TNF-α (tumour necrosis factor-α) levels, insulin resistance and body size [44]. Consistent with these observations, we found that KICA decarboxylation was decreased in patients with advanced stages (≥ II) of NASH. There was an inverse relationship between the mitochondrial decarboxylation capacity and the stage of liver disease in NASH patients. The more advanced the stage of NASH, the lower was the exhalation of $^{13}$CO$_2$ deriving from KICA metabolism, indicating that the test may also be helpful for staging purposes and for the assessment of disease progression. It may also be hypothesized that the extent of mitochondrial impairment, as assessed by KBT, may predict the risk of evolution towards end-stage liver disease in NASH patients. In fact, by using a retrospective multiple logistic regression model, KBT was able to significantly discriminate according to fibrosis stage within NASH patients. In particular, although significantly related, KBT appeared to be superior to serum hyaluronate, which did not differentiate below stage III of NASH. Moreover, the regression analysis showed that the presence of NASH and insulin resistance, but not obesity, were independent factors associated with the alteration of KBT.

Apparently, obesity in patients without steatohepatitis does not affect KICA decarboxylation, and it could be speculated that ‘uncomplicated’ fat accumulation in the liver is not deleterious to mitochondrial function. Previous reports, employing KICA and octanoate respectively as breath test substrates for mitochondrial function, also indicated a normal mitochondrial amino acid metabolism [39] or an even accelerated mitochondrial β-oxidation of medium-chain fatty acids [45] in patients with liver steatosis and in obese women without features of NASH [46].

In contrast with obese patients with simple fatty liver and overweight healthy individuals, the inverse relationship observed between BMI and KBT values in NASH patients and the finding that obese NASH patients had lower mitochondrial KICA metabolism compared with lean NASH patients requires consideration. Moreover,
regression analysis showed that NASH and insulin resistance were independently associated with KBT impairment. As KICA decarboxylation was not impaired in patients with simple steatosis and obesity, it could be speculated that ‘uncomplicated’ fat accumulation in the liver and obesity are not deleterious to mitochondrial function. Obesity is known to increase the risk of liver disease [47] and fatty infiltration of hepatocytes is known to worsen the prognosis of viral hepatitis [48]. Obesity plays an important role in the development of NASH, probably acting as a factor favouring the perpetuation and amplification of the cellular functional derangement [37]. The more progressive forms of NASH, in fact, are strongly associated with obesity [49,50]. However, according to our present results, obesity appears to act more as an additional damaging factor than a self-promoting cause of subcellular functional derangement in NASH patients.

In conclusion, the present study shows that both microsomal and mitochondrial functions are disturbed in NASH patients as assessed by two different breath tests. Therefore stable isotope breath tests may usefully contribute to a better and non-invasive characterization of NAFLD by discriminating NASH with simple steatosis and by having predictive power regarding the stage of NASH.

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