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• CLINICAL RESEARCH •

Measurement of hepatic functional mass by means of ^{13}C-methacetin and ^{13}C-phenylalanine breath tests in chronic liver disease: Comparison with Child-Pugh score and serum bile acid levels

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Abstract

AIM: To evaluate and compare the clinical usefulness of ^{13}C-phenylalanine and ^{13}C-methacetin breath tests in quantitating functional hepatic mass in patients with chronic liver disease and to further compare these results with those of conventional tests, Child-Pugh score and serum bile acid levels.

METHODS: One hundred and forty patients (50 HCV-related chronic hepatitis, 90 liver cirrhosis patients) and 40 matched healthy controls were studied. Both breath test and routine liver test, serum levels of cholic and chenodeoxycholic acid conjugates were evaluated.

RESULTS: Methacetin breath test, expressed as 60 min cumulative percent of oxidation, discriminated the hepatic functional capacity not only between controls and liver disease patients, but also between different categories of chronic liver disease patients. Methacetin breath test was correlated with liver function tests and serum bile acids. Furthermore, methacetin breath test, as well as serum bile acids, were highly predictive of Child-Pugh scores. The diagnostic power of phenylalanine breath test was always less than that of methacetin breath test.

CONCLUSION: Methacetin breath test represents a safe and accurate diagnostic tool in the evaluation of hepatic functional mass in chronic liver disease patients.

Key words: Chronic hepatitis c; Liver cirrhosis; Breath Tests; Hepatic functional mass

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INTRODUCTION

Evaluation of liver function is crucial in the overall management of patients with liver diseases[1]. In clinical practice, diagnosis of liver disease is based on the results of physical examination, imaging techniques (ultrasonography, computed tomography, magnetic resonance, *etc*.) and biochemical investigations[2]. As far as the latter is concerned, several tests are available, each reflecting a specific function of hepatocytes and/or a specific liver damage. However, these biochemical parameters are not sufficiently sensitive to evaluate the complex biological events occurring within the hepatocytes (biosynthesis, biotransformation and catabolism of xenobiotics, *etc*) as well as the alterations induced by the disease on these events. Furthermore, no single liver biochemical test of the liver is endowed with the diagnostic accuracy of tests used in the evaluation of other organs, such as creatinine for kidney function. In fact, no single biochemical test can be considered as a sensitive index of the overall hepatic function and is able alone to predict the severity and prognosis of hepatic diseases, whether acute or chronic[3].

To improve the diagnostic efficacy of biochemical tests, several quantitative tests have been proposed to measure the residual hepatic function and numerous substrates have been used in the assessment of liver function, such as sulfobromophthalein dyes, indocyanine green, and sorbitol[4] . However, these tests, although accurate in evaluating hepatic functional mass, have been shown to be unpractical in clinical setting for several reasons: need of repeated blood samples, need of prolonged catheterization, risk for anaphylactic reactions, elevated costs, *etc*. These problems have, in part, been overcome by the use of carbon-labeled compounds and by the evaluation of the kinetics of carbon excretion in breath[5]. The rate of ^{13}C excretion in breath is determined by the rate-limiting step in the overall process used, and the rate limiting step is located at the site of the impaired organ or enzyme function[6].

Different substrates have been proposed, each exploring a specific hepatic function. Aminopyrine which was the first studied compound[7], is useful in the evaluation of hepatocyte microsomial function[8]. Other substrates include phenacetin[9], caffeine[10], lidocaine[11], methacetin[12] and erythromycin[13]. Phenylalanine[14] and galactose[15] are used to explore the cytosolic enzymatic activity, while methionine and ketoisocaproic acid have been proposed in the study of the mitochondrial function[5,16].

Although clinical application of breath test has been in use for several years, no general agreement has been reached concerning its application in the clinical setting[4,6,17,18].

As a consequence, the Child-Pugh classification[19,20], which

was proposed several years ago and represents a concerted evaluation of clinical criteria and laboratory data, still remains the most widely accepted predictor of the severity of liver diseases.

Recently, the clinical utility of some breath tests, namely methacetin and phenylalanine breath tests, has been re-proposed[21,22], even if their diagnostic power did not completely discriminate between different types of chronic liver diseases.

Furthermore, it has been suggested[21] that methacetin might be preferable to aminopyrine because of its rapid metabolism and lack of toxicity in small doses[23]. The test dosage is lower than therapeutic levels and no adverse reactions have been reported.

The aims of this study were to comparatively explore the clinical usefulness of breath tests using these two different substrates, ^{13}C-phenylalanine and ^{13}C-methacetin breath tests (PBT and MBT), in the assessment of functional hepatic reserve or function in patients with chronic liver disease and to verify the presence of a relationship, in terms of disease severity, between PBT and MBT results and those obtained with conventional liver function tests, Child-Pugh score, and serum bile acid levels. The latter comparison was performed since serum bile acid levels, especially serum levels of primary bile acids, are considered a sensitive index of liver function and disease prognosis[24,25].

MATERIALS AND METHODS

Study population

A total of 140 patients with chronic liver diseases were studied: 50 with histologically diagnosed hepatitis C virus (HCV)-related chronic hepatitis and 90 with clinically or histologically diagnosed liver cirrhosis.

Patients with liver diseases of unknown etiology and with cancer or who were heavy smokers and those aged <18 years or >80 years were not taken into consideration. The presence of factors known to potentially influence endogenous carbon dioxide production (no recent food ingestion and physical activity, respiratory diseases, thyroid dysfunctions, fever)[26] was investigated and, if found positive, those patients were excluded from the study. Furthermore, patients having recently used drugs being potentially able to interfere with hepatic cytochrome P450, such as corticosteroids, cimetidine, benzodiazepines, and omeprazole, were also excluded from the study[27].

For control purposes, 40 subjects with no clinical and biochemical evidence of hepatic, gastro-intestinal, endocrine or respiratory diseases, and no history of chronic alcohol consumption or drug use, were enrolled.

Demographic and clinical characteristics of the study population are shown in Table 1.

The study was approved by the local ethics committees and all individuals provided written informed consent prior to enrollment in the study.

Biochemical and ultrasonographic evaluation of the liver

Liver function tests and hepatic ultrasonography (US) were performed on the first day of the study period, prior to carrying out the breath test. Routine liver function tests [alanine and aspartate transaminase, total proteins, serum albumin and gamma-globulins, prothrombin activity, γ-glutamyltransferase (γ-GT), total and conjugated bilirubin, alkaline phosphatase and blood ammonia] were performed. Furthermore, serum levels of cholic acid (CCA) and chenodeoxycholic acid (CDCA) conjugates were evaluated using the enzyme-linked immunosorbent assay (ELISA)[28]

US was performed in all the patients in order to detect the presence of parenchymal structural alterations and/or ascites. Portal echo-colour Doppler and endoscopy of the upper gastrointestinal tract were performed in cirrhotic patients in order to assess the portal hypertension grade and the presence of oesophageal varices. Child Pugh scores were calculated for cirrhotic patients[19,20]. Patients were classified by their score into either class A (scores 5-6), class B (scores 7-9) or class C (scores 10-15).

^{13}C -Methacetin and ^{13}C phenylalanine breath tests

Breath tests were carried out using 100 mg of ^{13}C-phenylalanine (99%^{13}C, Cambridge Isotope Laboratories, Andover, MA, USA) and 75 mg of ^{13}C-methacetin (99%^{13}C, Cambridge Isotope Laboratories, Andover, MA, USA) on two different days in fasting subjects. Duplicate baseline breath samples were collected before administration of the substrates, which were dissolved in 50 mL of water. Duplicate breath collections were also taken every 10 min for 2 h using glass vacutainers. Breath samples were stored at 4 ℃ until analysis, which was performed within 15 d. During the tests, subjects were required to stay at rest, without eating, drinking and smoking.

Analytical methods

The $^{13}CO_2$ enrichment in breath, expressed as cumulative percent of oxidation of a dispensed dose, was measured with a stable isotope mass spectrometer (Europe Scientific Tracermass, Crewe, UK).

To quantify the rate of hepatic substrate oxidation, analytical data were expressed as percentages of the $^{13}CO_2$ recovery per hour using an area under curve (AUC) method, assuming a CO_2 production rate of 5 mmol/min/m^2 body surface area, as described by Schoeller *et al*[29].

Analysis of the elimination kinetics of ^{13}C-labeled isotope (expressed as parts per million) related to time (expressed as minutes) allowed the study of other parameters, such as the isotopic peak excretion (parts per million), and the AUC of max. percent of oxidation.

Statistical analysis

Categorical variables were summarized as means of frequencies and proportions, continuous variables were summarized as mean±SD. Differences in PBT and MBT scores and in CCA and CDCA serum levels between controls, chronic hepatitis and cirrhotic patients were compared with one way analysis of variance (ANOVA). Data was expressed as mean and 95%

Table 1 Patient characteristics

	CTR n = 40	CH n = 50	LCA n = 30	LCB n = 30	LCC n = 30
Gender male/female	25/15	27/23	23/7	20/10	22/8
Age mean (range)	50 (30-80)	57 (38-78)	60 (33-80)	63 (32-80)	59 (39-76)
BSA Mean	1.76	1.75	1.78	1.74	1.72
Etiology Viral/Alcoholic	0	50/0	22/8	25/5	24/6
Child score Mean (range)	0	0	5.38 (5-6)	8.0 (7-9)	10.54 (10-15)

CTR: Controls; CH: Chronic hepatitis; LCA: Child A cirrhosis; LCB: Child B; LCC: Child C; BSA: Body surface area.

confidence interval (95% CI).

Multiple linear regression was performed. The dependent variable was the Child score, and independent variables were the first hour percent of oxidation of MBT and PBT, CCA and CDCA serum levels and liver function tests. Ability to discriminate between the different groups (controls, chronic hepatitis and cirrhosis patients) was quantified by using the area under the receiver operating characteristic curve (ROC area)[30]. The ROC area was a reliable measure to summarize the discriminative power of a diagnostic model. A test that correctly classified all subjects had an area of 1.0 (perfect discrimination) and a test with no discriminatory value had an area of 0.5 or less. A value of 0.7-0.8 was considered to represent reasonable discrimination, and a value >0.8 to represent good discrimination[31].

All two-tailed P-values less than 0.05 were considered statistically significant. Statistical calculations were carried out using the statistical software Stata (Release 7, Santa Monica, CA, USA).

RESULTS

No side effects were observed after administration of the isotopes.

Results of MBT are shown in Figure 1A. MBT percent of oxidation at 60 min significantly discriminated between controls and patients with chronic hepatitis and cirrhosis of all Child-Pugh classes ($P<0.001$). Statistically significant differences were also found between patients with chronic hepatitis and those with Child-Pugh A cirrhosis and between the latter and Child-Pugh B and C cirrhosis ($P<0.001$). The same results were obtained by evaluating the cumulative percent of oxidation after 120 min.

Peak MBT excretion (Table 2) was different in controls with respect to all patients ($P<0.001$). Differences were observed between patients with chronic liver diseases and those with Child-Pugh B and C cirrhotic patients ($P<0.001$), but not with Child-Pugh A cirrhotic patients. Similar results were obtained when MBT area under the curve of the max. percent of oxidation was considered (Table 2).

Results of PBT are shown in Figure 1B. PBT cumulative percent of oxidation calculated at 60 min was able to discriminate between controls and chronic hepatitis patients with respect to Child-Pugh B and C patients ($P<0.001$), but not between chronic hepatitis patients and Child-Pugh A cirrhotic patients (Figure 1B); the same results were obtained by evaluating the cumulative percent of PBT oxidation 120 min after ingestion of the labeled substrates. No statistical differences were found between the studied groups when PBT peak excretion and AUC of max. percent of oxidation was considered (Table 2).

Serum CCA and CDCA levels are shown in Figure 2. Both CCA and CDCA levels were significantly higher in liver disease patients than in controls; furthermore, differences were observed between chronic hepatitis patients and cirrhotic patients, as well as between Child-Pugh A, B and C cirrhotic patients ($P<0.001$).

Comparison between MBT and PBT results and liver function tests failed to reveal any significant correlation in chronic liver disease patients, while significant correlations were present in cirrhotic patients. In fact, the percent of PBT oxidation at 60 min was significantly related to serum levels of albumin ($r = 0.35$, $P<0.01$), total ($r = -0.40$, $P<0.001$) and conjugated bilirubin ($r = -0.33$, $P<0.05$), and to serum CCA ($r = -0.33$, $P<0.05$) and CDCA levels ($r = -0.28$, $P<0.05$). No significant correlation was found between PBT and prothrombin time, AST, ALT or alkaline phosphatase. The same correlations were found when 120-min cumulative percent of PBT oxidation was considered. No correlation was found between PBT, expressed as excretion peak and maximal AUC percent of oxidation and liver function tests.

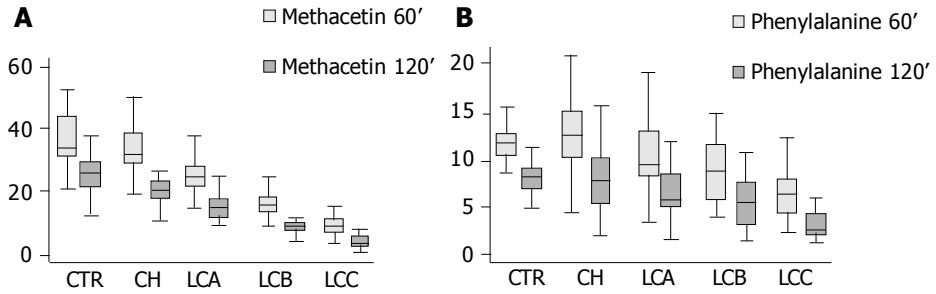

Figure 1 Methacetin and phenylalanine breath tests: cumulative percent of oxidation at 60 and 120 min. A: Methacetin breath test: cumulative percent of oxidation at 60 and 120 min. CTR = controls; CH = chronic hepatitis; LCA = liver cirrhosis Child A; LCB = liver cirrhosis Child B; LCC = liver cirrhosis Child C. CTR *vs* CH, LCA, LCB, LCC: $P<0.001$ CH *vs* LCA, LCB, LCC: $P<0.001$ LCA *vs* LCB, LCC: $P<0.001$ LCB *vs* LCC: $P<0.001$. B: Phenylalanine breath test: cumulative percent oxidation at 60 and 120 min. CTR = controls; CH = chronic hepatitis; LCA = liver cirrhosis Child A; LCB = liver cirrhosis Child B; LCC = liver cirrhosis Child C. CTR *vs* LCB, LCC: $P<0.001$ CH *vs* LCB, LCC: $P<0.001$ LCA *vs* LCC: $P<0.05$.

Table 2 $^{13}CO_2$-peak and area under the curve (AUC) of $^{13}CO_2$ maximal scores in all groups of subjects studied

Tests	CTR Mean (95% CI)	CH Mean (95% CI)	LCA Mean (95% CI)	LCB Mean (95% CI)	LCC Mean (95% CI)
^{13}C-phenylalanine					
$^{13}CO_2$-peak	12.68 (10.94-14.42)	16.11 (13.58-18.63)	11.85 (9.41-14.29)	10.45 (8.07- 12.83)	7.11 (2.74-11.47)
AUC$^{13}CO_2$max	5.40 (2.32-7.62)	6.85 (2.83-12.80)	5.81 (2.64-10.36)	4.60 (2.13-9.25)	5.40 (2.77-10.65)
^{13}C-methacetin					
$^{13}CO_2$-peak	31.03[b] (25.61-36.46)	22.80[d] (20.74-24.85)	19.19[f] (15.60-22.77)	10.18 (8.52-11.83)	7.02 (5.03-9.00)
AUC$^{13}CO_2$ max	16.76[h] (7.35-28.04)	14.69[d] (5.41-31.27)	9.44[e] (2.76-29.01)	4.91 (3.13-8.83)	5.32 (2.87-7.17)

CTR: Controls, CH: Chronic hepatitis, LCA: Child A cirrhosis, LCB: Child B, LCC: Child C [b]$P<0.001$ *vs* All groups [d]$P<0.001$ *vs* Child B,C. [f]$P<0.01$ *vs* Child B,C. [h]$P<0.001$ *vs* Child A,B,C. [e]$P<0.05$ *vs* Child B.

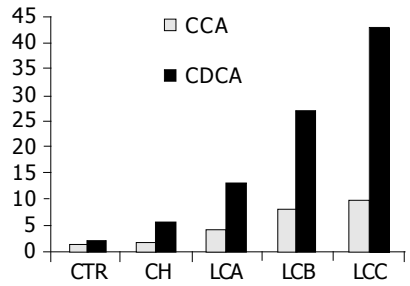

Figure 2 Serum levels of cholic acid (CCA) and chenodeoxycholic acid (CDCA) conjugates. CTR = controls; CH = chronic hepatitis; LCA = liver cirrhosis Child A; LCB = liver cirrhosis Child B; LCC = liver cirrhosis Child C. [a]$P<0.05$ *vs* CH, LCA, LCB, LCC [b]$P<0.001$ *vs* LCA, LCB, LCC [d]$P<0.001$ *vs* LCB, LCC.

As far as 60-min percent of MBT oxidation was concerned, it was significantly related to prothrombin time ($r = 0.43$, $P<0.001$), total ($r = -0.47$, $P<0.001$) and direct bilirubin ($r = -0.49$, $P<0.01$), serum albumin ($r = 0.41$, $P<0.001$), serum CCA ($r = -0.50$, $P<0.001$) and CDCA levels ($r = -0.44$, $P<0.01$). No significant correlation was found between MBT and transaminase or alkaline phosphatase levels. The same correlations were documented when 120-min MBT oxidation was considered. Moreover, MBT oxidation was significantly correlated ($r = -0.52$, $P<0.001$) with portal vein calibre measured at US. No correlation was found between MBT, expressed as excretion peak and maximal AUC percent of oxidation, and liver function tests.

The results of the areas under the ROC curves for PBT and MBT evaluated to discriminate between chronic hepatitis and cirrhotic patients, depending on collecting time, are summarized in Table 3. The best results were obtained at 60 min for PBT and MBT (area under the ROC curve of 0.72 and 0.93 respectively).

Table 3 Areas under receiver operating characteristic (ROC) curves for phenylalanine breath test (PBT) and methacetin breath test (MBT) in chronic hepatitis and cirrhotic patients

PBT collecting time	Area	SE	MBT collecting time	Area	SE
10 min	0.45	0.07	10 min	0.83	0.04
20 min	0.54	0.07	20 min	0.86	0.04
30 min	0.59	0.07	30 min	0.89	0.03
40 min	0.64	0.06	40 min	0.91	0.03
50 min	0.69	0.06	50 min	0.92	0.03
60 min	0.72	0.05	60 min	0.93	0.03
70 min	0.70	0.06	70 min	0.93	0.02
80 min	0.70	0.06	80 min	0.93	0.02
90 min	0.70	0.06	90 min	0.93	0.02
100 min	0.71	0.05	100 min	0.93	0.02
110 min	0.72	0.06	110 min	0.92	0.03
120 min	0.72	0.05	120 min	0.92	0.03

The comparative evaluation between MBT and PBT in terms of areas under the ROC curves documented higher and significant values for MBT in all the comparisons, except for that related to controls and chronic hepatitis patients (Table 4). Figures 3A and 3B illustrate the behaviours of 60-min MBT and PBT, in comparison between controls and liver disease patients, and chronic hepatitis and Child-Pugh A cirrhotic patients, respectively. MBT always showed higher values with respect to PBT, thus confirming its higher diagnostic power.

Table 4 Comparison between area under the receiver operating characteristic (ROC) curves for methacetin breath test (MBT) and phenylalanine breath test (PBT)

Group	Time (min)	MBT		PBT		χ^2
		Area	SE	Area	SE	
CTR *vs* all patient	60	0.86	0.04	0.73	0.04	0.02
CH *vs* C	60	0.89	0.03	0.69	0.05	0.003
CTR *vs* CH	60	0.67	0.07	0.56	0.008	ns
CH *vs* C-Child A	60	0.79	0.05	0.60	0.07	0.02

CTR : Controls; CH: Chronic hepatitis; C: Cirrhosis (Child A,B,C).

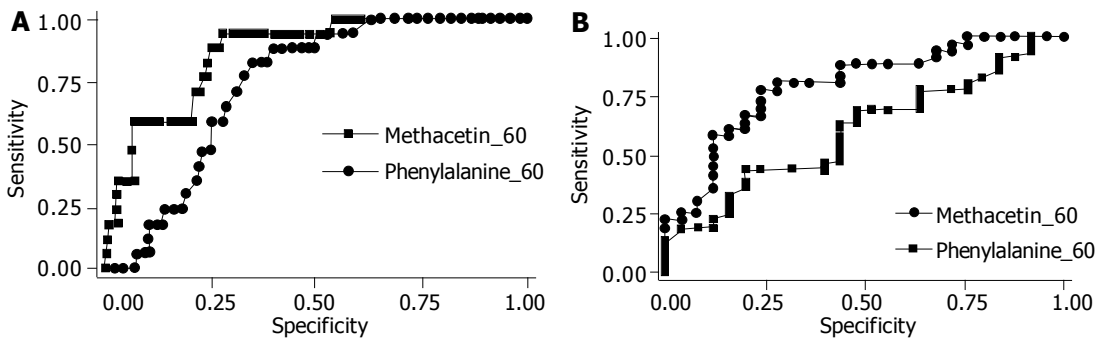

Figure 3 Area under the receiver operating characteristic (ROC) curves compared to the methacetin and phenylalanine breath tests between control subjects and liver disease patients as well as between chronic hepatitis and Child-Pugh A cirrhosis patients. A: Area under the receiver operating characteristic (ROC) curves compared to methacetin and phenylalanine breath tests between control subjects and liver disease patients. --M60 = cumulative percent oxidation of methacetin at 60 mins; -P60 = cumulative percent oxidation of phenylalanine at 60 min; B: Area under the receiver operating characteristic (ROC) curves compared to methacetin and phenylalanine breath tests between chronic hepatitis and Child-Pugh A liver cirrhosis patients. -M60 = cumulative percent oxidation of methacetin at 60 mins; --P60 = cumulative percent of oxidation of phenylalanine at 60 mins.

The results of the multiple linear regression analysis performed to evaluate whether MBT and PBT, serum CCA and CDCA levels and standard liver tests could predict the Child-Pugh scores are shown in Tables 5 and 6. CCDA and CCA, together with MBT, showed the highest regression coefficient value (Table 5). Similar results, but with lower regression coefficients, were obtained when PBT was considered (Table 6).

Table 5 Multiple regression analysis of ^{13}C-methacetin breath test, serum levels of cholic acid (CCA) and chenodeoxycholic acid (CDCA) conjugates

Variable	Beta	*P*
^{13}C-methacetin		
(60 min % oxidation)	-0.544	0.000
CCA	0.576	0.0016
CDCA	0.855	0.001

$r^2 = 0.660$.

Table 6 Multiple regression analysis of ^{13}C-phenylalanine breath test, serum levels of cholic acid (CCA) and chenodeoxycholic acid (CDCA) conjugates

Variable	Beta	*P*
^{13}C-phenylalanine		
(60 min % oxidation)	-0.252	0.057
CCA	0.487	0.070
CDCA	1.010	0.00

$r^2 = 0.545$.

DISCUSSION

Results from the present study show that MBT and to a lesser extent PBT, could discriminate hepatic functional capacity both between healthy subjects and liver disease patients, and between the different categories of chronic liver disease patients. The diagnostic role of MBT was further confirmed by the correlation between MBT and liver function tests and, in particular between serum CCA and CDCA levels. The most useful expression of MBT kinetic parameters is the cumulative percent of oxidation at 60 min, since other modalities of expressed data (peak, AUC of the max percent of oxidation) are less accurate from a diagnostic point of view. Furthermore, MBT as well as serum bile acids, are highly predictive of Child-Pugh scores, as shown by the results of logistic regression. In the present study, the diagnostic power of PBT was always less than that of MBT.

In patients with liver diseases, various laboratory tests and indicators are used to grade liver damage. Conventionally, the degree of injury is assessed using tests which reflect hepatic structure (biopsy), hepatocyte permeability (transaminases) and synthetic activity (albumin, bilirubin and prothrombin time)[1].

These tests are static measurements based on the evaluation of the serum concentration of a particular substance at a given time[3] They do not quantitate functional hepatic reserve, but only hepatocellular damage[2]. In fact, evaluation of enzyme activity has not been considered adequate for the evaluation of hepatocyte function and reserve[8], which can be more accurately measured by dynamic tests.

Several quantitative tests have been proposed to evaluate the functional hepatic mass and numerous substrates have been used in the assessment of liver function, such as sulfobromophthalein dyes, indocyanine green, sorbitol. However, these tests, although accurate in evaluating hepatic functional mass, are found to be unpractical in the clinical setting for several reasons: need of repeated blood samples, prolonged catheterization, risk for anaphylactic reactions, elevated costs, *etc*.

Breath tests with carbon-labeled compounds have been proposed as sensitive and accurate dynamic tests, being useful for the non-invasive measurement of hepatic function[6]. However, although several studies have demonstrated the usefulness of breath tests as hepatic function tests, there is no general agreement regarding their application in the clinical setting[5,17,18].

Thus, in patients with liver diseases, Child-Pugh classification still represents the most widely used marker of liver function. This classification, however, does not strictly reflect the quantitative functional hepatic reserve, and measurement thereof could be influenced by the subjectivity of some parameters (i.e., degree of ascites or hepatic encephalopathy) and by modifications induced by concomitant treatments (i.e., albumin infusion).

The diagnostic reliability of MBT and /or PBT in chronic liver diseases has been proposed and evaluated by various authors, but with controversial results.

Using MBT, Klatt *et al*[32] revealed significant differences between chronic hepatitis and cirrhotic patients, but not between controls and chronic hepatitis patients. Burke *et al*[14] on the other hand, showed more encouraging results using PBT, and in particular, a good correlation with the Child-Pugh scores, but their findings have not been confirmed by others. Perri *et al*[21] in a comparative evaluation of MBT, PBT and aminopyrine breath tests, in a small group of chronic liver disease patients, failed to show any differences between the results obtained with the different substrates.

In the present study, we demonstrated that evaluation using MBT but not PBT, percent of oxidation could discriminate between different groups of chronic liver disease patients, and in particular, between chronic hepatitis and Child-Pugh A cirrhotic patients, and was related to Child-Pugh score status. The correlation between MBT and serum levels of primary bile acids considered as a sensitive index of liver function[25,33,34] further supports the diagnostic role of MBT.

Different results have been recently obtained by Lara Baruque *et al*[22] who demonstrated a high sensibility of both MBT and PBT for the diagnosis of hepatic dysfunction. The specificity, however, was very low. In addition, these authors did not find significant differences in the test results, between chronic hepatitis and Child-Pugh A patients.

This is an important aspect since the evolution from chronic hepatitis to cirrhosis represents a crucial moment in the natural history of chronic liver diseases. Furthermore, the differentiation between chronic hepatitis patients and cirrhotic patients, as we obtained using both MBT and serum bile acid levels, is also important to define the diagnostic strategy to be adopted (i.e., to proceed with liver biopsy or use a clinical-biochemical score).

We showed that MBT had a greater diagnostic capacity than PBT. We could not explain this observation and in particular the lower diagnostic power of PBT than expected. A possible explanation concerning the lower solubility and the slower metabolism of phenylalanine compared to methacetin, was reported also by Lara Baruque *et al*[22].

Other important results emerging from the present study were related to the timing of breath collection and the expression of the isotope breath kinetics. The best discrimination capacity was obtained on the basis of the areas under the ROC curves, for both MBT and PBT 60 min after substrate ingestion. This finding, which is in agreement with that of some studies[14, 22] , but not of others[21,32,35], is of practical importance, since it suggests that further (up to 60 min) breath samples are not necessary. In our experience, additional parameters, such as isotope excretion peak and maximal AUC, are not necessary,

since they do not increase the diagnostic accuracy of the cumulative percent of oxidation. However, further studies are needed to confirm this observation.

To date, the important unsolved question is the usefulness and possible superiority of breath tests in predicting the prognosis of liver disease and the rate of disease progression compared with Child-Pugh classification[19,20]. According to several authors, the use of breath tests for the prognosis of liver disease patients should be considered only when their superior accuracy with respect to Child-Pugh scores is demonstrated. Merkel *et al*[36] published a study on 125 patients with chronic liver diseases, who were followed for 48 mo, demonstrating that aminopyrine breath test was superior to Child-Pugh scores in predicting fatal cirrhotic-correlated events. Similar results were obtained by Figg[37] and by Herold[38], while other investigators[39,40] were unable to document a superiority of quantitative function tests over Child-Pugh classification. Recently Zipprich *et al*[41] have suggested that the possibility of overlapping values in cirrhotic patients could be due to the influence of anaemia and oxygen supply to the cirrhotic liver.

Although our study is not a prospective study, it shows that MBT and serum primary bile acids are the only parameters found to be predictive of Child-Pugh scores. Furthermore, in our previous prospective study[41] performed on cirrhotic patients awaiting liver transplantation, we showed that percent of oxidation of MBT strictly followed the clinical course of liver diseases. In fact MBT progressively decreased until liver transplantation was performed and then increased to reach normal values in patients whose liver transplantation was successful.

In conclusion, MBT and PBT represent a safe, simple and accurate test useful not only in diagnosing chronic liver disease in patients, but also in differentiating between different stages of chronic liver diseases. Furthermore, this study confirms the clinical usefulness of serum primary bile acid measurement in the diagnosis of chronic liver disease in patients.

If further longitudinal studies confirm the MBT diagnostic and prognostic values, this evaluation could represent an important tool for the overall diagnostic and therapeutic management of liver disease patients.

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