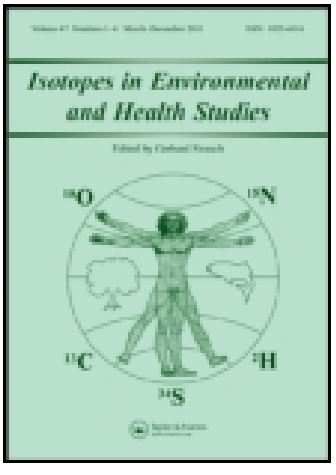

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L-[1-^{13}C]phenylalanine breath test in patients with chronic liver disease of different etiologies

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L-[1-^{13}C]phenylalanine breath test in patients with chronic liver disease of different etiologies

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The aim of this study was to compare the oxidation of L-[1-^{13}C]phenylalanine (^{13}C-PheOx) in patients with chronic liver failure due to different etiologies using L-[1-^{13}C]phenylalanine breath test. Breath samples were collected before the administration of 100 mg L-[1-^{13}C]phenylalanine, and every 10 min thereafter until completion of 1 h. Control subjects ($n = 9$) presented a larger cumulative percentage of ^{13}C dose recovery (CPDR) than patients ($n = 124$) with chronic liver disease, regardless of the etiology (7.5 ± 0.7 vs. 4.2 ± 0.2, $p = 0.001$). No differences in CPDR were found considering the Child-Pugh (CP) class or etiology: alcoholic (CP $A = 7.7 \pm 0.7$, CP $B = 4.1 \pm 0.5$, CP $C = 2.0 \pm 0.3$), hepatitis C virus (CP $A = 5.4 \pm 0.5$, CP $B = 4.0 \pm 0.2$, CP $C = 2.2 \pm 0.3$), hepatocellular carcinoma (CP $A = 5.5 \pm 1.6$, CP $B = 3.6 \pm 1.8$, CP $C = 2.2 \pm 1.0$); or cryptogenic cirrhotic patients (CP $A = 7.4 \pm 1.5$, CP $B = 4.4 \pm 0.4$, CP $C = 2.1 \pm 0.7$). Results confirm that ^{13}C-PheOx decreases in patients with cirrhosis with respect to controls, notwithstanding the etiology.

Keywords: breath test; carbon-13; cirrhosis; diagnostic isotope application in medicine; liver disease; men; phenylalanine; stable isotope tracer techniques

ACM Codes: A.1; E.5; J.3

1. Introduction

L-[1-^{13}C]phenylalanine breath test (^{13}C-PheBT) is a non-invasive, easy to perform test that allows for the distinction of patients with various degrees of liver disease from otherwise healthy persons. Some studies have shown that the severity of liver cirrhosis correlates with the suppression of $^{13}CO_2$ recovery after a dose of phenylalanine [1–5]. Phenylalanine oxidation is a valuable indicator of liver function: it represents the cytosolic enzyme activity. The liver is the main site where phenylalanine is oxidised; thus, a decrease on this capacity reflects disease of the liver itself. L-[1-^{13}C]phenylalanine has a carboxyl carbon atom which is irreversibly oxidised; its oxidation

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may be quantitatively calculated by the appearance of labelled carbon in breath [6]. The characterisation of this specific sub-function of the liver can be distinctively altered by the specific pathophysiological changes corresponding to the different etiological factors of liver disease [4–5,7]. However, the fact that phenylalanine oxidation may differ among the various etiologies of liver disease is yet to be established. Therefore, the aim of this study was to compare the oxidation of L-[1-^{13}C]phenylalanine (^{13}C-PheOx) in patients with chronic liver failure of different etiologies using ^{13}C-PheBT.

2. Materials and methods

2.1. *Patients*

The study was performed at the Laboratory of Gastro-Hepatology at Centro Médico Nacional Siglo XXI. Patients with chronic liver diseases of different etiologies and control subjects were studied.

Patients included were adults, older than 18 years, with documented history of liver disease – regardless of the etiology – and without any respiratory or gastrointestinal disease which could possibly alter breathing or cause malabsorption, respectively. Nine healthy volunteers without history of chronic alcohol consumption served as controls. All controls had normal blood chemistry and liver function tests (aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AP), total bilirubin (TB), albumin). In 29 patients, cirrhosis was confirmed by histological examination; the remaining patients were diagnosed on grounds of clinical and laboratory findings. The etiology of liver disease included: hepatitis C virus (HCV), diagnosed by positive anti-HCV antibodies; alcoholic liver disease, when chronic alcohol consumption was $\geq$30 g/d for two or more years; hepatocellular carcinoma and cryptogenic liver disease.

All subjects were studied following the same protocol. Demographic, clinical, biochemical and ultrasonographic data were collected. The hepatic function was evaluated by ^{13}C-PheBT, Child-Pugh (CP) score and standard liver blood tests. Other collected biochemical data were blood creatinine and glucose. Patients with chronic liver disease were classified, according to the CP score, into class A (score 5–6), class B (score 7–9) or class C (score 10–15) [8].

The study was approved by the institutional review board of the hospital and all individuals provided written informed consent prior to their enrollment in the study.

2.2. ^{13}*C-phenylalanine breath test*

^{13}C-PheBT was measured following an overnight fast with no control of prior dietary intake [9]. In accordance to previous studies [5,10–12], patients were orally administered a 100 mg dose of L-[1-^{13}C]phenylalanine (isotopic purity 99 % ^{13}C; Isotec™ Inc, OH, USA) dissolved in 50 ml water. Alveolar breath samples were collected while in a resting position and following a normal exhalation. At each sample time, patients were asked to blow directly into a 10 cc Exetainer tube (Labco Limited™, Buckinghamshire, UK) through a straw. Duplicate breath samples were collected before administration of the ^{13}C-phenylalanine dose (basal), and every 10 min thereafter until completion of 1 h. $^{13}CO_2$ enrichment was determined by isotope ratio mass spectrometry (BreathMat-plus™, Finnigan Bremen, Germany). The rate of hepatic ^{13}C-phenylalanine oxidation (^{13}C-PheOx) at each time point was calculated from the appearance of $^{13}CO_2$ on exhaled air and assuming a CO_2 production rate of 300 mmol/m^2 body surface area per hour, as described by Schneider *et al*. [13]; the ∂ of ^{13}C values reported by the mass spectrometer were converted to percentages of ^{13}C dose recovery per hour of the amount initially administered. For this calculation,

the following equation was used:

$$\%\text{dose/h} = \frac{\text{mmol excess}^{13}\text{C (a)}}{\text{mmol excess}^{13}\text{C administered (b)}} \times 100,$$

where (a) is $(\%^{13}C_t - \%^{13}C_{t_0})/100 \times CO_2$ production

$$\%^{13}C = \frac{(\partial_t/1000 + 1) \times 0.0112372}{[(\partial_t/1000 + 1) \times 0.0112372] + 1},$$

where $\%^{13}C_t$ is the relative concentration, expressed as percentage of ^{13}C on time t after ingestion of the substrate; $\%^{13}C_0$ is relative concentration, expressed as percentage of ^{13}C at time 0, i.e. before ingestion of the substrate; ∂_t is ∂-value at time t; and 0.0112372 is $^{13}C/^{12}C$ of Pee Dee Belemnite standard and where (b) is

$$(\%^{13}C_s - \%^{13}C_{t_0}) \times \frac{m}{M} \times n,$$

where $\%^{13}C_s$ is the relative concentration of ^{13}C on the administered substrate; $\%^{13}C_{t_0}$ is relative concentration of ^{13}C at time 0, i.e. before ingestion of the substrate; M is molar mass of the substrate; m is amount of substrate administered; and n is the number of ^{13}C labelled atoms in the substrate.

Cumulative oxidation was calculated at 60 min and expressed as cumulative percentage ^{13}C dose recovery (CPDR) [3–5,13,14].

Table 1. Demographic data, clinical and biochemical characteristics of the control subjects and patients.

	Control group ($n = 9$)	Patients ($n = 124$)
Age, year[a]	47.3 ± 5.8	52.7 ± 0.9
Gender, n(%)		
Male	6 (66.6)	66 (53.3)
Female	3 (33.3)	58 (46.7)
CP class n(%)		
A	–	43 (34.6)
B	–	42 (33.4)
C	–	39 (31.8)
CP score	–	8.08 ± 0.2
Cumulative ^{13}C-phenylalanine oxidation, CPDR[a]	7.5 ± 0.7	4.2 ± 0.2
Cumulative ^{13}C-phenylalanine oxidation by CP class, CPDR[a]		
A	–	6.1 ± 0.4 a,b
B	–	4.1 ± 0.2 a,c
C	–	2.2 ± 0.2 b,c
AST, U/l[a]	24.1 ± 0.5	77.9 ± 5.9
ALT, U/l[a]	21.0 ± 2.3	79.2 ± 7.4
AP, U/l[a]	66.4 ± 5.4	255.0 ± 16.7
Albumin, g/dl[a]	4.2 ± 0.1	3.12 ± 0.1
TB, mg/dl[a]	0.9 ± 0.1	4.2 ± 0.1
Prothrombin time, seconds above control[a]	–	4.0 ± 0.3
Creatinine, mg/dl[a]	0.9 ± 0.1	1.2 ± 0.1
Ascites, n(%)	–	57 (45.9)
Encephalopathy, n(%)	–	26 (20.9)

CPDR: cumulative percentage ^{13}C dose recovery.
[a]Results expressed as mean ± standard error of the mean.
Similar letters indicate a statistically significant difference between values (Bonferroni post-test comparisons).

2.3. *Statistical analysis*

Data are expressed as proportions, means ± standard error of the mean or intervals. Student's *t* test was used for comparisons between pairs of groups. χ^{-2} test and one-way analysis of variance (ANOVA) and Bonferroni post-test comparison were used for comparing multiple groups. A p value of ≤ 0.05 was considered as significant. Statistical analysis was performed using STATA V 8.0 (Stata Corporation, College Startion, TX, USA) statistical software.

3. Results

Demographic, clinical and biochemical characteristics of patients with chronic liver failure ($n = 124$) and control subjects ($n = 9$) are shown in Tables 1 and 2. The cause of cirrhosis was alcoholic liver disease in 39, HCV in 60, hepatocellular carcinoma associated to HCV in eight and cryptogenic liver disease in 17 patients. The parameters of hepatic liver function were significantly different among all groups of patients, except for albumin, AST and cumulative oxidation of phenylalanine (Table 2).

Both the subjects in the control group and the patients with liver disease showed a peak oxidation of phenylalanine at 30 min, followed by a decline along the remainder of the test period. Liver disease patients tended to maintain a low phenylalanine oxidation rate with respect to the control subjects (Figure 1).

Table 2. Characteristics of the patients according to the etiology of their liver disease.

	Alcoholic ($n = 39$)	VHC ($n = 60$)	Hepatocellular carcinoma ($n = 8$)	Cryptogenic ($n = 17$)
Age, year[a]	49.5 ± 1.6	53.4 ± 1.3	57.8 ± 4.6	55.5 ± 2.4
Gender, *n*(%)				
Male	37 (94.8)	17 (28.3)	5 (62.5)	7 (41.1)
Female	2 (5.2)	43 (71.7)	3 (37.5)	10 (58.9)
CP class				
A, *n*(%)	9 (23.0)	27 (4.0)	3 (37.5)	4 (23.5)
B, *n*(%)	10 (25.6)	22 (36.6)	2 (25.0)	8 (47.0)
C, *n*(%)	20 (51.2)	11 (18.4)	3 (37.5)	5 (29.5)
CP score	8.9 ± 0.4	7.3 ± 0.3	8.3 ± 1.2	8.5 ± 0.6*
Cumulative ^{13}C-phenylalanine oxidation, CPDR[a]	3.9 ± 0.4	4.3 ± 0.3	3.8 ± 0.8	4.6 ± 0.6
Cumulative ^{13}C-phenylalanine oxidation by CP class, CPDR[a]				
A	7.7 ± 0.7	5.4 ± 0.5	5.5 ± 1.6	7.4 ± 1.5
B	4.1 ± 0.5	4.0 ± 0.2	3.6 ± 1.8	4.4 ± 0.4
C	2.0 ± 0.3	2.2 ± 0.3	2.2 ± 0.6	2.1 ± 0.7
AST, U/l[a]	81.7 ± 12.5	79.1 ± 7.7	116.8 ± 29.2	47.0 ± 8.8
ALT, U/l[a]	48.5 ± 4.8	104.3 ± 13.7	95.5 ± 23.8	53.1 ± 6.5*
AP, U/l[a]	261.6 ± 25.8	233.2 ± 25.2	444.5 ± 87.8	227.5 + 30.4*
Albumin, g/dl[a]	3.0 ± 0.1	3.2 ± 0.1	2.7 ± 0.1	2.9 + 0.1
TB, mg/dl[a]	7.3 ± 1.6	1.8 ± 0.2	7.2 ± 3.8	3.9 ± 2.1*
Prothrombin time, seconds above the control[a]	4.9 ± 0.5	2.9 ± 0.4	6.5 ± 2.5	4.1 ± 1.4[a]
Creatinine, mg/dl[a]	1.1 ± 0.1	0.9 ± 0.0	2.8 ± 1.2	1.7 ± 0.7*
Ascites, *n*(%)	23 (59.0)	21 (35.0)	2 (25.0)	11 (64.7)*
Encephalopathy, *n*(%)	14 (36.0)	7 (11.7)	2 (25.0)	3(17.6)**

CPDR: cumulative percentage ^{13}C dose recovery.
[a]Results expressed as mean ± standard error of the mean.
Statistically significant difference among groups (*ANOVA analysis and **χ^{-2} test).

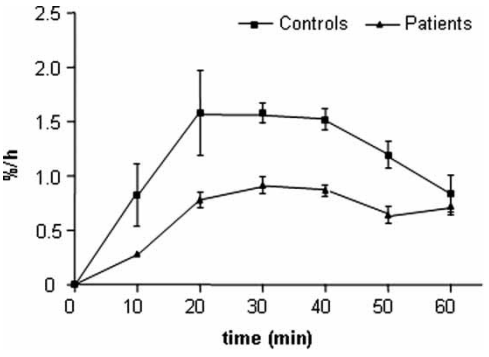

Figure 1. Percentage ^{13}C dose per hour of phenylalanine oxidation as a function of time. Each time-point represents the mean ± standard error of the mean oxidation for control subjects and patients.

The participants in the control group had a greater cumulative oxidation than the patients with chronic liver disease (7.5 ± 0.7 vs. 4.2 ± 0.2, $p = 0.001$). Cumulative oxidation at 60 min was able to discriminate CP *A* from CP *B* and CP *C* patients ($p = 0.000$); however, this test did not allow for the distinction of control subjects from CP *A* patients ($p = 0.43$) (Table 1). There were no differences in cumulative ^{13}C-PheOx among the different CP groups within the alcoholic, VHC, hepatocellular carcinoma or cryptogenic groups of patients (Table 2).

4. Discussion

Our results confirm that the ^{13}C-PheBT distinguishes patients with various degrees of liver disease from otherwise healthy persons [5,11,12]. However, no significant differences in the CPDR in ^{13}C-PheBT during the first 60 min among the different groups of liver disease could be detected. These results are consistent with those obtained by previous studies, which have documented that the CPDR in ^{13}C-PheBT is significantly lower in patients with liver cirrhosis than in healthy adults and that the estimation of activity of phenylalanine hydroxylase in patients with cirrhosis is 20% of the normal value, therefore suggesting that a reduced enzymatic activity may account for the decreased metabolism of phenylalanine in these patients [3]. Other studies have reported that, as liver function worsens – as defined by the CP score – so does the phenylalanine metabolism [5].

The peak of phenylalanine oxidation in the ^{13}C-PheBT occurred at 30 min, both in patients and in controls. These findings are similar to those reported in other studies, where the peak of phenylalanine oxidation occurs between 10 and 30 min, decreasing thereafter until the end of the test period. Furthermore, it has been shown that the peak of oxidation and the total oxidation curve of phenylalanine in patients with liver cirrhosis remain lower than in the control subjects [4,11,12].

Statistically significant differences were found for a variety of clinical and biochemical findings among all the liver disease groups; however, no significant differences were found regarding the CPDR even when considering the CP class, which may in turn lead us to think that the ^{13}C-PheOx is a robust indicator which may allow for the assessment of the functional liver capacity as a result of progressive liver damage, and that the characterisation of the liver specific sub-functions is not altered in a particular way by a specific cause of liver disease. Furthermore, there is evidence which supports that the reduction in ^{13}C-PheOx depends on the primary hepatocellular damage, the physiopathological changes, the course of the liver disease, the total number of cells, the individual cellular function and the low enzymatic activity of phenylalanine hydroxylase and *p*-hydroxyphenylpyruvate hydroxylase [3,5,7,15].

On the other hand, our group found that the CPDR obtained by the ^{13}C-PheBT is a strong predictor of survival in patients with chronic liver disease. Values lower than 5.0% for CPDR are associated with an elevated probability of dying, and values greater than 5.0% are associated with a better outcome [16].

In conclusion, the results of this study show that phenylalanine oxidation at 60 min is similar in different liver parenchymal diseases, and that it decreases with respect to the degree of liver dysfunction. Furthermore, our data confirm that ^{13}C-PheBT is a useful indicator to estimate the functional liver mass and may distinguish patients with liver disease from persons with healthy liver in a non-invasive fashion.

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