¹³C breath tests for measurement of liver function

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The use of stable isotopes is meant to allow a more pathophysiological approach to liver disease by using a battery of breath tests that examine the integrity of different metabolic pathways. Several tests are being investigated. These are: ¹³C aminopyrine breath test (ABT),¹ ¹³C methacetin breath test (in both of which the substrate is metabolised by the cytochrome P450 enzyme system), ¹³C phenylalanine breath test (PBT), and, finally, ¹³C galactose breath test (GBT).

The galactose elimination capacity (GEC) test was proposed several years ago as a quantitative test to measure liver function.² However, the multiple blood samples needed to establish the decrease of galactose concentration make the test difficult in clinical practice and has led to the investigation of the possible use of a [¹³C] GBT in the management of chronic liver diseases.

The metabolism of galactose occurs via a cytosolic pathway independent of the cytochrome P450 system. Thus, there is less variation of its metabolism due to drug induction or inhibition, or genetic polymorphism. The fact that this carbohydrate has a high extraction ratio, however, makes the metabolism of galactose dependent on liver blood flow and hepatic functional mass. To overcome this problem the metabolic pathway must be saturated by giving a large dose of galactose: in this case, its metabolism is directly correlated to the hepatic functional mass.³⁻⁶

Theoretically, a combination of breath tests (using galactose and aminopyrine, for example) will enable the investigation of total liver functional capacity, and would be applicable in the prognosis of liver disease and also in the follow up assessment of liver transplant patients.

In abstract 1 the GBT (after intravenous substrate administration) is compared with the classic GEC test in controls and patients with liver disease. Good correlation was obtained in all cases, thus showing that the GBT is a sensitive method to detect impairment of liver function. Furthermore, abstract 2 shows that the GBT is significantly correlated to the degree of liver fibrosis in a study on patients with chronic hepatitis C infection and no cirrhosis.

It will be clearly advantageous if oral rather than intravenous tests can be done and abstract 3 compares the results obtained in children with acute or chronic liver disease after ¹³C galactose has been given either intravenously or orally. Good correlation was obtained, but when comparisons were made between GBT and the results from the ABT, PBT, and the MEGX test (lidocaine metabolite formation: monoethylglycineylidide) it resulted in weak or negative correlation.

Abstract 4 evaluated whether the ¹³C phenylalanine or ¹³C methacetin breath tests could replace the ¹³C ABT in the assessment of liver function and it concluded that the two former tests are safer alternatives.

For future work it is important to determine factors which account for variability within and between individuals, to develop tests for specific functions (in particular to differentiate between periportal and centrilobular function), and to assess the limits of the range of normality, age, and sex differences. With this baseline information to hand, the usefulness of applying the tests to clinical practice can then be determined.

- 1 Schoeller DA, Baker AL, Monroe PS, *et al.* Comparison of different methods of expressing results of the aminopyrine breath test. *Hepatology* 1982;2:455-62.
- 2 Lindskov J. The quantitative liver function as measured by the galactose elimination capacity. *Acta Med Scand* 1982;212:295-302.
- 3 Henderson JM, Kutner MH, Bain RP. First order clearance of plasma galactose: the effect of liver disease. *Gastroenterology* 1982;83:1090-6.
- 4 Tygstrup N. The urinary excretion of galactose and its significance in clinical intravenous galactose tolerance tests. *Acta Physiol Scand* 1961;51:263-74.
- 5 Tygstrup N. The galactose elimination capacity in control subjects and in patients with cirrhosis of the liver. *Acta Med Scand* 1964;175:281-9.
- 6 Tengström B. An intravenous galactose tolerance test and its use in hepatobiliary diseases. *Acta Med Scand* 1968;183:31-40.

(1) ¹³C galactose breath test: a sensitive test to measure liver function

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Aim: We investigated the possible use of a [¹³C] galactose breath test (GBT) in the management of chronic liver diseases.

Patients: The GBT was performed in six healthy volunteers and 46 patients with chronic liver diseases. In cirrhotic patients, the severity of the liver disease was assessed according to the clinicobiological classification of Child-Pugh. In patients with chronic hepatitis, the severity of histological lesions was analysed based on semiquantitative assessment of activity, hepatocyte necrosis, fibrosis, and steatosis (on a scale: absent, mild, moderate to severe).

Methods: After an overnight fast, five mg/kg of body weight of [¹³C] galactose (GLP [¹³C] galactose, isotopic purity 99% (from Isotec, Miamisburg, Ohio, USA) was injected intravenously, together with a load of 500 mg/kg of unlabelled galactose, dissolved in water at a final concentration of 25%. Breath samples were obtained before the galactose was given, and then every 10 minutes for 180 minutes, and the isotopic enrichment was measured by GC-IRMS. Blood samples to determine galactosaemia (galactose oxidase method) were also obtained every five minutes between 20 and 75 minutes after the galactose infusion, to determine the galactose elimination capacity (GEC) according to Tygstrup.

Results: The results of the GBT were analysed as the percentage of the dose given recovered in breath in one hour because the latest part of the ¹³CO₂ excretion curve could be more directly related to glucose oxidation than to galactose metabolism.

Figure 1 Correlation between the results of GBT and GEC.

We found a good correlation between the results of the GBT and of the GEC, especially for the lower values of both tests (fig 1). With regard to the severity of the liver disease, we found a significant difference between the values of the GBT in controls and in patients with chronic hepatitis ($p < 0.01$). There was also a significant difference between chronic hepatitis and child A patients ($p < 0.01$), and between child A and child C patients, although this difference was less pronounced ($p < 0.05$). Similar results were obtained with the GEC, except that the difference between controls and chronic hepatitis did not reach statistical significance, possibly owing to the small number in the control group. Furthermore, owing to the rapid disappearance of galactose in blood in controls, measurement was less accurate in this group.

Finally, in the chronic hepatitis group we found a significant correlation between the GBT and the grade of fibrosis on liver biopsy specimens ($p < 0.01$). This correlation was also found for the GEC ($p < 0.01$).

Conclusions: The GBT appears to be a sensitive test to detect liver function impairment. Its use seems particularly promising in mild liver diseases such as chronic active hepatitis, and could be of clinical help in the follow up of these patients and in the assessment of the efficacy of antiviral treatment. More results are needed to evaluate the possible oral administration of galactose instead of taking it intravenously. Measurements of labelled galactose and glucose in blood would also be useful for a better understanding of the isotopic breath data.

(2) ^{13}C galactose breath test: early indicator of liver fibrosis in hepatitis C infection

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Background: Simple, non-invasive tests are still needed to measure liver function mass. In cirrhotic patients, we have previously shown that the ^{13}C galactose breath test (GBT) is well correlated with the severity of liver disease.

Aims: The aim of this study was to measure liver function mass by GBT in patients with hepatitis C virus (HCV) chronic hepatitis and no cirrhosis, and to correlate the results of the GBT with the severity of fibrosis in liver biopsy specimens.

Methods: GBT was performed in six healthy volunteers (mean age: 44 (range: 32–63), four men and two women) and 16 liver biopsy proved chronic virus C hepatitis patients (mean age: 48 (range: 25–64), 12 men and four women). GBT was done after an overnight fast: galactose was given intravenously in < 10 minutes (0.5 g/kg of body weight of unlabelled galactose + 5 mg/kg of body weight of ^{13}C galactose). Breath samples were obtained before galactose was given (basal) and every 10 minutes thereafter until

210 minutes. $^{13}\text{C}/^{12}\text{C}$ isotopic ratio was determined in each breath sample by GC-IRMS, and the amount of galactose oxidised to CO_2 per unit of time ($\mu\text{mol}/\text{min}$) was calculated. On liver biopsy specimens, the degree of inflammation, necrosis, and steatosis was assessed as mild, moderate, or severe, whereas the intensity of fibrosis was measured according to the Knodell fibrosis score (1 or 3). Statistical analyses were done by regression analysis and one way ANOVA.

Results: GBT was significantly lower in patients with HCV compared with controls (85 (SD 5) v 141 (SD 10) $\mu\text{mol}/\text{min}$, $p < 0.0001$). In patients with HCV the results of GBT were independent of the levels of serum alanine aminotransferase and aspartate aminotransferase, the severity of hepatic steatosis, necrosis, and inflammation. They were significantly lower in the group with a Knodell fibrosis score of 3 (11 patients) than in the group with a Knodell fibrosis score of 1 (five patients): 77 (SD 5) v 102 (SD 9) $\mu\text{mol}/\text{min}$, $p < 0.05$.

Conclusion: GBT is altered early in the course of HCV chronic hepatitis, and is significantly correlated with the degree of liver fibrosis. This test could thus be useful in monitoring the efficacy of anti-viral treatment in HCV chronic hepatitis.

(3) ^{13}C galactose breath test for dynamic measurement of liver function in children

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Introduction: The ^{13}C aminopyrine breath test (ABT) or the MEGX test (lidocaine metabolite formation: monoethylglycinexylidide) are usually applied dynamic liver function tests. However, the metabolism of aminopyrine and lidocaine depends on the cytochrome P450 enzyme system. In contrast, galactose is metabolised by a cytosolic pathway, and reflects functional liver cell mass. Our main interest was whether $^{13}\text{C}_1$ galactose breath tests (GBT) will give comparable results as the intravenous test of galactose elimination capacity (GEC), and to evaluate simplifications of the GBT with respect to its use in children.

Methods: Ten patients (two girls, eight boys, age: 7–17 years) with acute or chronic liver diseases were investigated. After an overnight fast, 5 mg/kg of body weight $^{13}\text{C}_1$ galactose were either injected intravenously or given orally together with an intravenous or oral load of 500 mg/kg of body weight of unlabelled galactose. During the GBT intravenous blood samples were obtained every five minutes between 20 and 75 minutes after galactose infusion. GEC (mg/kg/min) was calculated according to the linear model of Tygstrup. The galactose elimination constant k_{el} (h^{-1}) of each blood galactose curve was calculated according to the exponential model of Tengström, respectively. The results of the intravenous or oral application of ^{13}C galactose (cumulative one hour, one and a half hour, two hour, and

Abstract 3, Table 1 Correlations between intravenous GBT and oral GBT and GEC and k_{el}

Mode	GBT (%t-h)	GEC ^a (mg/kg/min)	k_{el} ^b (/h)
Oral	1 hour	0.533	0.649*
	1.5 hours	0.606	0.723*
	2 hours	0.646*	0.734*
	3 hours	0.641*	0.729*
Intravenous	1 hour	0.846**	0.863**
	1.5 hours	0.844**	0.870**
	2 hours	0.836**	0.868**
	3 hours	0.830**	0.860**

* $p < 0.05$; ** $p < 0.01$; ^a = linear model by Tygstrup; ^b = exponential model by Tengstrom.

Abstract 4, Table 1 Median values

	Chronic hepatitis (interquartile range)	Cirrhosis (interquartile range)	p Value
ABT			
% Cumulative dose at two hours	12.9 (10.7)	5.4 (1.3)	0.0018
% Cumulative dose at four hours	25.6 (18.5)	11.0 (2.3)	0.0061
DH peak	8.2 (4.8)	3.9 (1.3)	0.0028
PBT			
% Cumulative dose at two hours	12.9 (4.3)	7.4 (3.8)	0.0028
% Cumulative dose at four hours	16.6 (4.7)	8.8 (5.9)	0.0215
DH peak	12.6 (7.8)	6.6 (5.5)	0.0008
MBT			
% Cumulative dose at two hours	21.7 (11.2)	14.5 (9.1)	0.0200
% Cumulative dose at four hours	31.6 (17.5)	22.0 (11.4)	0.0369
DH peak	19.6 (17.2)	13.6 (9.9)	0.0318

three hour ^{13}C elimination rates) were compared with each other and with the results of the ^{13}C aminopyrine breath test (ABT) (2 mg/kg oral) and with the ^{13}C -phenylalanine breath test (PBT) (1.5 mg/kg oral) and with the MEGX test (1 mg/kg intravenous lidocaine; serum concentration in $\mu\text{g/l}$ 30 minutes after injection). A second point of interest was to evaluate a reduction of the number of breath samples to make the test more practical, especially in children. The cumulative one hour, two hour, and three hour ^{13}C elimination rates of 25 breath tests were calculated therefore according to two different modes: 30 minutes sampling mode versus the usual extended test protocol (10 minute intervals up to 90 minutes, thereafter twice in 15 minutes, and twice in 30 minute intervals).

Results: The ^{13}C elimination rates at one hour, two hours, and three hours of the GBT intravenous and the GBT oral showed a significantly linear correlation ($r=0.829$, $p<0.01$; $r=0.875$, $p<0.001$; and $r=0.860$, $p<0.01$ respectively). Table 1 summarises the correlations between GBT given intravenously and GBT given orally and GEC and k_{el} .

Between the cumulative ^{13}C elimination rates at one, two, and three hours calculated either with the 30 minute sampling mode or with the usual test protocol, highly significant linear correlations were found ($r=0.996$, $p<0.001$; $r=0.999$, $p<0.001$; and $r=1.00$, $p<0.001$ respectively).

Only the results of the GBT after the oral mode showed significant correlations with other dynamic liver function tests, whereas there were no significant correlations at all with the intravenous mode. However, there was a negative correlation between oral GBT and PBT.

Conclusions: The results indicate that GBT may be used after an oral application of ^{13}C galactose together with an oral load of unlabelled galactose. GBT breath sampling can be reduced to 30 minute intervals and the test period can be reduced to two hours or even one hour. These simplifications meet the needs, especially those of children. The lacking, weak, or even negative correlations with conventional dynamic liver function tests suggest that functional liver cell mass is neither related to microsomal liver function nor to phenylalanine metabolism. The prognostic relevance of GBT has to be elucidated.

(4) ^{13}C breath tests to evaluate liver functioning mass

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Introduction: The aminopyrine breath test (ABT) has been classically proposed as a non-invasive clinical tool to evaluate liver functioning mass. Although the ABT correlates with the degree of severity of liver disease, the aminopyrine is not completely safe for the risk of agranulocytosis.

Aims: The aim of this work was to evaluate whether the phenylalanine (PBT) or the methacetin (MBT) breath tests could replace the ABT in assessing liver function.

Methods: Twenty four outpatients (mean (SD) age: 39.7 (14.1) years, M/F: 1:12) referred for liver biopsy for persistently raised alanine aminotransferase were studied. Patients who were taking drugs with modulating capacity on P450 cytochrome activity were excluded from the study. Diagnosis of chronic hepatitis or cirrhosis was made on liver histology. Child Pugh score was calculated in all cirrhotic patients. After an overnight fast, each subject received 2 mg/kg of body weight of the ^{13}C substrate (CIL, Andover, MA, USA) dissolved in 50 ml of water. The three breath tests were done in a random order on three different days. Breath samples were taken before the substrate and every 15 minutes for four hours after ingestion of the aqueous solution. At each sampling time, patients blew directly into 10 ml tubes (Exetainers, Europa Scientific, Crewe, UK) through a straw. The ^{13}C enrichment was determined by isotope ratio mass spectrometry (ANCA-NT, Europa Scientific, Crewe, UK). The analytical data were expressed as percentages of $^{13}\text{CO}_2$ recovery each hour of the given dose (%DH) at any time. By trapezoidal rule, the percentages of $^{13}\text{CO}_2$ cumulative values at two and four hours (percentage of cumulative dose at two hours and four hours) were calculated. The maximal percentage of DH at any time (DH peak) was also recorded.

Results: Fifteen patients were affected by chronic hepatitis C and nine by post-hepatitis cirrhosis. All cirrhotic patients were Child Pugh A. Table 1 shows the median values (interquartile range) of percentage of cumulative dose at two and four hours and DH peaks. A significant difference in all these variables between patients with chronic hepatitis and cirrhosis was found ($p<0.05$, Wilcoxon rank sum test) (Table 1).

Conclusions: Both PBT and MBT can be used as liver function tests alternatively to ABT. No side effects were observed during the tests. Like the ABT, these tests can differentiate between chronic hepatitis and first stage post-necrotic cirrhosis and could be proposed as non-invasive tests to assess the presence or the absence of cirrhosis.