PRELIMINARY REPORT

Human Hepatic Macrovesicular Steatosis: A Noninvasive Study of Mitochondrial Ketoisocaproic Acid Decarboxylation

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Differentiating between alcoholic and nonalcoholic hepatic steatosis is often a difficult clinical task. However, decreased fatty acid mitochondrial oxidation appears as the main factor for alcoholic steatosis, whereas nonalcoholic steatosis may be due to other causes. We studied mitochondrial function, based on a ^{13}C-ketoisocaproic acid (^{13}C-KIC) breath test, in nine alcoholic and 12 nonalcoholic steatosis patients and 10 healthy volunteers. Our results showed a 42% ^{13}C-KIC decarboxylation decrease in alcoholic steatosis patients, but not in nonalcoholic steatosis patients. This noninvasive breath test appears helpful for the diagnostic work-up of hepatic steatosis.

ALCOHOLIC AND NONALCOHOLIC cases of hepatic macrovesicular steatosis are often difficult to differentiate based on clinicobiologic data and liver biopsy specimens.[1,2] Decreased mitochondrial oxidation of lipids is one of the major factors of ethanol-induced fatty liver,[2] whereas nonalcoholic hepatic steatosis may be due to other factors such as increased triglyceride availability or decreased hepatic lipoprotein secretion.[3] Lauterburg et al[4] have shown that a ^{14}C-ketoisocaproic acid (^{14}C-KIC) breath test is a relevant in vivo marker of mitochondrial function, and that the results of the KIC breath test were decreased in patients with chronic alcoholism. The goal of our study was to develop a nonradioactive ^{13}C-KIC breath test for the etiologic diagnosis of hepatic macrovesicular steatosis.

SUBJECTS AND METHODS

Twenty-one patients with histologically proven hepatic macrovesicular steatosis without cirrhosis agreed to participate in the study: nine chronic alcoholics and 12 patients with no evidence of alcohol abuse (Table 1). Results of the KIC breath test in the two groups were compared with those obtained in 10 healthy volunteers (seven men and three women), none of whom were alcoholics.

The breath test was performed after an overnight fast. One milligram per kilogram body weight of [1-^{13}C]-KIC (99% atom percent [AP]^{13}C, Tracer Technologies, Somerville, MA) was administered orally together with 20 mg/kg L-leucine dissolved in 200 mL 0.1N citric acid solution. Breath samples were obtained before administration of the labeled molecule (basal sample) and 10, 15, 20, 25, 30, 60, 90, 120, and 150 minutes after. Enrichment of $^{13}CO_2$ in breath was measured by an on-line system coupling gas chromatography (HP 5510A, Les Ullis, France) and isotopic ratio mass spectrometry (VG Isotec SIRA 10, Middlewich, UK).[5] The following kinetic parameters of the test were analyzed: time of appearance of $^{13}CO_2$ peak and percentage of administered ^{13}C recovered in breath between 0 and 60 minutes.[6] Results were analyzed by one-way ANOVA, and statistical differences between groups were assessed using the Scheffé test, with P less than 0.05 as the significance threshold.

RESULTS

In control subjects, a rapid increase of $^{13}CO_2$ excretion was noted as early as 10 minutes after oral administration of ^{13}C-KIC; the peak of $^{13}CO_2$ excretion was obtained at 28 ± 1 minutes (mean ± SEM), with a slow decline thereafter (Fig 1). In the alcoholic steatosis group, the $^{13}CO_2$ peak was obtained later (50 ± 6 minutes, $P < .05$), and the ^{13}C recovery in 60 minutes was decreased by 42% (5.5% ± 0.4% *v* 9.5% ± 0.5% in controls, $P < .05$). In the nonalcoholic steatosis group, the peak of $^{13}CO_2$ excretion was observed significantly earlier than in the alcoholic steatosis group (31 ± 3 minutes). Similarly, the percentage of ^{13}C recovery in 1 hour was identical to the value for the control group (8.9% ± 0.5%), but was significantly greater compared with the value for the alcoholic steatosis group (Fig 2).

DISCUSSION

This study shows a significant decrease of KIC decarboxylation as measured by the ^{13}C-KIC breath test in patients with alcoholic hepatic steatosis, but not in nonalcoholic steatosis patients.

[1-^{13}C]-KIC is mainly decarboxylated by the branched-chain ketoacid dehydrogenase (EC 1.2.4.4), an enzyme located within mitochondria. In humans, a large portion of the branched-chain ketoacid dehydrogenase is found in the liver,[7] where the rate-limiting step for branched-chain amino acid metabolism is transamination.[8] However, [1-^{13}C]-KIC can be transaminated back to [1-^{13}C]-leucine: in this case, a substantial amount of the labeled molecule could be decarboxylated with production of $^{13}CO_2$ outside the liver, mainly in skeletal muscle.[8] To increase the availability of labeled KIC for hepatic mitochondrial decarboxylation, an L-leucine load was added. Leucine administration significantly inhibits KIC transamination,[4] allowing an indirect measure of hepatic mitochondrial function by the KIC breath test. Furthermore, all our patients were ambulatory, none had cirrhosis, and body weights were similar in both groups (Table 1), making unlikely the possibility of decreased muscle KIC decarboxylation or liver functional mass in the alcoholic group as a cause of the decreased results of the KIC breath test.

Besides the KIC breath test, serum aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) values were higher in the alcoholic steatosis group, and serum

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Table 1. Clinical and Biological Characteristics of Hepatic Steatosis Patients

Characteristic	Alcoholic Steatosis	Nonalcoholic Steatosis
Age, years (range)	50 (38-63)	47 (35-56)
Sex (F/M)	3/6	5/7
Body weight (kg)	70 ± 5	74 ± 2
GGT (N < 45 IU/L)	267 ± 80	64 ± 9*
Alkaline phosphatase (N < 100 IU/L)	91 ± 10	80 ± 9
AST (N < 45 IU/L)	90 ± 12	50 ± 7*
ALT (N < 45 IU/L)	77 ± 19	67 ± 14
Cholesterol (mmol/L)	5.8 ± 0.8	6.1 ± 0.6
Triglycerides (mmol/L)	1.0 ± 0.2	1.9 ± 0.3*
Albumin (g/L)	42 ± 2	46 ± 2

NOTE. A significant difference between the 2 groups (*$P < .05$) is noted for serum AST and GGT activities and triglyceride levels.

Abbreviations: ALT, alanine aminotransferase; N, normal value.

triglyceride levels were increased in nonalcoholic steatosis patients (Table 1). Variables such as GGT,[1] the alanine aminotransferase to AST ratio,[9] or the PGA (prothrombin time, gamma-glutamyl transpeptidase, apolipoprotein AI) index[10] have been proposed for the etiologic diagnosis of hepatic steatosis, but it is unlikely that one single test may perfectly discriminate between alcoholic and nonalcoholic macrovesicular steatosis. The noninvasive KIC breath test is thus another diagnostic tool that may decrease the need for liver biopsy in the classification of hepatic steatosis.

In conclusion, we suggest that the [1-^{13}C]-KIC breath test could be of clinical use for the diagnosis of fatty liver, in addition to clinical and standard biochemical data.

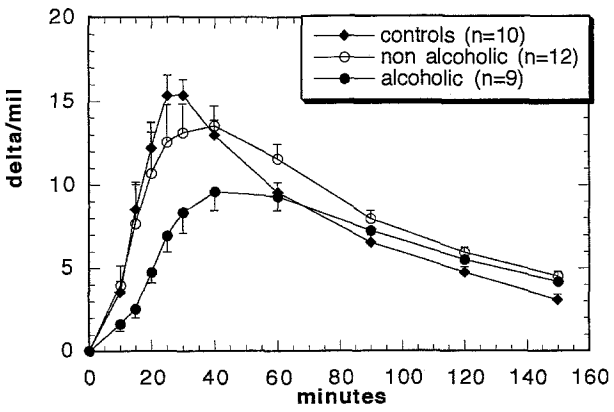

Fig 1. $^{13}CO_2$-exhalation curves after oral administration of ^{13}C-KIC in healthy controls and patients with alcoholic or nonalcoholic macrovesicular steatosis. Results were expressed as Δ% of ^{13}C enrichment over basal values, and the error bars represent ± SEM.

Fig 2. Percentage of the administered dose of ^{13}C-KIC recovered in the breath in 1 hour. Each circle is the value of the breath test for one individual; (■) mean value for each group.

REFERENCES

1. Diehl AM, Goodman Z, Ishak KG: Alcohol-like liver disease in nonalcoholics. Gastroenterology 95:1056-1062, 1988

2. Lieber CS: Alcohol and the liver: Metabolism of ethanol, metabolic effects and pathogenesis of injury. Acta Med Scand [Suppl] 703:11-55, 1986

3. Dianzani MU: Reactions of the liver to injury: Fatty liver, in Farber E, Fischer MM (eds): Toxic Injury of the Liver. New York, NY, Dekker, 1979, pp 281-331

4. Lauterburg BH, Liana D, Schwarzenbach FA, et al: Mitochondrial dysfunction in alcoholic patients as assessed by breath analysis. Hepatology 17:418-422, 1993

5. Guilluy R, Billion-Rey F, Pachiaudi C, et al: On-line purification and carbon-13 isotopic analysis of carbon dioxide in breath: Evaluation of on-line gas chromatography–isotope ratio mass spectrometry. Anal Chim Acta 259:193-202, 1992

6. Schoeller DA, Klein PD, Watkins JB, et al: ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for $^{13}CO_2$ breath tests. Am J Clin Nutr 33:2375-2385, 1980

7. Khatra BS, Chawla RK, Sewell CW, et al: Distribution of branched-chain alpha-keto acid dehydrogenases in primate tissues. J Clin Invest 59:558-564, 1977

8. May M, Mancusi VJ, Aftring RP, et al: Effects of diabetes on oxidative decarboxylation of branched-chain keto acids. Am J Physiol 239:E215-E222, 1980

9. Redlich CA, West B, Fleming L, et al: Clinical and pathological characteristics of hepatotoxicity associated with occupational exposure to dimethylformamide. Gastroenterology 99:748-757, 1990

10. Poynard T, Aubert A, Bedossa P, et al: A simple biological index for detection of alcoholic liver disease in drinkers. Gastroenterology 100:1397-1402, 1991