ORIGINAL ARTICLE

^{13}C-METHACETIN BREATHE TEST IN EARLY DIAGNOSTICS OF NON-ALCOHOLIC FATTY LIVER DISEASE

DOI: 10.36740/WLek202103119

Helen E. Sklyarova
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

The aim: Of the study was to evaluate the early utility changes of the ^{13}C methacetin breath test parameters in patients with NAFLD.

Materials and methods: There were included 50 subjects in the study, among them 35 patients had steatosis and 15 patients had steatohepatitis, including 35 (70.0%) male subjects and 15 (30.0%) female subjects. The control group included 17 apparently healthy volunteers, among them 10 (58.8%) subjects were male and 7 (41.2%) subjects were female.

Results: It was determined that metabolism kinetics in case of liver steatosis was significant decreased more than 30% compared to the control group ($p = 0.0001$) and in case of steatohepatitis that decrease was more than 65% ($p = 0.00001$) compared with normal values. It resulted in less cumulative dose accumulation in steatosis ($p = 0.00001$) and steatohepatitis ($p = 0.00001$). Among the reasons for the decrease in the kinetics of metabolism in steatosis, there were insufficient response of hepatocytes on 10 minutes (<10 dose/h,%) in 40% of cases and reduction of metabolism rate amplitude at 20-40 minutes following methacetin administration.

Conclusions: The results of ^{13}C-methacetin breath test demonstrate that in patients with NAFLD there is a gradual slowing of metabolism rate in hepatocytes, which leads to a decrease in cumulative dose.

KEY WORDS: non-alcoholic fatty liver disease (NAFLD), ^{13}C-methacetin breathe test, steatosis, steatohepatitis

Wiad Lek. 2021;74(3 p.I):487-491

INTRODUCTION

With the implementation of breath tests in the clinical practice to determine the liver function, it became possible to quantify its functional capacity. It is mainly associated with the need for early diagnosis of non-alcoholic fatty liver disease (NAFLD), which is one of the predictors of cardiovascular diseases and type 2 diabetes mellitus [1,2].

Systematic intake of fatty food and physical inactivity are leading to critical increases in obesity and metabolic syndrome frequency in industrialized countries. As a result, NAFLD has become an important public health issue [3,4].

The intense accumulation of fat in the liver eventually leads to the occurrence of steatosis, which is found in 80-90% of cases in patients with obesity, and subsequently it leads to steatohepatitis, which is diagnosed in 10-20% of patients [5,6].

In majority of cases, obese patients consider themselves to be healthy individuals, although they may report fatigue and sensation of heaviness in the right hypochondriac region. It is found hepatomegaly in 75% of cases based on results of direct examination. Laboratory data indicate the transaminases levels elevation only in 30% of cases; however, ultrasonography examination of the internal organs reveals hyperechogenicity of the liver, as well as computed tomography data demonstrate homogeneous decrease in the density of the liver structure [5].

In general, ultrasound diagnostic methods allow to detect NAFLD in the cases of at least 30% fat accumulation in hepatocytes [7].

Therefore, the results of static biochemical markers analysis and instrumental examination allow to make only indirect conclusions regarding the liver function and extent of the liver damage, whereas the needle biopsy is associated with significant risk of complications [8].

In this aspect, the use of breath tests based on the metabolism of orally administered substrates labeled with a stable carbon isotope ^{13}C makes it possible to evaluate the functional status of the liver in normal and pathological conditions [2,9].

Currently available breath tests allow to determine microsomal (methacetin, phenacetin, aminopyrine, caffeine), mitochondrial (methionine) and cytosolic (galactose, phenylalanine) liver function [1].

In clinical practice, the ^{13}C methacetin breath test (^{13}C-MBT) is used most widely and cumulative dose and metabolic rate are analysed for determination of the liver functional capacity. In apparently healthy subjects, the metabolic capacity of the liver presented as a percentage of the accumulated dose during 120 minutes ranges from 20% to 35% that corresponds to 100% of functioning hepatocytes and is the main constant for the evaluation of the results of methacetin test. The second parameter for analysis of the metabolism kinetics of

^{13}C labeled methacetin is the time from the substrate intake to a peak of $^{13}CO_2$ elimination in exhaled air. A shift in the peak of the metabolism rate from 10 to 50 minutes indicates the development of fatty liver infiltration [10,11,12].

In general, a decrease in ^{13}C-MDT parameters relative to normal values evidences of impaired liver microsomal function, which may be characteristic of steatosis and fibrosis [13,14].

However, the diagnosis of the early stages of NAFLD is not yet fully understood. The proposed criteria for identifying the liver steatosis, steatohepatitis and cirrhosis need clarification.

THE AIM

The aim of the study was to perform a comprehensive comparison of changes in ^{13}C-MDT, biochemical and ultrasonographic features in patients with steatosis and steatohepatitis.

MATERIALS AND METHODS

There were included 45 subjects in the study, among them 30 patients had steatosis and 15 patients had steatohepatitis, including 32 (71.1%) male subjects and 13 (28.9%) female subjects. The control group included 17 apparently healthy volunteers, among them 10 (58.82%) subjects were male and 7 (41.18%) subjects were female.

The diagnosis of NAFLD was based on clinical examination data (overweight/obesity, ultrasound parameters, and hepatic transaminases). Subjects with history of viral hepatitis, hemochromatosis, autoimmune hepatitis, Wilson's disease and subjects who consumed alcohol at a daily dose of 30 mg for men and 20 mg for women or received hepatotoxic drugs were excluded from the study. There were also excluded from the study patients who took drugs that affect CYP1A2 activity and anticoagulants.

The complete clinical examination included anthropometric characteristics, such as height, body weight, body mass index, waist and hip circumferences. Hepatic transaminases, blood lipids, glucose and insulin were also determined with the calculation of insulin resistance index.

The methacetin breath test was performed in the morning after 12 hours of fasting and 30 minutes of resting in sitting position. Patients were advised that pineapple, kiwi fruit or mineral water should be avoided 48 hours before the test. The test breakfast consisted of 75 mg of ^{13}C-methacetin dissolved in unsweetened fruit tea. Breath samples were collected in aluminum bags before the administration of methacetin and then every 10 minutes during the first hour of the test and every 20 minutes

Table I. Characteristics of study groups

		Control group (Group 1), (n=17)	Steatosis (Group 2), (n=30)	Steatohepatitis (Group 3), (n=15)	p-value
Gender	Males	10 (58.82 %)	19 (67.86 %)	12 (75 %)	–
	Females	7 (41.18 %)	9 (32.14 %)	4 (25 %)	
Age (years)		58.24±2.31	57.14±2.04	53.75±2.85	–
BMI (kg/m²)		26.88±1.60	31.61±0.76	30.00±1.02	p_{1-2}=0.00007 p_{1-3}=0.0039 p_{2-3}=0.3307
Total cholesterol (µmol/L)		4.91±0.19	5.56±0.26	5.63±0.21	p_{1-2}=0.1749 p_{1-3}=0.0425 p_{2-3}=0.5646
LDL (µmol/L)		2.90±0.20	3.51±0.23	3.28±0.26	p_{1-2}=0.123 p_{1-3}=0.2907 p_{2-3}=0.595
Triglycerides (µmol/L)		1.84±0.11	2.18±0.22	2.53±0.31	p_{1-2}=0.6039 p_{1-3}=0.064 p_{2-3}=0.1478
HDL (µmol/L)		1.13±0.05	1.14±0.05	1.02±0.05	p_{1-2}=0.9715 p_{1-3}=0.0945 p_{2-3}=0.1362
ALT (IU/L)		32.11±2.30	38.93±1.09	50.62±2.95	p_{1-2}=0.0013 p_{1-3}=0.0002 p_{2-3}=0.00001
AST (IU/L)		31.23±2.35	41.28±2.07	53.94±3.09	p_{1-2}=0.0024 p_{1-3}=0.00002 p_{2-3}=0.0004

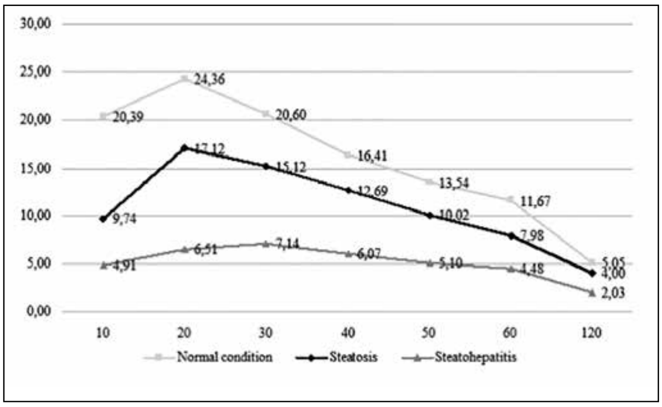

Fig. 1. Metabolism rate in normal condition, steatosis, and steatohepatitis

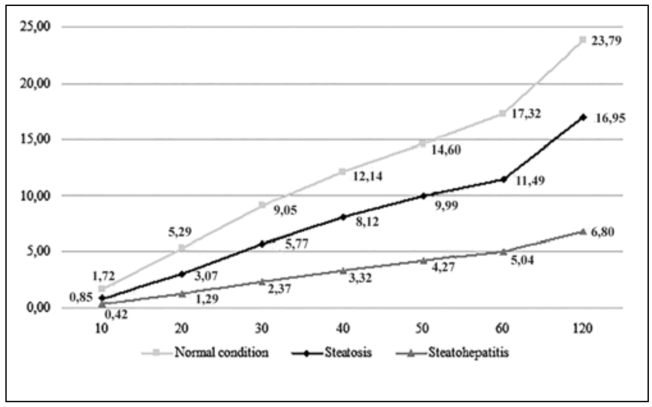

Fig. 2. Cumulative dose in normal condition, steatosis, and steatohepatitis

during the second hour of the test. No adverse events were reported by patients.

Measurement of ^{13}C-labeled CO_2 in exhaled air was performed using the infrared spectrometer IRIS (manufactured by Wagner Analysen Technik GmbH). The following parameters were analyzed: cumulative dose at 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, and 120 minutes of the test period. The metabolism rate was also measured at the same time points.

Statistical analysis of the received data was performed on a personal computer using software Microsoft Excel and Statistica v. 10.0, StatSoft Inc., USA. The reliability of difference between average values was estimated using the Student's paired t-test. The mean value (M) and standard error of the mean (m) also were evaluated. When investigating the relationship of normally distributed quantitative parameters, Pearson's correlation analysis (r) was used to assess their orientation and strength. The difference between the parameters was considered statistically significant at p-value < 0.05.

RESULTS

Following the examination of patients with NAFLD, the most common signs of metabolic syndrome are: overweight or obesity, dyslipidemia, increased over time the concentration of ALT and AST, depending on the presence of steatosis or steatohepatitis (Table I).

According to ultrasound examination data, hepatomegaly, hyperechogenicity of the liver parenchyma and heterogeneity of the liver structure due to steatosis were observed in 80% of cases. In addition, diameter enlargement of the portal vein and of the splenic vein was found in case of steatohepatitis.

During the ^{13}C-MBT, the features of the hepatocytes response were revealed depending on the stage of the course of NAFLD in steatosis and steatohepatitis.

In apparently healthy subjects, the metabolism rate interval after methacetin administration was between 24.36 ± 1.18 and 11.67 ± 0.82 dose/h,% during 60 minutes of the examination and was not lower than 10 dose/h,% (Figure 1).

In case of steatosis, the metabolism rate after methacetin administration was significantly decreased compared to the control group (p=0.0001). Among the features of the decrease in the metabolism rate in steatosis, there were insufficient response of hepatocytes to the intake of methacetin on 10 minutes (<10 dose/h,%) in 40% of cases and reduction of amplitude at 20-40 minutes in the kinetics of metabolism at a sufficient level instead of 60 minutes compared to normal.

In patients with steatohepatitis compared with the control group and liver steatosis group, there was observed a further decrease in the rate of metabolism, which did not reach 10.0 dose/h,% during the first 60 minutes of the examination.

Accumulation of the cumulative dose was significantly reduced in the case of steatosis and steatohepatitis compared to the control group for the first 10 minutes of the methacetin breath test. Thus, in apparently healthy individuals, the values at 10 minutes (Cum10) ranged from 1.4 to 2.8 dose/h,% with the mean value 1.72 ± 0.10 dose/h,%. However, the methacetin accumulation level at 10 minutes was lower than 1.0% in 64.3% of cases in the presence of steatosis, and it was more than 4 times lower than in the control group in the presence of steatohepatitis (Figure 2).

The results of the study showed that there is a decrease in the rate of metabolism resulting in a correspondingly reduced cumulative dose in steatosis of the liver. In the case of steatohepatitis, such changes are more pronounced throughout the examination. While the rate of metabolism was 68.5% in steatosis compared to the control values (p = 0.00001), then in steatohepatitis it was decreased in 32% compared to normal (p = 0.00001).

DISCUSSION

Despite the wide use of common methods of examination for the detection of NAFLD, none of them provide reliable diagnosis confirmation, including anthropometric parameters for the determination of the obesity stage, liver tests, blood lipid spectrum, liver echolocation and elastography [5]. Only in the presence of fibrosis, the best results are obtained by liver elastometry. However, indices of static biochemical markers allow only indirect conclusions to be drawn on the liver function in early stages of NAFLD [1,13]. Therefore, the use of breath tests to determine the metabolism of orally administered substrates, in particular methacetin, makes it possible to evaluate the functional status of the liver in dynamics in the normal condition and in NAFLD [1,12].

It is well known that there is a gradual decrease in the rate of metabolism and in the cumulative dose in steatosis and steatohepatitis [2,11,12]. Thus, in the presence of steatosis, it is observed the shift of the peak metabolism rate from 10 minutes to 20-30-40-50 minutes of the test [3,6]. The progression of the pathological process leads to a decrease in the rate of metabolism and in the cumulative dose that characterizes the "capacity" of the liver [9]. However, for early diagnosis of NAFLD, it is important to evaluate the cumulative dose accumulation during the examination [3,9]. Thus, the points for difference between normal condition and pathology are metabolism levels of 1.05 at 10 minutes, 4.15 at 20 minutes, 7.15 at 30 minutes, 9.00 at 40 minutes, and 20.00 at 120 minutes [6]. Among the generally parameters of metabolism assessment at 40 and 120 minutes, attention should be also drawn to Cum 10, as ^{13}C-dose/h,% at 10 minutes and 60 minutes coincide and have similar accuracy in the diagnosis of steatohepatitis [1].

This study showed the comparable significance of the obtained parameters of the metabolism rate and the accumulation of cumulative dose, which are specific in the normal condition and in steatosis or steatohepatitis. In apparently healthy subjects, the rate of metabolism is maintained at a high level, which provides sufficient cumulative dose accumulation and corresponds to approximately 100% of functioning hepatocytes [5]. In the presence of steatosis, decreased metabolism rate and accordingly significant decrease in the cumulative dose are caused by the reduction of functional activity of hepatocytes to 30-40 minutes instead of 60 minutes compared with apparently healthy individuals.

In the case of steatohepatitis, there is a sharp decrease in the rate of metabolism and accordingly the cumulative dose, which amounts to only a quarter of the functional capacity of hepatocytes in healthy persons.

CONCLUSIONS

1. Methacetin breath test (^{13}C-MBT) allows to detect impaired metabolic rate and accordingly changes in the metabolic capacity of the liver in dynamics at the early stages of NAFLD.
2. Steatosis of the liver is characterized both by decrease in the metabolism rate and by a reduction of the metabolism kinetics at a sufficient level to 20-40 minutes instead of 60 minutes in apparently healthy individuals.
3. The presence of steatohepatitis is evidenced by a sharp decrease in the rate of metabolism and metabolic capacity of the liver (less than 10 dose/h,%) compared with apparently healthy individuals and patients with steatosis of the liver.

REFERENCES

1. Rapoport S.I., Shubina N.A. Respiratory tests in diagnostics of liver disease. Clin med. 2016;94(12):885-892. doi:10.18821/0023-2149-2016-94-12-885-892.
2. Miele L., Marrone G., Cefalo C. et al. Potentian use of liver function breath tests in the clinical practice. Eur Rev Med Pharmacol Sci. 2013;17(2):82-89.
3. Stepanov Yu.M. Results of the observational cross-over PRELID 2 study (2015–2016). Part 1. The prevalence of non-alcoholic fatty liver disease, the characteristics of concomitant pathology, metabolic syndrome and its individual criteria in patients seeking general practitioners' and gastroenterologists' help in Ukraine. Gastroenterol. 2019;53(1):26-33. doi:10.22141/2308-2097.53.1.2019.163454.
4. Williams C.D., Stengel J., Asike M.I. et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterol. 2011;140(1):124-131. doi:10.1053/j.gastro.2010.09.038.
5. Gerok V., Blyum H.E. Zabolevanija pecheni i zhelchevydelitel`noi sistemy [Diseases of the liver and biliary system]. Moscow: MEDpress-inform;2009.199 p.(In Russian).
6. Razlan H., Marzuki N.M., Tai M.L. et al. Diagnostic value of the C methacetin breath test in various stages of chronic liver disease. Gastroenterol Res Pract. 2011;2011:235796. doi:10.1155/2011/235796.
7. Chalasani N., Younoss Z., Lavine J.E. et al. The diagnosis and Management of non-alcoholic fatty liver disease: practice guideline by the American gastroenterological association, American association for the study of liver disease, and American college of gastroenterology. Gastroenterol. 2012;55(6):2005-2023. doi:10.1002/hep.25762.
8. Lock J.F., Taheri P., Bauer S. et al. Interpretation of non-invasive breath tests using (13)C-labeled substrates – a preliminary report with (13) C-methacetin. Eur J Med Res. 2009;14:547-550.
9. Musialik J., Jonderko K., Kasicka-Jonderko A. et al. (13)CO2 breath tests in non-invasive hepatological diagnosis. Prz Gastroenterol. 2015;10(1):1-6. doi:10.5114/pg.2014.47501.
10. Gorowska-Kowolik K., Chobot A., Kwiecien J. 13C methacetin breath test for assessment of microsomal liver function: methodology and clinical application. Gastroenterol Res Pract. 2017;7397840. doi:10.1155/2017/7397840.
11. Grattagliano I., Lauterburg B.H., Palasciano G. et al. 13C-breath tests for clinical investigation of liver mitochondrial function. Eur J Clin Invest. 2010;40(9):843-850.
12. Kempiński R., Neubauer K., Wieczorek S. et al. 13C-Methacetin Breath Testing in Patients with Non-Alcoholic Fatty Liver Disease. Adv Clin Exp Med. 2016;25(1):77-81. doi:10.17219/acem/60878.
13. Aksentiychuk K., Kurlyak N., Sklyarov E. Monitoring of liver function in patients with fatty liver disease in combination with coronary heart disease and type 2 diabetes mellitus. Curr Iss Pharm Med Sci. 2013;26(4):376-379. doi:10.12923/j.2084-980X/26.4/a.04.
14. Moran S., Mina A., Duque X. et al. The utility of the 13C-methacetin breath test in predicting the long-term survival of patients with decompensated cirrhosis. J Breath Res. 2017;11:036011. doi:10.1088/1752-7163/aa7b99.

This article is a fragment of scientific research work "Features of the respiratory, cardiovascular, digestive system in patients with diabetes mellitus and obesity: features of pathogenesis, clinic, diagnostics".

ORCID and contributionship:
Helen E. Sklyarova – 0000-0003-3667-6304 A,B,C,D,E,F

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR
Helen E. Sklyarova
Danylo Halytsky Lviv National Medical University
3/1 Nizynska st., 79010 Lviv, Ukraine
тel.: +380636210005
e-mail: elena505skl@gmail.com

Received: 21.04.2020
Accepted: 27.11.2020

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis,
D – Writing the article, **E** – Critical review, **F** – Final approval of the article