

ORIGINAL PAPER

Clinical features of the impact of eating disorders on the results of ¹³C-methacetin breath test for assessment of liver function in girls with anorexia nervosa

Katarzyna Górowska-Kowolik¹, Agata Chobot², Katarzyna Bąk-Drabik³, Jarosław Kwiecień³

¹Department of Pediatric Gastroenterology and Hepatology, Clinical Hospital No. 1 in Zabrze, Medical University of Silesia in Katowice, Zabrze, Poland

²Department of Pediatrics, Institute of Medicine, University of Opole, Opole, Poland

³Department of Pediatrics, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Zabrze, Poland

ABSTRACT

Introduction: This study was designed to assess the influence of chosen aspects of anorexia nervosa eating disorders (AN ED) on ¹³C-methacetin breath test (MBT) results.

Material and methods: We investigated a group of 81 girls, including 41 patients with confirmed diagnosis of AN ED. The study group was divided in two subgroups. According to the DSM-5 classification, 25 underweight patients met the criteria of anorexia nervosa (AN), and 16 girls with BMI value > 5th percentile for age and sex were diagnosed as atypical anorexia nervosa (AAN). Laboratory tests assessing the liver function and the MBT were performed in all the participants of the study.

Results: In all healthy females the values of anthropometric parameters and laboratory results concerning the liver function were normal. Girls with AN ED achieved higher cumulative ¹³CO₂ dose salvage during the MBT than healthy controls. Also, DOB (delta over baseline) values were significantly higher in the study group, as well as in both ANN and AN subgroups, compared to controls. Comparison of the patients' subgroups revealed higher elimination of the cumulative ¹³CO₂ dose in females with AN compared to those with AAN. Among the study group there was a negative correlation between the DOB values in all time points of the test and the weight, weight percentile, BMI, and BMI percentile.

Conclusions: Girls with weight deficiency in the course of AN achieved higher cumulative ¹³CO₂ dose recovery during the MBT than healthy controls and other AN patients. The recovery of ¹³CO₂ during the MBT was negatively correlated with parameters describing patients' weight status. The obtained results suggest a significantly faster ¹³C-methacetin metabolism in girls with AN ED, which is probably a consequence of the characteristics of the primary disease. The presented observations suggest that MBT is not useful in monitoring liver function in patients with AN ED.

KEY WORDS:

anorexia nervosa, liver function, atypical anorexia nervosa, ¹³C-methacetin breath test, P450 cytochrome.

INTRODUCTION

The breath test using methacetin labelled with the ¹³C carbon isotope (¹³C-methacetin breath test – MBT) allows

a non-invasive, repeatable assessment of the functional liver capacity. The test's methodology is based on the evaluation of the hepatic functional reserve by determining the amount of ¹³CO₂ eliminated with the exhaled air.

ADDRESS FOR CORRESPONDENCE:

Katarzyna Górowska-Kowolik, Department of Paediatric Gastroenterology and Hepatology, Clinical Hospital No. 1 in Zabrze, Medical University of Silesia in Katowice, 13-15 3 Maja St., 41-800 Zabrze, Poland,
ORCID: 0000-0003-2439-0931, e-mail: k.gkowlolik@gmail.com

¹³CO₂ is formed in the liver after dealkylation of methacetin by the CYP1A2 cytochrome [1]. The usefulness of the MBT was described in many acute as well as chronic liver diseases [2–5]. Even though numerous studies indicate the potential utility of the MBT in various diseases, its application in clinical practice remains limited. Afolabi *et al.* indicated, among others, the need to determine the diagnostic value of the test in various groups of patients and the potential influence of factors other than liver disease on the obtained results [6]. In the course of anorexia nervosa eating disorders (ED AN) many metabolic complications occur. Progressing malnutrition and specific behaviours of these patients may result in hepatopathy of diverse stages [7]. The reasons given above led to the design of this study being aimed at assessing the influence of different types of AN ED on the MBT results.

MATERIAL AND METHODS

The study included females aged 12 to 17 years, with diagnosed restrictive type AN ED, who were hospitalised in the Department of Endocrinology, Clinical Hospital No. 1 in Zabrze, Poland. Patients with cachexia from organic diseases other than AN ED, history of chronic liver diseases, acute infection, and respiratory tract obstruction and diseases that may impact the motility of the gastrointestinal tract other than AN ED were excluded from this investigation. Anorexia nervosa (AN) was diagnosed based on the DSM-5 criteria when all of the following conditions were met:

- A. Restriction of energy intake relative to requirements leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health.
- B. Intense fear of gaining weight or becoming fat, or persistent behaviours affecting weight loss even though underweight.
- C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight [8].

If a patient met criteria B and C and there was a significant weight loss, but the present weight was within the normal range for age and sex, atypical anorexia nervosa (AAN) was diagnosed [9]. Inclusion criteria were met in total by 44 patients, but three of them did not complete the MBT (two cases of intentional, provoked vomiting after taking the substrate and one case of a hysteria episode during the test). The remaining patients ($n = 41$), who met the inclusion and exclusion criteria, comprised the study group.

Subsequently, based on the body mass index (BMI) values, the group was divided into two subgroups: AN – underweight (BMI \leq 5 percentile for age and sex) patients ($n = 25$ [61%]), AAN – 16 patients (39%) who were not underweight (with BMI $>$ 5th percentile for age and sex).

Figure 1 presents a flowchart showing how the study group was comprised and divided into the subgroups. Information concerning the course of the main disease: the total weight loss from the beginning of the disease (in kilograms), the total weight loss (as a percentage of initial weight), speed of weight loss (in kilograms per month), duration of the secondary amenorrhea (in months, calculated based on the date of the last menstruation), and medications were also collected.

The control group ($n = 40$) consisted of age-matched females who were hospitalised for diagnostic reasons in other paediatric departments of Clinical Hospital No. 1 in Zabrze, Poland. These girls were characterised by adequate nutritional status and fulfilled inclusion criteria. Based on their medical history, physical examination, and additional diagnostic tests for eating disorders, menstrual cycle disorders, acute infections, respiratory tract obstruction, hepatopathy, or organic diseases of the gastrointestinal tract were ruled out in these children.

Weight and height were assessed in all participants of the study. The measurements were performed in the morning, after fasting, on the day preceding the MBT. Weight was expressed in kilograms with 0.1-kg precision. Height was measured with 0.1-cm precision using a stadiometer. Based on the obtained data for each patient the BMI was calculated (using the formula: weight/height² [kg/m²]). To plot weight, height, and BMI we used percentile charts for the population elaborated from the OLA and OLAF projects, which concerned Polish children aged 3–18 years. BMI equal to or lower than the fifth percentile for age and sex was considered as underweight.

The characteristics of the study and control group are presented in Table 1. In all healthy females the values of anthropometric parameters and laboratory results concerning the liver function were normal, which reflects the initial inclusion criteria. Variance analysis of both subgroups of patients and the control group revealed significant heterogeneity in terms of weight ($p < 0.001$), weight percentile ($p < 0.001$), BMI ($p < 0.001$), and BMI percen-

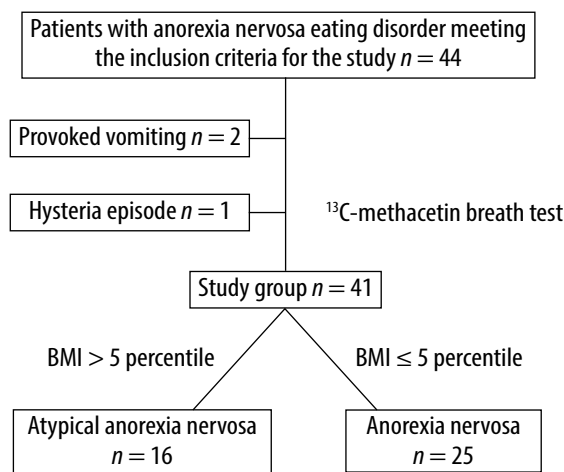


FIGURE 1. The design of the study group and its division into subgroups

TABLE 1. Age and anthropometric parameters among study and control groups (data presented as median \pm IQRs)

Parameter	AN <i>n</i> = 25	AAN <i>n</i> = 16	Controls <i>n</i> = 40
Age (years)	15.0 \pm 1.00	13.5 \pm 0.75	15.0 \pm 1.5
Weight (kg)	38.5 \pm 2.85	45.0 \pm 2.88	55.05 \pm 4.48
Weight (percentile)	2 \pm 1.95	21 \pm 12.25	58.0 \pm 16.5
Height (cm)	161.5 \pm 2.9	160.9 \pm 5.38	164.25 \pm 3.0
Height (percentile)	41 \pm 13	51.5 \pm 28.5	59.0 \pm 19.75
BMI (kg/m ²)	15.1 \pm 1.05	17.15 \pm 0.7	20.25 \pm 1.18
BMI (percentile)	0.1 \pm 0.95	14.5 \pm 8.5	49 \pm 16.25

AN – anorexia nervosa, AAN – atypical anorexia nervosa

tile ($p < 0.001$) – as a consequence of the applied inclusion criteria. Median weight percentile of girls with AN was 2.0 ± 1.95 and BMI percentile 0.1 ± 0.95 , which was significantly lower when compared to the control group (respectively, 2.0 ± 1.95 vs. 58 ± 16.5 , $p < 0.001$ and 0.1 ± 0.95 vs. 49 ± 16.25 , $p < 0.001$) as well as the AAN subgroup (respectively, 2.0 ± 1.95 vs. 21 ± 12.25 , $p < 0.001$ and 0.1 ± 0.95 vs. 14.5 ± 8.5 , $p < 0.001$). The BMI centile of girls with AAN remained within the broad normal range, but was significantly lower than that of controls (14.5 ± 8.5 vs. 49 ± 16.25 kg/m², $p < 0.001$). There were no differences in the percentage of weight loss and duration of secondary amenorrhoea between females with AN and those with AAN (respectively, 26 ± 8.6 vs. 26 ± 8.7 , $p = 0.83$ and 6.2 ± 3.45 vs. 5.3 ± 2.64 , $p = 0.42$).

All of the study's participants had the following laboratory tests carried out: activity of aminotransferases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) and gamma glutamyltransferase, international normalised ratio, total bilirubin, and haemoglobin concentration. In general, AST and ALT activity was significantly higher in patients than in healthy controls (24.0 ± 14.1 vs. 17.6 ± 3.06 , $p = 0.007$ and 25 ± 22.1 vs. 11.87 ± 3.9 , $p < 0.001$, respectively). Hypertransaminasaemia was revealed in five (12%) girls from the study group. Elevated activity of both aminotransferases was found in three patients with AN, and two patients (one with AAN and one with AN) had isolated elevated ALT values. In each of the subgroups the highest values of the liver enzymes did not exceed more than three times the upper normal limit. In females from the study group heart insufficiency was ruled out based on the echocardiograph.

MBT was performed in patients and controls using an IRIS device according to the methodology recommended by the manufacturer [10]. The investigation was carried out in the morning, after fasting. Patients were given orally 75 mg of ¹³C-methacetin (Eurisotop, France) dissolved in 200 ml of a neutral fluid at room temperature. Once the test's methodology was explained to the patient, she was asked to take a deep breath, withhold it for five sec-

onds, and exhale the air slowly through a mouthpiece into an aluminium-covered bag so that it was fully inflated. Sample bags and mouthpieces were part of the equipment of the device. We applied the following scheme of collecting the breath samples: a “null” sample before the ingestion of ¹³C-methacetin followed by nine samples 10, 20, 30, 40, 50, 60, 80, 100, and 120 minutes after the substrate was swallowed. During the test the patients were asked to remain fasting and restrain from physical activity. All samples were analysed by means of the IRIS device in order to assess the content of ¹³CO₂ relative to ¹²CO₂ in the exhaled air. Compared to the baseline, the increase of ¹³CO₂ concentration was determined by DOB (delta over baseline) value. Based on the DOB results the software of the IRIS device calculated two basic parameters describing the kinetics of ¹³C-methacetin metabolism:

- 1) ¹³C-methacetin cumulative dose (% CD) – recovered with the exhaled air, defined as the percentage of exhaled ¹³CO₂ relative to the amount of ¹³C-methacetin that was taken;
- 2) time to peak (TTP) – time from taking ¹³C-methacetin to the peak elimination of ¹³CO₂ with the exhaled air.

Because there are no normal ranges of MBT results for different age groups, the curves describing the kinetics of the ¹³C-methacetin metabolism were referred to the normative MTB ranges for healthy adults given by the device's manufacturer.

The basic parameter to assess if the MBT result is correct was the ¹³CO₂ cumulative dose in the 120th minute of the test (% CD120). Its normal values range between 20.8 and 37.3% (of the ¹³C isotope recovery from the initially taken dose). The kinetics of ¹³C-methacetin metabolism was regarded normal when TTP equalled 10–20 minutes.

The research project was accepted by the Ethical Board of the Medical University of Silesia in Katowice, Poland (KNW/0022/KBI/74/16). Written consent was obtained from all caregivers of the participants and the participants themselves if they were aged 16 years or more.

STATISTICAL ANALYSIS

Statistical analysis of the collected data was performed using the Statistica (StatSoft Polska Sp. z o.o.) software and Excel Microsoft Office (Microsoft Poland) worksheets. Results were considered as significant at $p < 0.05$. For some of the statistical calculations the variables were initially standardised. At the beginning descriptive statistics were performed. Distribution normality was estimated by Shapiro-Wilk test. Depending on the normality of the distribution, values were presented as mean and standard deviation or median and IQR. In the case of non-normal distribution and/or lack of variance homogeneity, the hypothesis distribution concordance between groups was verified using the range non-parametric ANOVA Kruskal-Wallis test and Mann-Whitney *U* test. For comparative analysis of normally distributed variables

the standard ANOVA algorithm was used. Depending on the distribution of the analysed continuous variables, Pearson's or Spearman's correlation coefficients were used to estimate the associations between two variables.

RESULTS

¹³C-METHACETIN BREATH TEST RESULTS

Neither patients nor healthy controls had decreased cumulative dose elimination of ¹³CO₂ in the 120th minute of the test (% CD120 < 20.8%). Results exceeding the assumed upper normal limit of the cumulative ¹³CO₂ recovery (% CD120 > 37.3%) were found in 12 girls with AN (48%) and in six with AAN (37.5%), as well as in two controls (5%). Patients achieved higher cumulative ¹³CO₂ dose recovery than controls. In the case of girls with AN the difference was significant in measurements at all time points of the test. In the AAN group a significantly higher recovery was observed since the 30th minute of the MBT. Also DOB values were significantly higher in the study group as well as in both ANN and AN subgroups compared to controls. Comparison of the patient subgroups revealed higher elimination of the cumulative ¹³CO₂ dose in females with AN compared to those with AAN. Figures 2 to 4 present the comparison of cumulative dose values between both study subgroups and controls (ANOVA variance analysis).

In patients and controls the highest mean ¹³CO₂ elimination with the exhaled air (dose/h) was found in the 20th minute of the test and equalled 32.463 ± 6.573% in AN girls, 29.624 ± 8.842% in the AAN subgroup, and 24.61 ± 6.59% in controls. Maximal values of ¹³CO₂ recovery with the exhaled air in the 20th minute of the test or later were revealed in 80% of patients with AN and

in 87.5% girls with AAN, as well as in 95% of healthy females. Among patients with AAN as many as 37.5% reached TTP after ≥ 30th minute of the MBT, and the highest value of TTP was 50 minutes. A similar situation was observed in 12% of patients (highest TTP in this subgroup was 40 minutes).

¹³C-METHACETIN BREATH TEST RESULTS VS. ANTHROPOMETRIC PARAMETERS

Among the study group there was negative correlation between the DOB values in all time points of the test and

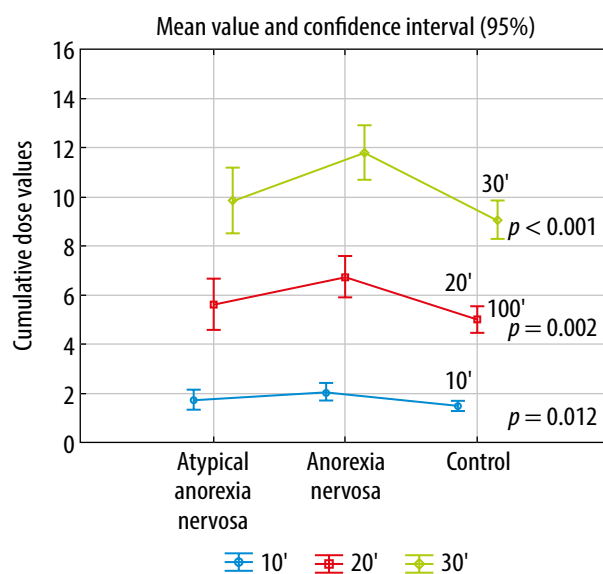


FIGURE 2. Graphic interpretation of the values of the cumulative dose in the 10th, 20th, and 30th minute of the methacetin breath test in the anorexia nervosa and atypical anorexia nervosa subgroups and controls; variance analysis

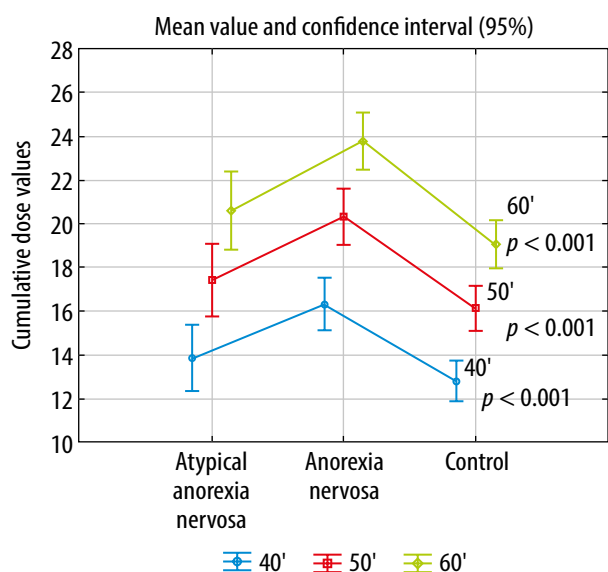


FIGURE 3. Graphic interpretation of the values of the cumulative dose in the 40th, 50th, and 60th minute of the methacetin breath test in the anorexia nervosa and atypical anorexia nervosa subgroups and controls; variance analysis

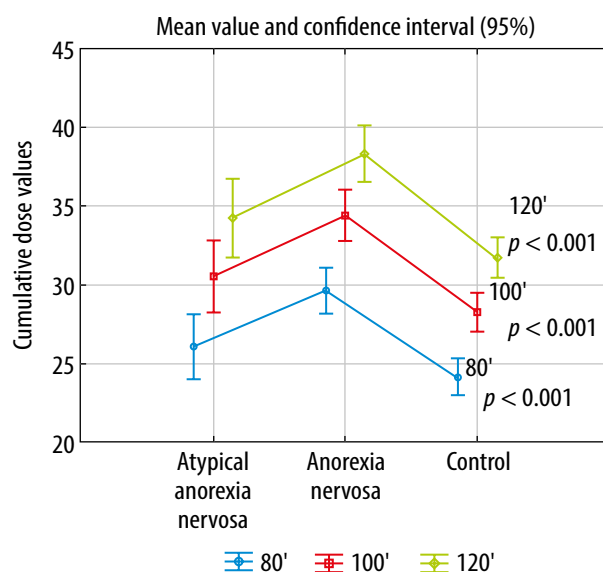


FIGURE 4. Graphic interpretation of the values of the cumulative dose in the 80th, 100th, and 120th minute of the methacetin breath test in the anorexia nervosa and atypical anorexia nervosa subgroups and controls; variance analysis

the weight, weight percentile, BMI, and BMI percentile. In the AN subgroup and controls such an association was revealed only in single time points of the test. There was no correlation between anthropometric parameters and MBT results in the AAN subgroup.

¹³C-METHACETIN BREATH TEST RESULTS AND BIOCHEMICAL PARAMETERS

Within the study group the activity of aminotransferases was not related to the cumulative dose of ¹³CO₂. Among AN patients there was a weak inverse correlation between AST activity and the methacetin cumulative dose between the 50th and 100th minute of the MBT as well as between GGTP activity and the total recovery of ¹³CO₂ starting from the 60th minute of the test. The cumulative dose from the 10th to 50th minute showed – in patients with AN – a weak relation with the INR value. Within the AAN group there was no association between biochemical parameters and the test results.

¹³C-METHACETIN BREATH TEST RESULTS VS. PARAMETERS DESCRIBING THE COURSE OF ANOREXIA NERVOSA EATING DISORDERS

In both patient subgroups (AN and AAN) the methacetin cumulative dose recovery was not associated with data from the patients' medical history concerning weight loss, rate of weight decline, or the duration of secondary amenorrhea.

DISCUSSION

To our knowledge, this is the first study to investigate the influence of the current weight status of AN patients on MBT test results. Previous observations carried out in patients with AN ED revealed a significantly faster ¹³C-methacetin metabolism in these individuals than controls [11, 12]. However, concerns were raised by the heterogeneous weight of the studied individuals, which may influence the interpretation of the obtained results. To assess the impact of the clinical type of AN ED on the MBT results we divided the study group as described in the materials section. In the subgroup of girls with AN both DOB values and the cumulative recovery of ¹³CO₂ were higher than in controls (in all time points of the test), and for the cumulative dose values also in females with AAN (beginning from the 20th minute of the test). Patients with AAN had also higher ¹³CO₂ recovery compared to controls; however, for cumulative doses statistically significant differences were noted starting from the 30th minute of the MBT. This result may be related to the clinical picture of AAN, which is different than in AN patients. AAN patients are characterised by higher BMI, milder clinical course of the disease, and faster advances in treatment [13]. According to Sawyer *et al.* patients with

AAN were often previously either obese or overweight, and they lose more weight during the course of the disease than patients with AN, but over a longer period of time [14]. This allows the suspicion that, despite severe dietary restrictions, the organisms of these patients are exposed to milder metabolic consequences of this behaviour than in the case of AN.

Both weight and BMI are significant parameters determining the course of disease of individual patients. We found a negative correlation between this parameter and MBT results in our study group. Additionally, in the AN subgroup there was an inverse relation between the cumulative dose of methacetin and the AST activity. What is worth noticing is that none of the investigated girls with AN presented signs of liver failure, and the revealed hypertransaminasaemia was mild. Rautou studied 12 patients with acute liver failure in the course of AN and showed a discrepancy between the high activity of aminotransferases as well as the organ's dysfunction and the lack of necrotic changes in the histological examination. In one third of the biopsies the cytoplasm of the hepatocytes included numerous autophagosomes [15]. During autophagocytosis the permeability of the cellular membrane increases, which may explain the increase of the serum activity of the aminotransferases [15, 16]. Their levels do not reflect the organ's functional capacity, but they provide indirect information concerning the destruction of hepatocytes. Autophagocytosis, primarily related to defensive mechanisms, protects the cell from death resulting from energy insufficiency and can lead to the cell's destruction, but only when it progresses rapidly [14]. Due to this hypothesis, below a certain BMI level an uncontrolled increase of phagocytosis and injury of hepatocytes may occur [16]. It may be argued that in the studied girls with AN the phagocytosis processes taking place still have a protective role and are not associated with the damage of the cytochromal function of the liver. On the other hand, the tendency to a decrease in the cumulative ¹³CO₂ dose along with increasing AST activity, observed in AN patients, may suggest that if massive degradation of hepatocytes occurs, it might be reflected by a rapid deterioration of methacetin metabolism.

In studies conducted in children with causes of malnutrition other than AN ED, no increase of the microsomal activity of the liver was observed. Akinyinka *et al.* revealed decreased activity of the CYP1A2 cytochrome, assessed via the serum paraxanthine: caffeine index in a group of seven Nigerian children with kwashiorkor [17].

Similarly, Oshikoya *et al.* described significant differences of caffeine metabolism in the breath test in children with different types of chronic malnutrition (marasmus, kwashiorkor, marasmic kwashiorkor) – lower values before than after realimentation [18]. The same authors, employing also a breath test with caffeine, investigated

a group of children with weight deficiency and did not reveal significant differences of the metabolic activity of the liver initially and after 2–6 weeks of realimentation [10]. As was noted by the researchers, malnutrition includes a number of disorders leading to improper nutritional status, but with different mechanisms. Depending on the pathogenesis of the disease, the systemic consequences of malnutrition, including the impact on the hepatic metabolism, may also vary [10]. In the case of AN ED the weight loss is intentional and all behaviours of the patient aim to intensify the decline of weight [7].

It should be mentioned that both diet and physical activity, typical for AN patients, may significantly modify the MBT results by interfering with the test's methodology. To assess the amount of labelled ¹³CO₂ obtained with the exhaled air it is necessary to know the total amount of CO₂ (tCO₂) exhaled by the patient. Based on this the proportion of ¹³CO₂ to ¹²CO₂ is estimated in the analysed probes, and other parameters representing the results of the test are calculated [19]. The tCO₂ value is usually fixed from above at the level of 300 mmol CO₂/m²/h. Such a methodology means that many factors influencing the MBT result are omitted. The basic metabolic activity varies depending on gender, age, and different diseases [6]. The tCO₂ concentration may change in the case of respiratory tract infections, fever, thyroid diseases, increased physical exercise, or after consumption of foods or sparkling beverages [20]. If these factors are not taken into account an improper tCO₂ can be adopted and modify the obtained MBT results.

It should also be considered that the methacetin dose used in the test was estimated using the methodology recommended by the IRIS manufacturer, and it was the same for all the participants of the study – regardless of their weight. Such a dosage (75 mg) has been used in many other original papers considering the utility of MBT in different patients. To the best of our knowledge, there are no special recommendations regarding the methacetin dose in children and adolescents or suggestions that it should be adjusted to the patient's weight. On the other hand, it should be noticed that, taking into account the mean body weight in the study group, the methacetin dose per kilogram was about 30% larger than in controls. Therefore, we cannot definitely exclude that this phenomenon may be responsible for the higher cumulative dose recovery in anorexia patients. To our knowledge, this is the first study to investigate the influence of the current weight status of AN patients on MBT test results. The study has some limitations.

A larger recruited sample with a more even spread of illness severity is needed. Most of the girls in the study group had normal activity of transaminases, and there were no cases of acute liver insufficiency among our patients. Consequently, it was impossible to show if there were any changes in MBT results in hypertransaminasemia patients, and the trend of these variations.

CONCLUSIONS

Girls with weight deficiency in the course of AN achieved higher cumulative ¹³CO₂ dose recovery during the MBT than healthy controls and other AN patients. The recovery of ¹³CO₂ during the MBT was negatively correlated with parameters describing the patient's weight status. The obtained results suggest that the ¹³C-methacetin metabolism in girls with AN ED was significantly faster – potentially as a consequence of the characteristics of the primary disease. Further research is needed, to estimate the utility of the test in patients with AN ED and liver insufficiency.

DISCLOSURE

The authors declare no conflict of interest.

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