

**"VICTOR BABEȘ" UNIVERSITY OF MEDICINE AND PHARMACY
FROM TIMISOARA**

FACULTY OF MEDICINE

DEPARTMENT OF MICROSCOPIC MORPHOLOGY

LEP IULIA TEODORA



DOCTORAL THESIS

**EVALUATION OF THE FOOD PROFILE
AND THE IMPACT OF THE NUTRITIONAL INTERVENTION
ON PATIENTS WITH METABOLIC SYNDROME**

- SUMMARY -

PhD supervisor
PROF. UNIV. DR. PUIU MARIA

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is an emerging public health problem, closely related to the metabolic syndrome. This condition is characterized by the presence of risk factors such as insulin resistance, obesity, dyslipidemia and hypertension, which significantly contribute to the initiation and progression of the disease. The alarming increase in the prevalence of MASLD is of particular concern given its potential to progress to severe forms of liver pathology, including non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma. Thus, appropriate management of MASLD is crucial to reduce associated morbidity and mortality.

This research paper analyzes the impact of nutritional interventions on MASLD in patients with metabolic syndrome, highlighting the essential role of specific nutrients, such as betaine, choline, 5-methyltetrahydrofolate (5-MTHF), vitamin B12 and omega-3 fatty acids (α -linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) in preventing disease progression. In addition, non-invasive diagnostic methods such as Fibroscan technologies, which include Controlled Attenuation Parameter (CAP) and Transient Elastography (TE), are evaluated as crucial tools for monitoring liver health.

The main objective of this thesis is to explore the relationship between nutritional intake and metabolic syndrome, as well as to evaluate the effectiveness of nutritional interventions on liver health. To this end, three interconnected studies are presented. The first study investigates the eating habits and nutritional intake of people with metabolic syndrome, identifying possible deficiencies that could contribute to the worsening of the health condition. The second study validates the dietary assessment method used, ensuring the accuracy of the data collected. In the third study, the effects of a cocktail of micronutrients - betaine, choline, 5-MTHF, vitamin B12, alpha-lipoic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - on steatosis and liver fibrosis, non-invasive techniques to measure the effectiveness of proposed nutritional interventions.

By integrating these studies, we aim to deepen our knowledge of the dietary influences of metabolic syndrome and develop innovative nutritional strategies aimed at improving liver health and preventing the progression of MASLD in affected patients. The obtained results can fundamentally provide effective clinical recommendations, having the potential to improve the quality of life of patients and reduce the economic impact on the health system.

PERSONAL CONTRIBUTIONS

STUDY 1: Assessment of Nutritional Intakes in Individuals with Obesity under Medical Supervision. A Cross-Sectional Study

Objective

The aim of the study was to assess whether the dietary intake of adults with obesity who were receiving treatment for related health conditions in Romania was adequate in terms of macronutrients and micronutrients. The primary assumption was that without comprehensive nutritional assessments, structured dietary recommendations, and follow-up monitoring, patients are unlikely to achieve the recommended dietary allowances (RDAs) as defined by the United States Department of Agriculture (USDA) and the dietary reference values (DRVs) set by the European Food Safety Authority (EFSA).

Methodology

The study sample consisted of 421 adults with obesity, comprising 204 men and 217 women. All participants received medical supervision for obesity or its associated comorbidities. The study was conducted in Timișoara, Romania, as part of a larger research program. The study was conducted in accordance with the ethical standards set forth by the Scientific Research Ethics Committee Board of the Victor Babes University of Medicine and Pharmacy, which approved the study protocol. All participants provided informed consent before participating in the study. To ensure the exclusion of individuals who abuse alcohol, participants completed the Alcohol Use Disorders Identification Test (AUDIT-C). The dietary intake of participants was assessed through the administration of a 24-hour dietary recall questionnaire, completed on up to four occasions over a period of non-consecutive days, including weekends and weekdays, in order to account for potential variations in dietary habits. The collected data were subsequently analyzed employing the IBM-SPSS software. Nutrient intakes were compared against the RDAs and the DRVs

Results

The study's results indicated that the vast majority of participants did not achieve the recommended daily intakes for several micronutrients, despite receiving guidance from a qualified medical professional. Inadequacies in intake levels were observed for a number of nutrients, according to both the USDA and EFSA criteria. Notably low intake levels were recorded for vitamin D, fluoride, choline, vitamin E, potassium, linoleic acid and alpha-linolenic acid. Furthermore, it is notable that the study identified substantial discrepancies in nutrient intake adequacy between genders. The results indicated that males consumed higher amounts of most nutrients in comparison to females.

An analysis of macronutrient intake indicated a significant discrepancy in the proportion of energy derived from fats and carbohydrates. The data indicated that individuals with higher energy intakes exhibited increased fat consumption and decreased carbohydrate intake, a pattern deviating from the recommended dietary guidelines. Additionally, the study identified concerns regarding fatty acid intake. Specifically, there was a low consumption of polyunsaturated fatty acids (PUFAs) and a high intake of saturated fatty acids (SFAs). These findings are consistent with broader dietary trends observed in European and global populations.

Discussion

The findings of this study elucidate the complex nutritional difficulties confronted by individuals with obesity in Romania, notably within the context of inadequate dietary counsel. Observed deficiencies in micronutrients and imbalanced macronutrient intakes have the potential to exacerbate the health risks associated with obesity. This could result in further complications, such as cardiovascular disease and metabolic disorders. The lack of structured dietary advice and follow-up by qualified dietitians appears to be a significant contributing factor to these nutritional deficiencies, emphasizing the necessity for more comprehensive dietary interventions within the healthcare system.

Furthermore, the study's findings align with prior research indicating that individuals with obesity frequently exhibit deficiencies in micronutrients, which may not be sufficiently addressed, even with the guidance of medical professionals. This indicates that the dominant strategy for addressing obesity in Romania, with a primary focus on macronutrient intake, may prove insufficient for meeting the broader nutritional demands of patients.

To summarize, the findings of this study offer vital insights into the nutritional status of Romanian adults with obesity, revealing a prevalent deficiency of micronutrients and an imbalance in macronutrient consumption. These observations underscore the urgent need for a more comprehensive and integrated approach to obesity management that incorporates structured dietary guidance and long-term supervision by duly trained dietitians. Such an approach could help mitigate the associated health risks and contribute to a more effective reduction of the obesity burden in Romania.

STUDY 2: Intake Differences between Subsequent 24-h Dietary Recalls Create Significant Reporting Bias in Adults with Obesity

Materials and Methods

Subject Recruitment

A total of 197 men and 212 women were selected from the original cohort for inclusion in the study. In order to be included in the study, participants had to meet the following criteria: they had to be aged between 18 and 70 years, have a body mass index (BMI) of at least 30 kg/m², have an abdominal circumference of at least 84 cm for women and 90 cm for men, and have dyslipidemia. Individuals with a history of cancer, autoimmune diseases, psychiatric disorders, blood coagulation disorders, or substance abuse were excluded from participation. All participants were under medical surveillance and treatment for their conditions at the time of recruitment, which occurred between September 2016 and December 2018.

Dietary Intake Assessment

Dietary intake was assessed by trained personnel using a standardised 5-pass 24-hour recall method. This method required participants to recall their food and drink intake from the previous day, including details on the type, composition, quantity, and time of consumption. The initial 24-hour recall was conducted in person during the baseline visit, while subsequent recalls were conducted via telephone at various intervals without prior notice. A total of 1,587 24-hour dietary recalls were collected, encompassing up to four days of food intake assessment per participant. The median recall timeframe was 62 days. Nutrient intake data

were derived from the Nutritioapp, a web-based application utilising data from the USDA Food and Nutrient Database, in conjunction with European and Romanian databases. Throughout the recall period, subjects adhered to an ad libitum diet.

Anthropometry, Biochemistry and Diagnosis of Chronic Diseases

At the outset of the study, participants' weight, height, and abdominal circumference were measured in accordance with international standards.

Following an overnight fast of a minimum of eight hours, blood samples were collected and biochemical assessments, including total cholesterol and triglycerides, were conducted. The diagnosis of chronic diseases was previously established by specialist physicians and documented in the participants' medical records.

Data Analysis

The statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 25.0. The normality of the variables was assessed using the Kolmogorov-Smirnov test. The variables were expressed as either means with standard deviations or as medians with interquartile ranges. Comparisons of two-level factors were conducted using the Mann-Whitney test, while proportions of participants with chronic diseases and proportions of weekend/weekday days in each 24-hour recall set were compared using chi-square tests. The Bonferroni method was employed to adjust for statistical significance. Three distinct models were utilized to analyze repeated designs for energy, macronutrient, and micronutrient intakes. The first model employed four ordered 24-hour recalls. The second model compared weekend versus mean weekday intakes. The third model compared the mean of four 24-hour recalls with the mean of three 24-hour recalls, with one of the ordered recalls excluded.

In order to adjust for the multiple comparisons between the four ordered recalls, the Sidak method was employed. All results were further adjusted for the false discovery rate (FDR) using an online tool, with adjusted p-values less than 0.05 considered statistically significant. The effect size was interpreted using the criteria established by Cohen, whereby an *r* value of 0.10 to 0.30 indicated a small effect, 0.30 to 0.50 a medium effect, and greater than 0.50 a large effect.

Results

The study involved 388 adults with obesity who completed four 24-hour dietary recalls. The results are presented below.

The median age of the cohort was 56.20 years, with a median BMI of 35.2 kg/m². The analysis of the order of the 24-hour recalls and nutrient intakes revealed significant discrepancies in the reported energy and nutrient intakes across the four recalls. Notably, these differences were particularly pronounced for energy, carbohydrates, vitamin C, calcium, fibre, folates, potassium, and total sugar.

Subsequent analysis was conducted to investigate the potential influence of weekend versus weekday dietary intake. Although the fourth recall included a greater proportion of weekend days, no significant differences were observed in nutrient intake between weekend and weekday recalls. Furthermore, models that excluded the first, second, third, or fourth recalls from the analysis demonstrated that the exclusion of specific recalls could significantly alter the reported mean intake of various nutrients. These findings emphasise the importance of considering the order of recalls and the potential bias introduced by weekend days in dietary assessment studies.

Discussion

This study represents a significant contribution to the existing literature on the consistency of reported energy, macro-, and micronutrient intakes across multiple 24-hour recalls in adults with obesity.

The findings indicate that both under- and over-reporting are prevalent in this population, with underreporting being more common. The random errors associated with day-to-day variability in dietary intake can be reduced by averaging multiple 24-hour recalls. However, when systematic errors, which consistently shift measurements in one direction, are present, it is essential to consider known confounders, such as the day of the week, gender, and BMI status, with great care.

Furthermore, the study identified a reduction in reported intakes in subsequent recalls, which may reflect a training bias or reporting fatigue. Furthermore, the discrepancies between the initial face-to-face recall and subsequent telephone recalls prompt inquiries into the influence of data collection mode on reported intakes. These findings highlight the necessity for further research to investigate the impact of recall order, weekend versus weekday recalls, and the mode of data collection on the accuracy and consistency of dietary intake assessments in individuals with obesity.

In conclusion, the results of this study offer valuable insights into some of the challenges and considerations involved in using multiple 24-hour dietary recalls to assess nutrient intake in adults with obesity. The findings suggest that there may be systematic biases depending on the order in which the recalls are taken, which could significantly impact the accuracy of the nutrient intake estimates made. Further research is required in order to develop strategies to mitigate these biases and to improve the reliability of dietary assessment methods in this population.

STUDY 3 : Use of a micronutrient cocktail to improve metabolic dysfunction-associated steatotic liver disease (MASLD) in adults with obesity. A randomized, double-blinded pilot clinical trial

Aims

The primary objective of the present study is to evaluate the potential impact of micronutrient supplementation on metabolic syndrome in obese individuals. To this end, anthropometric measurements, lipid profiling, and FibroScan technology have been employed to assess controlled attenuation parameter (CAP) and transient elastography (TE) values. Our working hypothesis is that a defined combination of micronutrients, administered daily, may result in favourable modifications to CAP and TE parameters associated with metabolic syndrome in obese subjects.

Materials and Methods

This study employed data from the NutriGen project, a double-blind, randomised, pilot interventional trial conducted at the Victor Babes University of Medicine and Pharmacy in Timișoara, Romania, between 2016 and 2020. The NutriGen project was designed to test the hypothesis that a specific nutrient cocktail could improve parameters related to liver fibrosis in adults with metabolic syndrome and liver disease (MASLD) who are obese. Furthermore, the

project sought to establish a genetic signature model involved in methyl group donation and omega-6/3 unsaturated fatty acid metabolism, with high predictive value for classifying dyslipidaemia and insulin resistance in obese subjects. The study was conducted in accordance with the ethical standards of the Victor Babes University of Medicine and Pharmacy Ethics Committee.

The initial cohort comprised 400 adults with obesity and dyslipidaemia, who were subjected to a comprehensive biological evaluation. In order to be eligible to participate in the study, individuals were required to meet specific inclusion criteria, including an age range of 18 to 70 years, a body mass index (BMI) of 30 kg/m² or higher, and an abdominal circumference of 84 cm or more for women and 90 cm or more for men. Dyslipidaemia was confirmed by serum cholesterol levels of 200 mg/dL or higher, HDL cholesterol levels of 50 mg/dL or lower for women and 40 mg/dL or lower for men, serum triglycerides of 150 mg/dL or higher, or the use of antidyslipidaemic treatments. Individuals with a history of cancer, autoimmune diseases, psychiatric disorders, blood coagulation disorders, or a history of drug or alcohol abuse were excluded from participation.

The second phase of the study concentrated on nutritional intervention and comprised 240 subjects selected from the initial cohort, comprising 120 women and 120 men. The participants were randomly assigned to either the control group, which received a placebo, or the treatment group, which received the micronutrient cocktail. Due to a number of participants dropping out of the study, others were selected from the initial group by randomisation. The study ultimately included 196 participants who commenced the treatment phase, of whom 155 completed the study and were deemed to be compliant. At the outset of the intervention and following a three-month supplementation period, participants underwent anthropometric evaluations, provided blood samples, and received FibroScan evaluations.

The treatment group was administered a daily regimen of specific micronutrients, including 800 mcg 5-MTHF, 2 g betaine, 1 g alpha-linolenic acid (ALA), 700 mg eicosapentaenoic acid (EPA), 500 mg choline bitartrate, 280 mg docosahexaenoic acid (DHA), and 1000 mcg Vitamin B12. The placebo group received a placebo comprising a tablet containing low-GI (starch-based) ingredients and a capsule containing 1 g of corn oil. Following the three-month observation period, a reassessment of the participants was conducted. A total of 155 subjects were evaluated and provided with vouchers for further FibroScan assessments.

Transient elastography (TE) and controlled attenuation parameter (CAP) measurements were conducted on all participants who had redeemed their vouchers using a FibroScan® Compact 530 device. The reliability of the results was determined by calculating the interquartile range (IQR) to the median ratio (IQR/M), which was found to be less than 30% based on the median value of 10 valid measurements. Liver stiffness was quantified in kilopascals (kPa), and steatosis was quantified in decibels per meter (dB/m). Participants were classified as normal if the transient elastography (TE) measurements were below 6 kPa and the controlled attenuation parameter (CAP) values were below 248 dB/m. Otherwise, participants with CAP values above 248 dB/m were classified as having steatotic liver disease.

Results

The results of the study indicated that 155 of the 196 adults who participated in the baseline evaluation and received the initial supplementation completed the study. The participants were subjected to a three-month intervention period, during which they were provided with either the micronutrient cocktail or a placebo. Compliance was monitored by counting the number of unused capsules at the conclusion of each month. Following a three-month period, participants underwent a second assessment comprising anthropometric measurements, blood sampling, and FibroScan evaluations.

The analysis of the FibroScan data indicated that the treatment group, which received the micronutrient cocktail, exhibited notable improvements in both CAP and TE measurements in comparison to the placebo group. In particular, the treatment group exhibited a reduction in liver stiffness and steatosis levels, as evidenced by lower CAP and TE values. These findings indicate that micronutrient supplementation has a beneficial impact on liver health in obese adults with MASLD. Furthermore, lipid profiling revealed improvements in serum cholesterol and triglyceride levels among the treatment group. This reduction in dyslipidemia markers provides additional evidence of the potential benefits of the micronutrient cocktail in addressing the metabolic dysfunctions associated with MASLD. The observed improvements in liver health and lipid profiles in the treatment group emphasise the importance of targeted nutritional interventions in managing MASLD in obese individuals.

Discussions

The results of this study highlight the potential efficacy of targeted micronutrient supplementation in improving liver health in obese adults with MASLD. The considerable reductions in CAP and TE values observed in the treatment group indicate that the particular combination of micronutrients, including 5-MTHF, betaine, ALA, EPA, choline, DHA, and Vitamin B12, may play a pivotal role in modulating the metabolic pathways associated with liver steatosis and fibrosis.

The favourable alteration in lipid profiles provides further evidence to support the hypothesis that targeted nutritional interventions can address the underlying metabolic dysfunctions contributing to MASLD. These findings are consistent with previous research indicating that specific nutrients, such as choline and omega-3 fatty acids, are crucial for maintaining liver health and reducing the risk of metabolic disorders.

The utilisation of FibroScan technology in this study provided a reliable and non-invasive methodology for the assessment of liver health, facilitating the early detection of steatosis and fibrosis. The findings suggest that targeted micronutrient supplementation could offer a promising therapeutic approach for managing MASLD in obese individuals, particularly those struggling with long-term lifestyle changes. However, the study also highlights the need for further research to validate these findings and explore the long-term effects of micronutrient supplementation on liver health. Additionally, future studies should consider larger sample sizes and longer follow-up periods to assess the sustainability of the observed improvements.

LIMITATIONS OF THE DOCTORAL THESIS

This PhD thesis makes significant contributions to the understanding of MASLD, but has notable limitations.

In the first study, self-reported dietary intake data are at risk of underreporting, a common problem in studies based on 24-hour dietary recalls, and being a cross-sectional study, causal relationships between dietary intakes and current diagnoses cannot be established. Estimates of nutrient intake are influenced by the variability of foods and the accuracy of the databases used, and the lack of biomarkers hinders methodological validation. The data are limited to people who tried to lose weight without professional advice, restricting inferences for other conditions.

In the second study, the lack of direct measurements and biomarkers prevented the identification of the causes of the observed differences, and the absence of a control sample limits the assessment of the specificity of the differences for people with obesity and comorbidities.

In the third study, the small number of participants due to low compliance and voluntary withdrawals limited the full analysis of parameters and suggests the need for further research. The statistical significance of the results should be interpreted with caution, and assessment of the false discovery rate (FDR) was performed to minimize the risk of reporting false results in the context of limited stratification due to small sample size.

CONCLUSIONS AND PERSONAL CONTRIBUTION

The studies contained in this PhD thesis spanning six years of research have provided crucial insights into the management of MAFLD and related issues:

1. This research represents a first detailed assessment of the intake of micronutrients and fatty acids in a Romanian population affected by obesity, highlighting significant nutritional deficiencies even under medical supervision. The absence of specific dietary advice and professional guidance from qualified dietitians has led to imbalances in nutrient intake, emphasizing the need to integrate long-term dietary counseling into the care of people with obesity. The study emphasizes the importance of personalized nutritional strategies to ensure a balanced intake of micronutrients and macronutrients, adapted to individual needs. Furthermore, future research should focus on the long-term consequences of these dietary patterns and the development of targeted interventions that improve the nutritional status and overall health of this vulnerable population.

2. The second study identified notable differences in energy and nutrient intake between the first 24-hour face-to-face recording and subsequent sessions, suggesting possible bias associated with the data collection method. This bias can affect the accuracy of dietary assessments and the reliability of the results. To ensure the validity of data, it is essential to identify and correct potential biases associated with different collection methods. Future research should focus on standardizing these methods or adjusting for session-specific effects, thus ensuring a more accurate nutritional assessment.

3. The third study offers preliminary evidence in support of the potential benefits of targeted micronutrient supplementation for the improvement of liver health in obese adults with MASLD. The observed enhancements in CAP and TE values, along with those in the lipid profile, suggest that such interventions may play a crucial role in the management of this condition. However, further research is required to confirm these findings and to explore the broader implications of micronutrient supplementation in the context of MASLD and other metabolic disorders.

In conclusion, this PhD thesis makes an important contribution to the advancement of the field by analyzing some essential aspects of MASLD management. The obtained results provide the basis for the development of personalized care strategies and for the optimization of clinical outcomes in patients affected by this pathology.

FUTURE RESEARCH ENDEAVOURS

The following research efforts may facilitate further advancement in the understanding and treatment of MASLD, based on the findings and discussions presented in the thesis:

1. Long-term outcomes of dietary interventions:

The objective is to evaluate the long-term effects and safety of a micronutrient cocktail (including betaine, choline, 5-MTHF, vitamin B12, ALA, EPA, and DHA) on liver health in patients with MASLD. The investigation will focus on the sustained improvement of liver steatosis and fibrosis over longer time periods, such as 1-2 years.

2. The development and validation of biomarkers is a crucial area of research.

The development and validation of biomarkers that can accurately assess micronutrient deficiencies and metabolic dysfunction in MASLD patients is a crucial area of research. This could provide more objective measures of nutritional status and circumvent the constraints of self-reported dietary data.

3. Personalized Nutritional Interventions

It would be beneficial to investigate the potential of personalized nutritional approaches, which could be tailored to specific genetic and metabolic profiles. Further research could concentrate on the manner in which disparate genotypic or metabolic traits respond to particular nutrients or dietary habits, with a view to developing more efficacious personalized treatment plans.

4. Dietary Interventions in Diverse Populations:

To ascertain whether the benefits observed in Romanian adults with obesity and MASLD are generalizable to other groups, it is recommended that research be expanded to assess the effectiveness of nutritional interventions in diverse ethnic or socioeconomic populations.