English version

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Protocol

Title: Evaluation of the correlation between diet quality, weight status and mental health in children, with emphasis on the identification of plasma and urinary biomarkers.
Study phase: Initial Project
Type of study: Experimental, descriptive, study
Study Classification: According to the Regulations of the General Health Law on Health Research, Article No. 17 .Research with minimal risk.
Main objective: To investigate the correlation between diet quality, mental health status and weight status in Mexican children aged 5 to 11 years, identifying plasma and urinary biomarkers associated with diet quality that are related to mental health status and weight.
Secondary objectives:
● Determine the weight status of pediatric participants.
● To assess the mental health status of pediatric participants.
● To evaluate diet quality in pediatric participants.
● Identify plasma and urinary biomarkers associated with diet quality.
● Analyze the possible correlations between the biomarkers associated with diet quality, mental health status and weight status, in the study population.
● Analyze the confounding variables and evaluate their impact on the relationships between diet quality, mental health status and weight status of the participants.
Null Hypothesis :
There is no positive correlation between plasma and urinary biomarkers associated with diet quality, mental health status and weight status in Mexican children aged 5 to 11 years, once the identified confounding variables are controlled.
Alternate hypothesis:
There is a positive correlation between plasma and urinary biomarkers associated with diet quality, mental health status, and weight status in Mexican children aged 5 to 11 years, even after controlling for identified confounding variables.
Research Questions:
● ¿What are the biomarkers associated with diet quality in pediatric school patients?
● ¿Is there a correlation between biomarkers associated with diet and obesity, and mental health in pediatric school patients?
● ¿Can potential diet-associated biomarkers be used for early diagnosis of metabolic diseases in the pediatric population?
Population: Pediatric patients ages 5 to 11 years of age will be recruited from the Monterrey Football League (MFL), including those with obesity, those without obesity, and those at risk of developing metabolic disease. The following evaluations will be carried out:

● Anthropometric evaluation using an Inbody scale to measure weight, height, BMI, percentages and total kilos of fat, muscle, bone and water.
● Mental health evaluation through surveys carried out by psychiatry residents to assess anxiety and depression.
● Determination of diet quality using a 24-h dietary intake report.
● Analysis of blood and urine samples to measure the biochemical profile, thyroid, HOMA index, lipid profile, among others.
● Determination of plasma and urinary metabolites by untargeted metabolomics and targeted metabolomics of myo-inositol and hippurate.
● Evaluation of clinical history and potential variables of socioeconomic status, physical activity, family history of obesity and metabolic disorders, genetic factors, parental educational level, adverse childhood experiences, and prenatal health and breastfeeding history of the mother.
“N”: The N will be 120 patients divided into 2 groups of 60 patients each:
● Pediatric patients with Obesity, ● Pediatric patients without Obesity
Approximate duration of the study: 12 months

1.0 INDEX

INTRODUCTION

Mental illnesses are considered a global health problem. The COVID-19 Pandemic significantly impacted the mental health of the general population, which is why the incidence of diseases such as anxiety and depression have increased even in pediatric patients. The World Health Organization (WHO) reports that 1 in 7 between 10 and 19 years of age suffers from some alteration in cognition, emotion regulation or behavior. In Mexico, according to the INEGI, in 2021 there were 8,351 suicides, with a rate of 2.1 per 100,000 inhabitants in ages 10 to 14. While ENSANUT 2022, recorded that 7.6% of adolescents had suicidal thoughts; of these, 6.5% attempted suicide. In children under 10 years of age the evidence is scarce. In the United States, a 20% rate of mental health problems is reported among children aged 3 to 10 years.

Childhood is a stage of growth vulnerable to the development of mental illness. In children, the main mental health problems reported are mood disorders, attention deficit hyperactivity disorder, conduct disorders and anxiety disorders. It has been reported that stressful situations in this early stage of life can predispose to the development of mental illnesses in childhood and adolescence. However, mental health problems in childhood are not widely recognized, which is why there is little professional support and few approved pharmacological therapies.

Mental health and weight status are strongly related. In Mexico, a systematic review recently reported evidence of the association between a greater number of depressive symptoms and the state of overweight and obesity in children and adolescents (8-18 years). Overweight and obesity are health problems of increasing importance in Mexican children and adolescents. It is estimated that in Mexico 7.7% of preschool children (1-5 years) are overweight or obese. While at school age (6-10 years) it rises to 37.3%. A figure that remains high in adolescence and adulthood. These figures indicate that the transition between preschool age and school age is a key period for preventing the appearance of these diseases in later stages of life. It has been shown that the quality of the diet considerably influences both changes in body weight and mental health, where a poor quality diet can induce metabolic alterations that appear even before the development of overweight and obesity. Therefore, the identification of a poor quality diet in pediatric age is relevant to prevent the development of these pathologies. However, there are currently no clinical tools available to objectively identify the level of dietary quality of pediatric patients and its relationship with the risk of developing overweight, obesity, and mental illnesses. Therefore, studying the characteristics of children's nutrition and detecting deficiencies, as well as their relationship with mental health status, is a potential area for improving the quality of life in our population.

BACKGROUND

Diet quality and biomarkers

Both mental health and body weight are conditions significantly affected by the quality of the diet. Although the mechanisms of their relationship are not clearly known, observational studies in adults, adolescents and pre-adolescents have found a consistent relationship between the quality of diet and the state of mental health, regardless of the socioeconomic level and level of education. In this sense, diets based on the majority consumption of fruits, vegetables, grains, fish and foods rich in antioxidants are associated with better mental health and lower risk of developing overweight and obesity. While diets high in fats, sugars, refined grains and red meat are associated with a higher risk of obesity and mental health problems.

Differences in diet quality can lead to changes in the concentrations of various metabolites in blood and urine, which is why the identification of these metabolites has emerged in recent years as a promising strategy for the search for biomarkers that indicate the quality of diet and the relationship with various metabolic diseases.Untargeted metabolomics has emerged as an important tool in this field, allowing the identification and quantification of various metabolites present in biological samples without the need to predefine the compounds of interest. Various studies have addressed the effects of diet quality and its relationship with a certain metabolic profile using untargeted metabolomics, in which it has been determined that diet quality considerably influences the plasma and urinary metabolic profile. In this sense, specific metabolites associated with the consumption of fruits and vegetables, as well as with the consumption of processed foods rich in saturated fats, have been identified. The consumption of fruits and vegetables is mainly associated with the identification of higher concentrations of polyphenols such as flavonoids, tannins, lignans and phenolic acids, as well as carotenoids such as β-carotene, lutein, zeaxanthin and lycopene, compared to diets rich in saturated fats. For this reason, the identification of this type of compounds is mainly associated with the Mediterranean diet, which is characterized by a greater consumption of fruits and vegetables. However, despite the information available to date, it has not been possible to establish with certainty biomarkers associated with diet that have a clinical impact and that are validated. On the other hand, most studies focus on addressing diet quality and eating patterns in adults, while studies in children are scarce. Differences in diet-associated biomarkers between the pediatric and adult populations may reflect differences in metabolic development, eating habits, and nutritional needs specific to each age group. While children experience rapid growth and development, which can influence metabolism and nutrient absorption, adults have more established eating patterns and different nutritional demands. Therefore, understanding these differences is crucial to accurately evaluate the quality of the diet and its impact on health in the pediatric stage. In this study we will focus on evaluating metabolites not predefined by untargeted metabolomics in the pediatric population and the direct evaluation of the metabolites hippurate and myo-inositol, which according to multiple studies could have a relevant role as biomarkers associated with diet and its relationship with diseases such as obesity and mental health disorders, but which have not been widely addressed in the pediatric population.

Hippuratus, mental health and obesity

Recently, the exploration of hippurate in urine, a metabolite resulting from the metabolism of phenolic compounds, has gained relevance. It is formed through the conjugation of benzoic acid with glycine in the liver and is excreted in the urine. In various studies, the presence and concentration of hippurate in urine have been associated with diet quality. In the context of obesity, studies have shown associations between the intake of certain food groups and urinary hippurate levels. It has been observed that children who consume a greater amount of fruits and vegetables tend to have higher levels of hippurate in urine, suggesting that hippurate may be a sensitive biomarker of intake of these foods in this population. Conversely, a diet high in processed foods and low in fruits and vegetables may be associated with lower levels of hippurate and a higher risk of obesity in children. In a group of European children aged 6 to 11 years, through a urine metabolomics study, it was found that the metabolites hippurate, n-methyl-nicotinic acid, urea and sucrose allowed discrimination between the Mediterranean diet and the diet rich in super-processed foods. While in a study in adolescents it was found that hippurate levels in 24-h urine after adjustment with albumin resulted in a positive correlation with the intake of fruits and vegetables, while In children, a better correlation was found between the intake of fruit and vegetable juices and the concentration of hippurate in 24-h urine.

Regarding mental health, in the adult population an important association has been found between low levels of hippurate and depression. It has been shown that hippurate levels change with age. Nevertheless, the associations found in hippurate in urine and mental health in adults, may not reflect what happens in children. In pediatric patients, low levels of hippurate in urine have been found in autism compared to controls. However, more studies and larger samples are required to confirm the role of hippurate as a biomarker of this condition. Therefore, the study of hippurate levels in children offers a promising perspective to better understand the relationship between diet, obesity and mental health, which could lead to more effective preventive and therapeutic interventions in this vulnerable population.

Myo-inositol, mental health and obesity

Myo-inositol is a compound that in recent years has gained attention as a dietary supplement, since it is considered that its levels may be modified in certain diseases, which is why multiple clinical trials have addressed its use mainly in adults. Myo-inositol is widely distributed in nature and is present in a variety of common foods, including fruits, vegetables, nuts, grains, and dairy products. Some rich sources of myo-inositol include citrus fruits, stone fruits, berries, legumes, nuts, seeds, and whole grains. Additionally, it can also be synthesized endogenously in the body from glucose. Myo-inositol is synthesized from glucose-6-phosphate and its conversion to D-glucuronate, catalyzed by the enzyme glucuronate reductase. Through a series of reactions, D-glucuronic acid-1,4-lactone is obtained, which is subsequently converted into myo-inositol-1-phosphate by the enzyme inositol monophosphate synthase. Finally, myo-inositol-1-phosphate is hydrolyzed to produce free myo-inositol, which can be used for different cellular needs as a second messenger and form the structure of the plasma membrane. Endogenous synthesis of myo-inositol occurs especially in tissues such as the brain, kidneys and liver.

The combination of endogenous synthesis and dietary intake contributes to maintaining adequate levels of myo-inositol in the human body to support normal physiological

functions. Low concentrations of myo-inositol have been related to various pathologies, especially those associated with metabolic and nervous system disorders. In this context, it has been observed that low levels of myo-inositol are associated with insulin resistance and diabetes mellitus type 2, where a decrease in intracellular myo-inositol levels and its excessive urinary excretion have been observed. Additionally, myo-inositol levels have been associated with the disease of polycystic ovary syndrome (PCOS) and resistance to insulin in such patients. PCOS is a common endocrine disorder in women of reproductive age that is characterized by hormonal imbalances, anovulation, and clinical features such as hirsutism and menstrual irregularity. Recently, the efficacy of PCOS treatment with myo-inositol supplementation has been demonstrated. Importantly, some clinical studies suggest an association between low myo-inositol levels and mood disorders, such as depression and bipolar disorder. Therefore, it has been proposed that alterations in myo-inositol metabolism and cellular signaling could play a role in the pathophysiology of these mental disorders. However, there is controversy in the results of clinical protocols where myo-inositol supplementation is addressed.

Regarding fluctuations in myo-inositol concentration in pediatric patients with obesity or mental health disorders, information is scarce. A study in obese children conducted in Japan found that urinary myo-inositol (UMI) levels may be a potential marker to detect glucose intolerance in early stages. Children with obesity or glycosuria showed elevated levels of UMI in comparison to those with normal values. In adolescents, it has been found that there is an important correlation between exposure to air pollutants from car traffic, anxiety and brain levels of myo-inositol. This research suggests that changes in myo-inositol levels could be related to mental health in childhood. Regarding alterations in mental health, clinical studies have shown associations between myo-inositol levels and mood disorders, such as bipolar disorder. Bipolar patients have been observed to have altered levels of myo-inositol in certain brain regions, suggesting a possible role in the pathophysiology of these disorders. However, more research is needed to fully understand these relationships and their clinical relevance in pediatric patients.

JUSTIFICATION

Childhood obesity is a public health problem of growing concern in Mexico and around the world. The high incidence of obesity in the pediatric population has led to an increase in cases of associated metabolic diseases, such as insulin resistance, type 2 diabetes, dyslipidemia and high blood pressure. It is crucial to identify patients at risk for developing metabolic disease before clinical obesity manifests, as once obesity is diagnosed, management of these conditions becomes considerably more difficult and costly.

Early diagnosis and preventive intervention are essential to address this problem effectively. However, identifying patients at risk for metabolic disease before the development of clinical obesity is challenging. That is why this study focuses on identifying early biomarkers associated with metabolic disease in children aged 5 to 11 years.

Furthermore, it is important to highlight that the quality of the diet plays a crucial role in the metabolic and mental health of children. There are studies that explore biomarkers related to the intake of fruits and vegetables, but few investigate biomarkers indicative of diet quality associated with mental health status in the pediatric population. This research seeks to address this gap in knowledge, providing valuable information on how eating

habits can influence children's mental health and their risk of developing metabolic disease.

In summary, this study aims to contribute to the early identification of patients at risk of metabolic disease and to the understanding of the relationship between diet quality, mental health and the risk of metabolic disease in the pediatric population. The results of this research could have important implications for the development of more effective prevention and management strategies for obesity and metabolic diseases in children.

RESEARCH QUESTIONS

¿What are the biomarkers associated with diet quality in pediatric school patients?

¿Is there a correlation between biomarkers associated with diet and obesity, and mental health in pediatric school patients?

¿Can potential diet-associated biomarkers be used for early diagnosis of metabolic diseases in the pediatric population?

Main objective

The fundamental objective of this study is to investigate the correlation between diet quality, mental health status and weight status in Mexican children from 5 to 11 years of age.

The purpose of which is based on the identification of plasma and urinary biomarkers associated with diet quality that are related to mental health status and weight status in the study group. With the aim of improving the understanding of the factors that contribute to metabolic and mental health in children, and guiding the development of effective interventions to promote their general well-being.

Secondary Objectives

Objective No. 1. Determine the weight status of pediatric participants:

Calculate the percentile of the body mass index (BMI) of each participant according to the record of weight and height, sex and age.

Classify participants according to BMI percentile into patients without obesity and patients with obesity, according to the criteria established by the WHO.

Objective No. 2. Assess the mental health status of pediatric participants:

Apply standardized questionnaires (CDI and SCARED-R) to assess mental health status and emotional well-being in pediatric participants.

Analyze the data to stratify the population into participants with optimal mental health and suboptimal mental health.

Objective No. 3. Evaluate the quality of the diet in pediatric participants:

Apply to the participants a dietary evaluation questionnaire adapted to the Mexican child population (24-hour dietary intake report).

Analyze the data obtained to stratify the population into participants with an optimal diet and participants with a suboptimal diet.

Objective No. 4. Identify plasma and urinary biomarkers associated with diet quality:

Through a targeted metabolomics study, evaluate the concentration of myo-inositol and hippurate in blood/urine.

Identify plasma biomarkers associated with diet quality through untargeted metabolomics analysis.

Objective No. 5. Analyze the possible correlations between the biomarkers associated with diet quality, mental health status and weight status, in the study population:

Identify possible correlations between diet quality, weight status and mental health in pediatric participants.

To evaluate the correlation between identified and/or quantified biomarkers and diet quality in pediatric participants.

Identify biomarkers associated with diet quality that can serve as indicators of metabolic risk or mental health problems in the studied population according to the determination of correlations with these study groups.

Objective No. 6. Analyze the confounding variables and evaluate their impact on the relationships between diet quality, mental health status and weight status of the participants:

Collect information on potential confounding variables, such as socioeconomic status, physical activity, family history of obesity and metabolic disorders, genetic factors, parental educational level, adverse childhood experiences, and prenatal and mother's breastfeeding history.

To evaluate the impact of these confounding variables on the associations between diet quality, mental health status, and weight status using multiple regression analysis to control for the effect of the confounding variables.

Hypothesis

Null Hypothesis

There is no positive correlation between plasma and urinary biomarkers associated with diet quality, mental health status and weight status in Mexican children aged 5 to 11 years, once the identified confounding variables are controlled.

Alternate Hypothesis

There is a positive correlation between plasma and urinary biomarkers associated with diet quality, mental health status, and weight status in Mexican children aged 5 to 11 years, even after controlling for identified confounding variables.

STUDY DESIGN

Study description

Phase 1: MFL Evaluation

An evaluation will be carried out for all players aged 5 to 11 who are part of the MFL, where retrospective data will be collected on the weights and heights recorded by the MFL doctor at the beginning of the 2024 season. From this first phase, a nutritional diagnosis will be made based on the calculation of BMI in relation to weight and height relative to their age and gender.

Inclusion criteria:

- Pediatric patients aged 5 to 11 who belong to the MFL.

Exclusion criteria:

- Medical conditions that may affect metabolism or body composition.
- Use of medications that may influence metabolic biomarkers.

Phase 2: Subdivision of Research Groups

Sixty pediatric patients belonging to the MFL with obesity and sixty patients without obesity will be randomly selected. Selection will be based on BMI and the availability of the patient and guardian to continue in the second phase of the study. The mental health status of each patient will be assessed through surveys administered by psychiatry residents at Zambrano Hellion Hospital. The patient's medical history, physical examination, and anthropometric characteristics will be evaluated. A blood sample will be taken for laboratory studies related to obesity such as HOMA index and lipid profile. The blood sample and a urine sample will be used for targeted metabolomics evaluation of myo-inositol and hippurate metabolites, and for untargeted metabolomics evaluation to search for metabolites associated with diet quality and mental health.

Inclusion Criteria and Exclusion Criteria:

<u>Group 1: Obesity</u>

Inclusion criteria:

- Pediatric patients from 5 to 11 years old.
- Body Mass Index (BMI) greater than or equal to the 95th percentile for your age and sex, according to the standards of the World Health Organization (WHO) or the Centers for Disease Control and Prevention (CDC).

Exclusion criteria:

- Medical conditions that may affect metabolism or body composition.
- Use of medications that can influence metabolic biomarkers.

<u>Group 2: Without Obesity</u>

Inclusion criteria:

- Pediatric patients from 5 to 11 years old.
- BMI between the 5th percentile and the 94th percentile for your age and sex, according to WHO or CDC standards.

Exclusion criteria:

- Obesity or BMI above the 94th percentile.
- Medical conditions that may affect metabolism or body composition.

Suspension Criteria:

1. Patients who do not complete the surveys within the specified time frame.

2. Patients who do not undergo laboratory studies on the dates or places indicated.

Treatment choice: It is not an intervention study.

Placebo control: It is not an intervention study.

Informed consent forms and selection record

Written informed consent to participate in the study must be obtained prior to any study-specific screening tests or assessments.

The informed consent forms of the patients included and those who were subsequently not included will be kept at the study center.

All screening assessments must be completed and reviewed to confirm that patients meet all eligibility criteria prior to recruitment. The investigator will maintain a screening log to document details of all screened patients and confirm eligibility or record reasons if screening fails, as appropriate.

Medical history and demographic data

The collection of the medical history will be carried out indirectly through interviews with the parents and directly with the patient. During the interrogation, relevant aspects will be addressed such as clinically significant diseases, history of surgeries, oncological history, reproductive status, smoking habits, and all medications used by the patient in the year prior to the screening visit, ranging from medications prescription to vaccines, over-the-counter products, homeopathic or herbal remedies, and nutritional supplements. A first-degree family history (siblings, parents) of metabolic diseases such as obesity, hypercholesterolemia, type 2 diabetes, resistance will be specifically asked. to insulin.

Given the barriers associated with extensive food frequency questionnaires in the study of the relationship between diet and mental health in children and adolescents, it becomes imperative to simplify dietary assessment methods. Jacka et al. conducted a study focusing on adolescents ages 11 to 18, demonstrating that a simpler measure of nutrient-dense food groups, such as fruits and vegetables, yielded nearly identical results to a longer dietary questionnaire. This finding suggests that a shorter but comprehensive assessment of dietary intake can effectively capture key dietary components relevant to mental health outcomes.

Furthermore, considering the synergistic effects between nutrients. Jacka et al. proposed that assessing overall diet quality could serve as a superior indicator in investigating the impact of diet on mental health. By using a measure that encompasses various dietary factors, including fruit and vegetable intake, researchers can gain a holistic understanding of the relationship between diet quality and mental well-being in children and adolescents.

Incorporating this knowledge into dietary assessment methods can help mitigate burden on participants while capturing essential dietary information relevant to mental health outcomes. Using a simplified but comprehensive measure of dietary intake, focusing on nutrient-dense food groups such as fruits and vegetables, can streamline data collection and improve the feasibility of studying links between diet and mental health in pediatric populations.

The questionnaire used will be the Children's Diet Quality Index (CDQI): This scale evaluates the quality of the diet in children and adolescents based on the intake of various food groups and nutrients. It has been used in numerous research studies and is well validated in various childhood populations.

Assessing mental health in children ages 5 to 11 is a crucial component to understanding how diet can influence their psychological well-being. To address this complex relationship, it is necessary to use validated and reliable assessment tools that can accurately capture symptoms of depression and anxiety in this population. The Childhood Depression Scales (CDI) and Childhood Anxiety Scales-Revised (SCARED-R) are two widely used and

validated instruments to evaluate symptoms of depression and anxiety in children and adolescents. These questionnaires provide a standardized and objective measure of emotional symptoms, allowing for a systematic assessment of mental health in this population.

The CDI assesses the presence and severity of depressive symptoms, while the SCARED-R focuses on assessing anxiety symptoms in children. Both questionnaires address a wide range of emotional symptoms relevant to children's mental health, such as sadness, hopelessness, nervousness and excessive worry. By incorporating the Childhood Depression and Child Anxiety Scales-Revised into our research, we will be able to gain a more complete understanding of how diet relates to mental health in children ages 5 to 11 years. These tools will allow us to identify possible associations between eating habits and emotional symptoms in this population, providing valuable information for the design of interventions and strategies to promote mental health.

The CDI and SCARED-R Scales are evaluated through a questionnaire that participants complete by selecting the option that best describes their experience with respect to each item.

Childhood Depression Scale (CDI):

It consists of a series of statements about various aspects of the depressive experience, including 27 to 30 items.

Children and adolescents select the answer that best reflects how they have felt during the last two weeks in relation to each statement.

Each item is scored on a scale from 0 to 2, where 0 indicates that the symptom is not present, 1 indicates that the symptom is present sometimes, and 2 indicates that the symptom is present most of the time.

The CDI total score is calculated by summing the scores of all items, with higher scores indicating greater severity of depressive symptoms.

Children's Anxiety Scale-Revised (SCARED-R):

It consists of a series of statements related to different aspects of anxiety.

Participants select the option that best reflects their experience with each item over the past two weeks.

Like the CDI, the SCARED-R usually consists of around 27 to 30 items, although there may be variations in different versions.

Each item is scored on a scale from 0 to 2, where 0 indicates that the symptom is not present, 1 indicates that the symptom is present sometimes, and 2 indicates that the symptom is present most of the time.

The SCARED-R total score is calculated by summing the scores of all items, with higher scores indicating greater severity of anxiety symptoms.

Physical examinations

Regarding the clinical approach, a complete physical examination will be carried out, which will include an evaluation of the head, eyes, ears, nose and throat, as well as the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal and neurological systems. In addition, somatometry, nutritional evaluation with a 24-hour diet interrogation, and psychiatric evaluation will be incorporated. Any abnormalities identified at baseline will be recorded in the general history of baseline conditions.

At subsequent visits, or as clinically indicated, limited physical examinations directed at specific symptoms will be performed. Changes in abnormalities from baseline, as well as the results of somatometry and nutritional assessment, are recorded in the patient's notes. New or worsening clinically significant abnormalities should be recorded as adverse events. This comprehensive approach to clinical assessment will strengthen the understanding and approach to eating disorders, thereby contributing to the quality and practical relevance of the research.

Laboratory and cabinet studies

A blood sample (20 ml) will be taken, which will be processed for: A) analysis of markers associated with general/metabolic disease, and B) Analysis of biomarkers associated with diet quality. For group A), the laboratory studies considered are: complete blood count, HOMA index, 4 to 24-element blood chemistry, lipid profile, and thyroid profile. For group B), the concentration of myo-inositol and hippurate will be evaluated through a targeted metabolomics study, as well as the assessment of biomarkers identified by untargeted metabolomics. Additionally, a urine sample (10 ml) will be taken for a general urine examination and the determination of myo-inositol and hippurate metabolites.

The blood and urine samples will be taken at the External Laboratory of Hospital Zambrano Hellion by personnel experienced in pediatric patient sample collection. The blood sample will be processed for complete blood count, HOMA index, 4 to 24-element blood chemistry, lipid profile, and thyroid profile studies. The urine sample will be processed for a general urine examination. Additionally, the laboratory personnel of Hospital Zambrano will separate plasma and urine samples, which will be delivered in cryovials to Dr. Perla Pérez Treviño, who will be responsible for their transportation while maintaining the cold chain and custody chain of the corresponding sample. The samples will be stored at -80°C in the research laboratory of the School of Medicine of Tecnológico de Monterrey, CITES building, 3rd floor. This plasma sample will be used for both targeted and untargeted metabolomics studies and will be stored in cryovials.

Both untargeted and targeted metabolomics studies will be conducted using the UPLC-MS/MS technique at the Metabolomics Center - Metcore, Vice-Rectory of Research and Creation, University of Los Andes. Additionally, myo-inositol in plasma will be evaluated using an enzymatic assay based on the K-inositol assay by Megazyme.

Vital signs

Vital signs will include measurements of respiratory rate, heart rate, systolic and diastolic blood pressure (with the patient in a sitting position), and temperature.

INVESTIGATION METHODOLOGY

Patient recruitment will be carried out by researchers C.A. and P.P. through the Mexican Football League and reviewed at the Zambrano Hellion Hospital, specifically on the 5th floor of Wellness and Prevention of TECsalud.

Activity	Date	Activities to be Conducted
Formation of Research Team and Clinical Research Binomials at the Institute of Obesity Research	2023	Establishment of the research team and clinical research binomials.
Determination of Research Topic	December 2023	Selection and finalization of the research topic.
Contact with MFL Monterrey Foot Ball League	January 2024	Initiation of communication with the MFL Monterrey Foot Ball League.
Development of Clinical Protocol	January to May 2024	Creation and finalization of the clinical protocol.
Submission for Ethics Committee Approval	May 2024	Submission of the research protocol for approval by the Ethics Committee.
Data Collection (retrospective)	Upon Ethics Committee approval, ideally June 2024	Collection of retrospective data upon approval.

Data Analysis and Compilation in REDCap	June and July 2024	Analysis and compilation of data using the REDCap platform.
Patient Recruitment for Phase 2 of Research	June, July, and August 2024	Recruitment of patients for the second phase of research.
Scheduling of Research Data, Physical Examination, and Laboratory Tests	August 2024 to August 2025	Scheduling and conducting of data collection, physical examination, and laboratory tests.
Data Analysis	September to November 2025	Analysis of collected data.
Manuscript Writing	December 2025	Writing of the research article.
Presentation of Data and Dissemination of Information	January and February 2026	Presentation and dissemination of research findings.
Publication	Between January and June 2026	Publication of the research findings.

Schedule of Visits	**Date**	**Activities to be Carried Out**
Baseline Evaluation Visit	Second Saturdays of the second half of 2024	Collection of personal data, medical history, pediatric physical examination, administration of psychological tests, nutritional assessment on InBody scale, vital signs measurement, and collection of blood and urine samples.

Laboratory Results Delivery	After the visit	Laboratory test results associated with general/metabolic disease, along with their interpretation, will be emailed.

Variables

- BMI Weight
- Height
- Percentage of fat
- Fat in kilograms
- Blood pressure
- Family history of Type 2 Diabetes, hypercholesterolemia, obesity, and insulin resistance
- Total cholesterol
- Triglycerides
- HOMA index
- Myo-inositol
- Hippurate
- CDI (Clinical Dementia Rating)
- SCARED-R (Screen for Child Anxiety Related Emotional Disorders - Revised)
- CDQI

STATISTICAL ANALYSIS TECHNIQUES

Measuring instruments:

- Inbody scale 120 version 2022. Simultaneous Multi-frequency Bioelectric Impedance Analysis (SMF-BIA) with 20 kHz, 100 kHz with calibration in August 2023
- Stadiometer: Inbody brand with BSM 170 ultrasonic sensor
- Nutritional questionnaire (fruit and vegetable consumption) CDQI
- CDI Depression Screening Questionnaire
- SCARED R Anxiety Questionnaire

Methods and models of data analysis according to type of variables

The normality of the variables will be evaluated using the Shapiro-Wilk test. and ANOVA to compare baseline characteristics between participants. Linear regression analysis will be performed to investigate associations between baseline hormone levels, weight, and body composition, and Pearson correlation coefficients are reported.

Programs to use for data analysis.

JMP statistical database software (Pro version 14.0; SAS Institute, Cary, NC) will be used for statistical analyses, EXCEL, and Graph Pad Prism. REDcap regisration

Compliance with laws and regulations

This study will be carried out in full compliance with the ICH E6 guideline of Good Clinical Practice and with the principles of the Declaration of Helsinki or with the laws and regulations of the country (in which the research is carried out,) whichever provide the greatest protection to the individual.

Informed consent

A sample sponsor informed consent form (and any applicable ancillary sample informed consent form such as the Child Informed Consent Form or Nursing Home Informed Consent Form, if applicable) will be provided at all facilities. If applicable, it will be provided in a certified local language translation. The sponsor or designee must review and approve any proposed deviations from the sponsor's sample informed consent forms or any alternative consent forms proposed by the study site (collectively, the "consent forms") prior to submission to the IRB/EC. IRB/EC approved consent forms must be provided to the sponsor for submission to health authorities in accordance with local requirements.

If applicable, the informed consent form will contain separate sections for any optional procedures. The investigator or authorized designee will explain to each patient the objectives, methods, and possible risks associated with each elective procedure. Patients will be told that they are free to refuse to participate and that they can withdraw their consent at any time and for any reason. A specific, separate signature will be required to document a patient's agreement to participate in elective procedures. Patients who refuse to participate will not provide a separate signature.

The patient or legally authorized representative must sign and date the consent forms prior to participation in the study. Each patient's case history or clinical records should document informed consent processes and that written informed consent was obtained prior to study participation.

Consent forms should be reviewed when there are changes in study procedures or when new information becomes available that may affect the patient's willingness to participate. Final modified IRB/EC approved consent forms must be provided to the sponsor for submission to health authorities.

Patients must re-consent with the most recent version of the consent forms (or with an addendum of significant new information/findings in accordance with applicable laws and IRB/EC policies) during their participation in the study. For modified or updated consent forms, each patient's case history or clinical records must document that the informed consent process and written informed consent was obtained using the updated/modified consent forms for continued participation. in the study.

The patient or the patient's legally authorized representative must receive a copy of each signed consent form. All signed and dated consent forms must remain in each patient's study file or facility file and must be available for study monitors to verify at any time.

Confidentiality

Procedures to safeguard the privacy of the subjects participating in the research, as well as to preserve the results of the research.

The principal investigator maintains confidentiality standards by assigning a code to each patient included in the study using a unique patient identification number. This means that patient names are not included in the data sets that are transmitted.

Patient health information obtained in this study is confidential and may only be disclosed to third parties as permitted by the informed consent form (or separate authorization to use and disclose personal health information) signed by the patient, unless permitted or required by law. Medical information may be released to the patient's personal physician or other appropriate medical personnel responsible for the patient's well-being for treatment purposes.

The data generated in this study should be available for inspection upon request by representatives of national and local health authorities, and the IRB/EC, as appropriate.

Foreseeable and probable risks

Since it is a hands-off protocol, there are no risks associated with this type of patient management. Risks of vasovagal syncope may occur during blood sampling.

Protection against physical and/or emotional risk

To safeguard the integrity of the research participants, the following mechanisms will be implemented:

Informed Consent: Obtaining detailed informed consent, clearly explaining the objectives, procedures, risks and benefits, with the possibility of withdrawing at any time without adverse consequences.

Confidentiality: Guarantee of the confidentiality of information by assigning identifiers instead of real names and restricted access to personal data, presenting results in an aggregated and anonymous form.

Research Ethics: Conducting the study in accordance with the ethical principles of the Declaration of Helsinki, obtaining approval from the ethics committee, reporting any substantial changes, and complying with ethical standards in the presentation of results.

Ethical approval will be submitted to:

COMITÉ DE ÉTICA EN INVESTIGACIÓN HOSPITAL LA MISIÓN, S.A. DE C.V.

Dirección: Del Hospital No.112, 1° y 2° piso, Col. Sertoma. C.P. 64718, Monterrey, Nuevo León, México.

Presidente: Dr. José Francisco Islas Cisneros

Teléfono: 81 1492 4050

Correo electrónico: investigacion@hospitallamision.com

Comprehensive Care of Participants: Provide comprehensive care, including medical follow-up and psychological support during and after the study, with an established protocol to manage adverse events or emergency situations.

Continuous Monitoring: Conduct continuous monitoring to ensure compliance with protocols, safety of participants, and immediately address any significant deviations, taking corrective action as necessary.

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