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Effects of polyphenol supplementation on hepatic steatosis, intima-media thickness and non-invasive vascular elastography in obese adolescents: a pilot study protocol

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ABSTRACT

Introduction Non-alcoholic fatty liver disease (NAFLD) is increasingly prevalent in obese adolescents. Increased systemic inflammation and decreased gut microbial diversity linked to obesity affect the liver and are also associated with cardiovascular diseases in adulthood. However, NAFLD and vascular alterations are reversible.

Methods and analysis This pilot study evaluated the feasibility of a prospective open-label randomised controlled trial evaluating the effects of polyphenols on NAFLD and vascular parameters in obese adolescents. Children aged 12–18 years with hepatic steatosis (n=60) will be recruited. The participants will be randomised with a 1:1 allocation ratio to receive polyphenol supplementation one time per day for 8 weeks along with the clinician-prescribed treatment (group B, n=30) or to continue the prescribed treatment without taking any polyphenols (group A, n=30). The outcome measures will be collected from both the groups at day 1 before starting polyphenol supplementation, at day 60 after 8 weeks of supplementation and at day 120, that is, 60 days after supplementation. The changes in hepatic steatosis and vascular parameters will be measured using liver and vascular imaging. Furthermore, anthropometric measures, blood tests and stool samples for gut microbiome analysis will be collected. After evaluating the study's feasibility, we hypothesise that, as a secondary outcome, compared with group A, the adolescents in group B will have improved NAFLD, vascular parameters, systemic inflammation and gut microbiome.

Ethics and dissemination This study is approved by Health Canada and the hospital ethics. Participants and their parents/tutors will both provide consent. Trial results will be communicated to the collaborating gastroenterologists who follow the enrolled participants. Abstracts and scientific articles will be submitted to high-impact radiological societies and journals. ClinicalTrials.gov ID: NCT03994029. Health Canada authorisation referral number: 250 811. Protocole version 13, 2 June 2023.

Trial registration number NCT03994029.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study's feasibility can introduce a new treatment option for liver steatosis with minimal risks.
⇒ The relationship between vascular compliance and non-alcoholic steatohepatitis has not been studied in a randomised controlled trial in children.
⇒ The study covers only a small sample size of adolescents in a single institution; thus, the observations may not represent the general paediatric population with obesity.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) prevalence is 26% in obese children.[1] NAFLD is the deposition of fat in the liver. Non-alcoholic fatty liver (NAFL) is strongly associated with obesity and represents a risk factor for metabolic syndrome and diabetes.[2] NAFL increases the risk of morbidity and mortality from cardiovascular diseases.[3] Histologically, NAFLD presents degrees of severity, namely NAFL, less severe than non-alcoholic steatohepatitis (NASH). The diagnosis of NASH is made with a biopsy. Histology is less reliable in children than in adults.[4]

With obesity, there is decreased bacterial diversity in the gut microbiome. The imbalance between the microbiome and the intestinal immune system stimulates the secretion of pro-inflammatory cytokines. Therefore, an inflammatory bowel condition is linked to insulin resistance, NAFLD and obesity.[5 6]

Early changes in cardiovascular diseases are manifested by decreased vascular compliance and thickening of the intima-media of the carotid artery wall.[7 8] Studies have shown that vascular wall changes start very early and that there is an association between obesity in

childhood and cardiovascular diseases in adulthood. The intima-media thickness (IMT), defined as the distance between the vascular lumen-intima and media-adventitia interfaces, increases with age and with atherosclerosis.[9–11] All these changes can be reversible. Lifestyle modification can prevent the progression of vascular diseases. A meta-analysis in 2015 showed that only five articles evaluated the relationship between NAFLD and IMT in children. Children with NAFLD had significantly increased IMT.[12 13]

The nutritional intervention has been studied for the management of liver steatosis.[5 14 15] Studies have found that the gut microbiome of patients with hepatic steatosis differs from that of controls.[6 16 17] Anhê *et al* found that mice fed a high-fat diet with cranberry juice extract did not develop fatty liver disease, unlike mice fed a fat-only diet.[18] There is, therefore, a protective effect of the polyphenols contained in cranberry extracts on NAFLD and cardiometabolic health, as demonstrated in several studies on diabetic patients.[17] Five per cent of the effects of polyphenols are direct through its metabolites, and 95% are through the change in the gut microbiome.[19 20] Polyphenols are produced naturally by plants (red grapes, berries and peanuts) as a protective antimicrobial mechanism.[21] The preventive effects of polyphenols have been demonstrated in animal studies[22–24] but are controversial.[25–27] Polyphenols are antioxidants that decrease liver steatosis and cholesterol blood levels in adults.[18 28–37] However, their effects are controversial and have not been studied thoroughly in children.[18 38 39] Hepatic steatosis may influence the bioavailability of polyphenols.[38–40] Thus, systemic diseases should also be influenced by the effects of polyphenols.[41 42] A systematic review of resveratrol (a polyphenol) in adults demonstrated inconclusive effects on NAFLD.[43 44]

The gold standard for diagnosing NASH and fibrosis is biopsy. However, it is invasive and does not give a global view of the liver. Studies have demonstrated the high diagnostic value of the five imaging modalities used in our study. Unlike biopsy, radiological imaging modalities are more representative of the liver, which depends on the degree of steatosis in the specific segment from which it was taken. There is no gold standard imaging, and, often, studies have published the results of more than one imaging modality to increase validity.

Objectives

The main objective of this pilot study is to evaluate the feasibility of a larger open-label randomised controlled trial on the effects of polyphenol supplementation. The specific objectives are as follows: (1) assess the feasibility of a randomised controlled clinical study in terms of recruitment, compliance to polyphenol supplementation and the visit roadmaps, duration of the radiological examinations and participants' satisfaction and point of view on the experience; (2) test the relevance of using a food diary; (3) test the effectiveness of the data collection procedure during the visits; and (4) explore the obstacles encountered while performing the radiological examinations and the rate of adverse events (AE), if any. This will further provide the data necessary to calculate the sample size of the future randomised clinical study. As a secondary outcome, we will analyse the primary data to demonstrate the effectiveness of polyphenol supplementation in reducing NAFLD in obese adolescents with fatty liver disease. We will also examine the effects of polyphenols on vascular parameters, including IMT and vascular elastography, as well as on anthropometric measurements, insulin resistance, inflammation, lipid/lipoprotein profile, gut microbiome and liver function. Different imaging modalities will be compared between children. We hypothesise that the trial is feasible. Quantitative imaging modalities best correlate with liver biopsy and vascular markers of atherosclerosis and polyphenols decrease liver steatosis.

METHODOLOGY

Design

This is a prospective open-label randomised controlled feasibility trial. The study will occur at an academic paediatric hospital. As the study implies three visits to the hospital within a 120-day period, patients preferentially residing in the city area will be recruited. A sample of n=60 of obese (body mass index (BMI) >85th percentile) adolescents aged 12–18 years will be recruited at the hepatology liver steatosis clinic of the hospital. The participants will be randomised with a 1:1 allocation ratio to receive 5 mL of Immunia Synergy per os one time per day for 8 weeks in addition to receiving the treatment prescribed by the clinician (group B, n=30) or to continue the prescribed treatment by the clinician without receiving any supplementation or placebo (group A, n=30). Study participants will, therefore, not be blinded to the intervention group. However, the research team will be blinded to the assigned treatment groups as they will conduct and analyse the radiological examinations and the subsequent imaging analysis. The study started in July 2021 and is planned to be completed in December 2024.

Exclusion criteria

The exclusion criteria to be validated in the medical record are as follows:

- Known chronic systemic diseases.
- Any other serious conditions, which, according to the doctor's judgement, would prevent compliance and safe participation in the study until completion.

The exclusion criteria to be validated when contacting the participants and their parents/tutors:

- Being pregnant.
- Taking all kinds of prescription or over-the-counter natural health products/natural supplements/vitamins on an ongoing basis or within the next 4 months, excluding vitamin D.
- Weight loss of 5% to 10% of the usual weight in the last 6 months before recruitment or weight change of 5% in the last 3 months.

- Alcohol consumption >two drinks/day or >one day/week.
- Known peanut allergy and/or allergy to the medicinal ingredients contained in the active polyphenol supplement: elderberry, haskap, black chokeberry, blueberry, blackcurrant.
- Any contraindications for MRI.

Inclusion criteria

The inclusion criteria to be validated in the medical record:

- Adolescents aged 12–18 years and BMI percentile >85th for age and sex.
- Diagnosis of hepatic steatosis on imaging (ultrasound or magnetic resonance) or
- Diagnosis of hepatic steatosis, NASH or fibrosis on liver biopsy or
- Elevated alanine aminotransferase (ALT) enzyme level or
- Index of hepatic steatosis 8×ALT/aspartate aminotransferase (AST)+BMI (+2 for girls)>30

Participants will be recruited from lists of patients who are currently followed up at the hospital's fatty liver disease clinic. If the criteria are met, patients may be directly offered to participate in the study by their clinicians during their regular follow-up visits. When the inclusion/exclusion criteria are validated, the project manager contacts the parents/tutors by email or phone.

Procedures

Once the consent form is completed, a first medical imaging visit is organised to conduct the hepatic magnetic resonance spectroscopy (MRS) for one last eligibility check. If the liver MRS shows a fat burden <5.5%, the participant is excluded from the study. If the liver fat burden is >5.5%, the participant remains in the study and the research examinations are proceeded on the day of that same visit. For data collection, the three visits occur in the medical imaging department as follows (see figure 1).

For visit 1 on day 1, the participant arrives at the hospital on a 12-hour fast. The participant is guided to the MRI examination room and MRS is obtained on a Philips 1.5T device. MRS is performed three times. The same technologist performs both mDixon-Quant and MRI elastography. Afterwards, a double-energy X-ray absorptiometry (DXA) is performed. The following forms are then

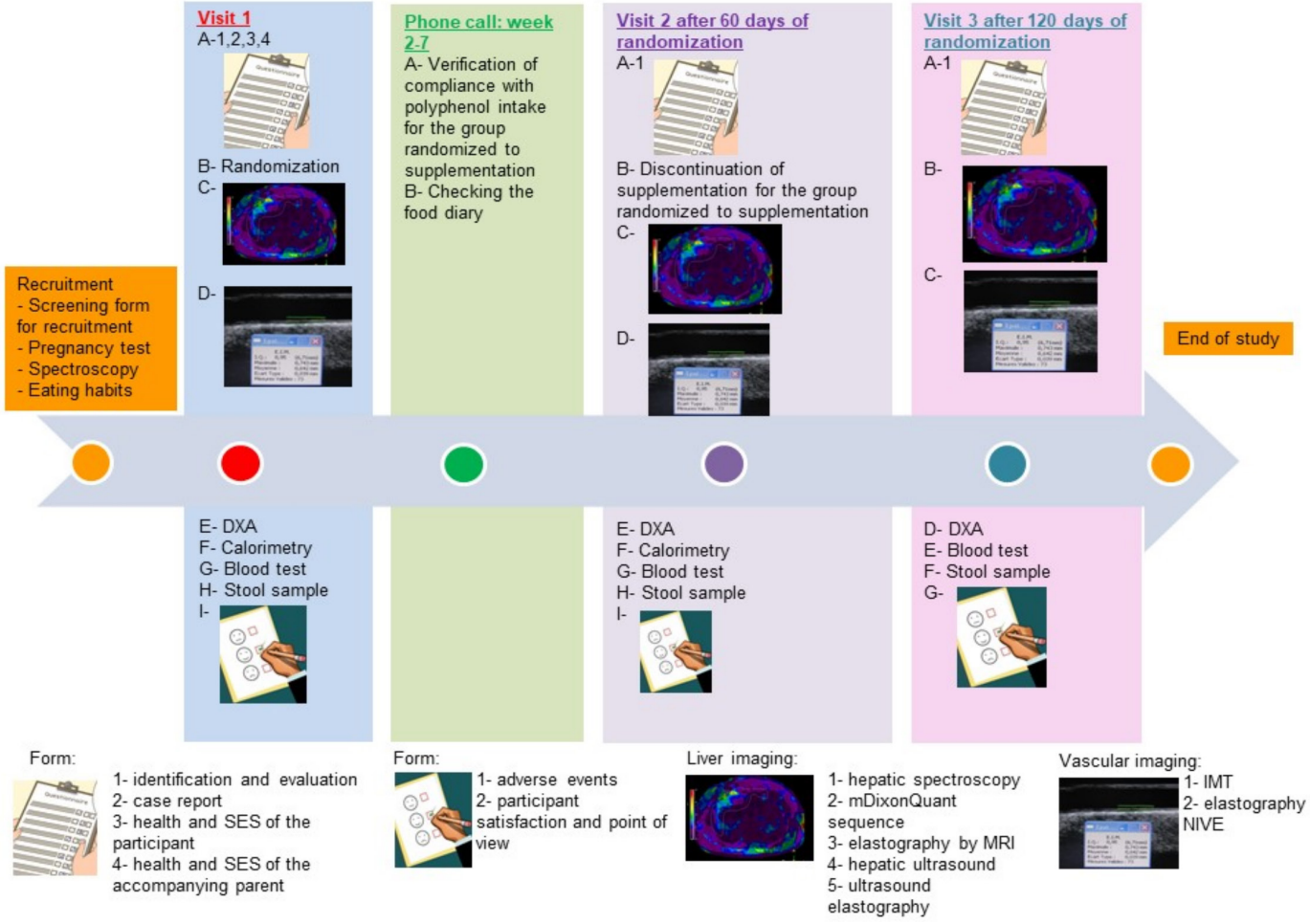

Figure 1 Schematic diagram depicting the overall schedule for trial participants in each study group during the 120-day intervention period.

completed by the participants and their parents/tutors: the consent form, the identification and evaluation form, the case presentation form, the health and socioeconomic status questionnaire forms, the compensation form ($C60/visit), the concomitant medication form and the participant satisfaction and point of view form at the end of the visit. The research assistant or the master student collects the following variables: date of birth, age, sex, weight, height and waist circumference. Tanner's stage is specified by self-assessment, and BMI is calculated in percentage for age and sex (BMI-for-age) according to the Centers for Disease Control and Prevention (CDC, BMI percentile calculator).

Then, conventional liver ultrasound and shear-wave liver elastography are performed. Next, an ultrasound device with a linear probe (Terason 3300) is used to capture images and record a 5 s video of the common carotid artery. A five-cardiac-cycle long video loop of radiofrequency-based ultrasound video for the evaluation of elastography of the carotid artery wall is recorded to calculate the axial strain (%), axial translation (mm), angular shear (%) and lateral translation (mm). Calorimetry is carried out by a student studying nutrition and dietetics. There are two calorimetry studies per participant, one initially at visit 1 and the other at visit 2. Finally, the participant is asked to urinate and blood pressure is measured using the average of three readings taken 1 min apart after a 10-min rest. A fasting blood test (12 hours) is performed. Four tubes are collected. The blood tests include electrolytes, creatinine and glomerular filtration rate (GFR), glucose, insulin, total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), complete blood count (CBC) and platelet, liver function test, thyroid-stimulating hormone (TSH), C-reactive protein (CRP) and sedimentation rate. The additional lipid metabolic markers measured are the following: apolipoprotein A1 (Apo AI), apolipoprotein B100 (Apo B-100) in plasma, the composition of lipoproteins and inflammatory markers ((high-sensitivity C-reactive protein (hCRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), adiponectin, leptin)). A stool sample for the gut microbiome analysis is collected at each of the three visits for the two groups. At the end of the first visit, group B participants are instructed to take 5 mL per day of Immunia Synergy® liquid polyphenol for 8 weeks. Both groups are invited to complete a food diary.

For visit 2 on day 60, similar to visit 1, the participant arrives at the hospital fasting (12 hours). Participants from groups A and B are instructed to bring the completed food diary and, for group B, the empty bottle of polyphenols. Liver MRS, mDixon-Quant and MRI elastography, liver ultrasound and shear-wave liver elastography, ultrasound of the common carotid artery, DXA study and calorimetry are performed as in visit 1. The same anthropomorphic measurements, fasting blood test and stool samples are collected according to the usual procedure. In addition, the following forms are completed: the identification and evaluation form, the case presentation form, the compensation form and the participant satisfaction and point of view form at the end of the visit. If any change is happening in the medication, the health of participant form, modified consent form and life habits are completed. Following this second visit, group B participants will stop taking polyphenols. Both groups continue the food diary.

For visit 3 on day 120, the participant brings the completed food diary from the previous 8 weeks. Visit 3 is similar to visit 2. The same steps are carried out and the same forms as visit 2 are completed.

The principal investigator or the Research Ethics Board can terminate a child's participation in the study without his/her consent if new findings or information indicate that the subject's participation in the project is no longer in his or her interest, if the instructions of the research project are not followed or if there are administrative reasons for abandoning the project. Any new knowledge acquired during the project that could impact the participant's or his/her tutor's decision to continue participating in this project is communicated to them quickly. If a participant demonstrates sustained non-compliance with the treatment or the filling of the food diary or fails to follow the instructions during the visits resulting in suboptimal examinations, the participant is withdrawn from the study. If side effects appear, the researchers may decide to remove the participant from the study while retaining and analysing the data already collected. However, if necessary, hepatology and gastroenterology follow-ups will be scheduled to assess the normalisation of the physical examination and the blood and hepatic tests.

The compliance to the intervention and strategies to improve adherence, defined by taking 5 mL of polyphenol per day, is measured by (1) a weekly call to assess compliance with polyphenol intake, completing the food diary and filing out the AE form if needed, (2) returning of the empty polyphenol bottle at week 8 (group B), (3) a weekly text message or letter that will be sent as a reminder to the compliance and (4) encouraging the group B participants to set the alarm every morning to remind themselves to take the supplement.

In case a participant withdraws from the study, the previously acquired data will be still used for the statistical analyses.

Patient and public involvement

Adolescents living with obesity do not have many treatment options other than lifestyle changes. The potential of polyphenols is, therefore, very well accepted especially by parents. Patients fill in a satisfaction questionnaire about their experience of research day, which we took into consideration in order to make the research visit easier, shorter and more accepted for future participants. Patients were not involved in the recruitment process. Letters showing the change in liver steatosis across the three visits and according to all imaging modalities will be sent to every participant at the completion of participation. The side effects of the polyphenols are explained to

participants, who are encouraged to report any symptoms for the duration of the study.

Analysis plan

The pilot study will assess the feasibility of a randomised controlled study based on the following outcome criteria: *Recruitment:* The objective of the study is to recruit at least one patient per week. Considering a loss of follow-up of 10%, a number of 60 patients for analysis will be aimed at. *Compliance with polyphenol supplementation:* Compliance with the intervention will be subjectively measured according to three criteria and will be calculated as a percentage for each participant for the duration of the 60 days during which supplementation is taken. The three criteria are the food diary, the report of weekly telephone calls and the verification of the empty bottle. *Retention*: The percentage of adolescents who agreed to participate in the study and the retention percentage will be calculated. *Effectiveness of the data collection procedure during the visits:* Subjective analyses of the obstacles to the various radiological examinations, including the waiting time (average (min) SD) to execute each examination, will be performed. The identification and evaluation form will be completed at each visit. *Satisfaction of the participants and their point of view on the experience:* Subjective analyses of the satisfaction form. *Rate of AE*: Objective analyses of the AE form and report of the Data and Safety Monitoring Board (DSMB). *Sample size calculation for a randomised controlled study:* A sample size for a future randomised controlled study will be calculated from the estimates obtained during this pilot study. This pilot study aims to observe the feasibility of the future study as well as to obtain estimates for the calculation of the future study's sample size.

The secondary outcome variable that will be monitored to document the effects of polyphenols on hepatic steatosis is the change in hepatic steatosis before randomisation (visit 1 at day 1; start of polyphenol supplementation for group B), after the intervention (visit 2 at day 60; end of polyphenol supplementation for group B) and 60 days after stopping the intervention (visit 3 at day 120). The percentage of absolute decrease or increase in steatosis on each of the modalities will be calculated between each of the three visits. A similar analysis will be performed for IMT and vascular elastography.

The secondary outcome measures include anthropomorphic measurements, insulin resistance evaluated by homeostatic model assessment for insulin resistance (HOMA-IR) and triglyceride glucose (TyG) index, inflammation, lipid/lipoprotein profile, liver function and gut microbiome composition. Each patient will be under his/her own control to minimise variation in the composition of the microbiome between subjects. A comparison of the change in the composition of the gut microbiome before, during and after treatment will be carried out for both groups. Change from baseline will be analysed for each of the outcomes by comparing the mean values on the three visits.

Randomisation

Considering a loss of follow-up of 10%, a sample of 67 adolescents will be recruited to ensure 60 patients for analysis (30 participants per arm and normal distribution). Participants will be randomly assigned to either the control or the experimental group with a 1:1 allocation as per a computer-generated randomisation schedule. This randomisation will be done at visit 1 using the GraphPad statistical system (http://www.graphpad.com/quickcalcs/index.cfm) by a collaborating research assistant who is not involved in the screening or recruitment of the participants.

The list of each participant's number and corresponding group will be kept at the central office of the pharmacy department without any researcher or project manager involved in recruiting having access to it.

Due to the inability to obtain a placebo for the liquid polyphenol supplement, participants will not be blinded to the study hypothesis. However, they will be instructed to conceal their respective group assignment from the researchers involved in collecting the outcome data. The principal investigator and coresearchers responsible for conducting the imaging investigation and analysing the imaging data will be blinded to the intervention groups.

Financial compensation

Each participant will receive an amount of $C60 for each of the three visits as compensation.

Data management and privacy protection

The principal investigator or the research assistant will enter information and data into the case report forms (CRF) for each visit. The CRF will be saved with a password on the principal investigator's computer. The forms completed during the three visits for each participant will be saved in a drawer with a key in that same office. Participant files will be maintained in storage for a period of 7 years after the completion of the study. A trained medical student will perform data entry. A double-entry check will be performed on selected participants. The full procedural information can be found in the project's procedure manual and in the detailed CRF. Access to the CRF will require a password which will never be sent by email among investigators. The research assistant will have access to the final trial dataset and can disclose the contractual agreement if present.

Statistical analysis

A descriptive analysis will be performed for all demographic variables and the criteria of interest presented by the groups. Differences in laboratory data and haematological markers will be represented by descriptive analyses with the number of abnormal results per group (%) and the number of subjects with abnormal results per group (%). The changes in hepatic steatosis over time from the first to the third visits will be analysed using the analysis of variance (ANOVA) or the Kruskal-Wallis test, depending on the distribution for the two groups. The percentage of

absolute decrease or increase in steatosis on each of the modalities will be calculated between each of the three visits. A similar analysis will be performed for IMT and vascular elastography.

Pearson's correlation will be used to assess the relationship between the levels of polyphenols and the five hepatic imaging modalities (MRS, quantitative MRI, hepatorenal index with the ultrasound, elastography by MRI and elastography by ultrasound) as well as blood pressure, IMT and vascular elastography variables. A correlation study (intraclass correlation coefficient) will be performed between liver biopsy performed no later than the previous year if available and the five imaging modalities. Bland-Altman plots will be performed to show the agreement of the different imaging modalities for fatty liver disease for each of the three visits. Line graphs will be constructed to show the change in hepatic steatosis over time for the two groups. The analyses will be performed using SAS software version 9.4.

Descriptive analyses (percentages) of AE will be carried out according to the DSMB.

Data monitoring committee

A data monitoring committee (DMC) has been established to oversee the project and inform the investigator of any concern related to the scientific integrity of the project. The DMC of the pilot study consists of one paediatrician, one clinician scientist and paediatrician, and one clinician scientist and endocrinologist, all three from the same hospital. The three DMC members are independent of the principal investigator and have declared no conflicts of interest. The committee met before the start of the project to inform the principal investigator of the questions related to participant safety regarding polyphenol supplementation, blood tests and the various radiological examinations. The principal investigator will make sure to act on the recommendations of the committee and to inform the ethics committee.

An interim analysis is performed on the primary endpoint when 50% of patients have been randomised. The primary endpoint for discontinuing the study will be the withdrawal of the supplement by Health Canada for another cause or the occurrence of known or unknown AE in more than 50% of the participants. Any side effects related to polyphenol supplementation will be monitored, such as hypersensitivity (allergy), diuretic effect, nausea, abdominal pain and diarrhoea or any unexpected serious side effects, such as deterioration of liver function. The severe adverse event form will be completed by the researcher and sent by internal mail to the DMC and faxed to Health Canada. It is the only time when unblinding is permissible. The principal investigator will communicate the abnormal results to participants' family physicians on request from the DMC committee.

Significance

Liver steatosis is a major problem related to obesity. It is an independent risk factor for early vascular diseases related to atherosclerosis. This pilot study will estimate the feasibility of the prospective clinical trial that evaluates the effects of polyphenols on liver steatosis, vascular compliance and gut microbiome. It can provide valuable scientific evidence of their usefulness in children who suffer from NAFLD and currently do not have any safe, approved medication to limit the progression of liver and vascular diseases.

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Contributors REJ: design, obtaining funds, analysing preliminary data, writing the draft and editing the final manuscript; J-BM: editing manuscript, conducting research; AC: recruitment and conducting research; EL: studying gut microbiome and lipoproteins; JD: editing manuscript; AD, EY and CT: data analysis; FA, MP, KG and PJ: recruitment and study conception.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by CHU Sainte-Justine Hospital ethics committee number 20202278. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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