Faculté de médecine
Département de nutrition
Université de Montréal

OFFRE POUR ÉTUDIANT DES CYCLES SUPÉRIEURS

MAÎTRISE X DOCTORAT ☐

Information	
Titre du projet	Effect of Low-Fat Compared to Low-Carbohydrate Diet on Fasting Lipids and Metabolic Profile in Subjects with Multifactorial Chylomicronemia: A Randomized Crossover Study
Professeur responsable	Dr Sophie Bernard (Département de Médecine, Université de Montréal)
Date de début prévue	Janvier 2019

Description du projet:

Voir le protocole ci-joint.

Domaines de recherche :

-Dyslipidémies
-Nutrition
-Métabolisme
-Prévention cardiovasculaire

Milieu de recherche :

- Institut de recherches cliniques de Montréal (IRCM)
  - Clinique de lipides, nutrition et prévention cardiovasculaire (https://ircm.qc.ca/fr/clinique/clinique-lipides)

Profil recherché :

- Le ou la candidat(e) doit :

  - être bilingue (français et anglais)
  -démontrer un intérêt pour la recherche

-avoir un esprit de synthèse et d'analyse
-aimer lire et écrire
-aimer le contact avec les patients et l'enseignement
-posséder des aptitudes pour le travail dans une équipe multidisciplinaire
-posséder des aptitudes pour la création de recettes et de menus standardisés
-faire preuve de créativité
-avoir une bonne capacité d'adaptation/gestion des imprévus
-être autonome

Exigences et conditions :

- Détenir un Baccalauréat en Nutrition et un permis de pratique valide en date de janvier 2019.

Documents demandés :

-CV
-Relevé de notes

Veuillez envoyer vos documents par courriel à:

Pour nous transmettre votre candidature ou bien pour plus de renseignements sur le projet, veuillez contacter :

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IRCM INSTITUT DE RECHERCHES CLINIQUES DE MONTRÉAL
AFFILIÉ À L'UNIVERSITÉ DE MONTRÉAL

Effect of Low-Fat Compared to Low-Carbohydrate Diet on Fasting Lipids and Metabolic Profile in Subjects with Multifactorial Chylomicronemia: A Randomized Crossover Study

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Introduction

The multifactorial chylomicronemia ((MCM), also known as type V hyperlipoproteinaemia or mixed hyperlipidaemia) is an oligogenic or polygenic disorder that is associated with a reduction in lipoprotein lipase activity which leads to chylomicronemia. In this disease, very high concentrations of serum triglycerides (≥10 mmol/l (≥880 mg/dL)) can be observed in the fasting state due to the accumulation of both VLDL-C and chylomicron. In patients with MCM, chylomicronemia typically occur in adulthood and is exacerbated by the presence of secondary factors such as a diet rich in dietary fats and simple sugars, obesity, alcohol intake and uncontrolled diabetes. It has been estimated that chylomicronemia can be found in 1:600 adults (Brahm, 2015). However, it is likely that the prevalence of MCM may increase in the future due to the increasing prevalence of obesity, metabolic syndrome and type 2 diabetes. This condition increases the risk of acute pancreatitis, which can be recurrent and potentially fatal. Indeed, the risk of acute pancreatitis is 10-20% for TG levels > 22.58 mmol/L (>2000 mg/dL) (Scherer, 2014). Furthermore, because MCM patients often present with other lipid disturbances as well as a worse metabolic profile, these patients are at increased risk of cardiovascular disease (CVD). A previous study showed that MCM patients present a 2-fold higher risk of CVD than normolipemic controls (Tremblay, 2011). Fortunately, MCM patients generally respond well to modifications in lifestyle, to treatment of secondary factors and to triglycerides lowering therapies such as fibrates. However, it is still unknown which kind of diet has the greatest effect on triglycerides level and on the metabolic profile in MCM patients.

The nutritional recommendations can be very different according to the nature of the patient's population to be treated. In order to reduce and manage triglycerides level in the general population, the American Heart Association guidelines recommend weight loss, reduction of simple carbohydrates (particularly fructose) intake, consumption of marine-derived omega-3 and limited alcohol intake (Miller, 2011). It has been estimated that a reduced sugar consumption combined with an increased unsaturated fat intake may reduce triglycerides levels by 10% to 20% (Miller, 2011). It is interesting to note that low-fat diets have been consistently associated with higher triglycerides levels (Tremblay, 2013; Liu, 1983). This is explained by the increased carbohydrate consumption associated with low-fat diets.

On the other hand, the nutritional intervention strategy is quite different for subjects affected by familial chylomicronemia syndrome (FCS), for which the treatment focuses on restriction of dietary fat. FCS is a very rare autosomal recessive disease that leads to a drastic reduction of chylomicrons clearance leading to chylomicronaemia. Therefore, a very strict lipid-controlled diet low in long-chain fatty acid (10-30g/day or 10%-15% of total energy intake) is required in order to lower chylomicron formation (Brahm, 2015).

MCM is a complex condition in which both an increased VLDL formation by the liver and a decreased chylomicrons and VLDL clearance are present. Furthermore, triglycerides values are fluctuating from day to day but generally remain very high. Therefore, the best dietary approach for these patients remains to be elucidated.

Interestingly, a study conducted in 17 nonobese men with fasting serum triglyceride concentrations > 2.3 mmol/L (>177mg/dL) tested the effect of a low-fat vs high-fat diet according to a randomized crossover design. The authors observed that the high-fat diet lowered triglyceride concentrations more effectively in all subjects with a baseline triglyceride concentration < 4.5 mmol/L (< 354 mg/dL), whereas the low-fat diet lowered triglyceride levels more effectively in the majority of subjects with a baseline value > 4.5 mmol/L (> 354 mg/dL) (Jacobs, 2004).

Based on these results, we can hypothesize that the low-fat (high-carbohydrate) diet would be more suitable for MCM patients than the low-carbohydrate (high-fat) diet. However, to date, no study has been conducted in MCM subjects in order to compare the triglycerides-lowering efficacy of low-fat vs low-carbohydrate diets.

Hypothesis

In this randomized crossover study, the 3-week low-fat diet will lower triglycerides levels to a greater extent than the 3-week low-carbohydrate diet in a sample of 20 MCM patients.

Primary Objective

The primary objective of this study is to compare the effects of low-fat vs low-carbohydrate diets on fasting serum triglyceride concentrations.

Secondary Objectives

1) To compare the effects of low-fat vs low-carbohydrate diets on other fasting cardiometabolic parameters: measured LDL-C, total cholesterol, HDL-C, glucose, insulin, HbA1c, HOMA-IR, Lp(a), apoB, non-HDL-C, hs-CRP, PCSK9 and free fatty acids (FFA).

2) To compare the effects of low-fat vs low-carbohydrate diets on SBP, DBP and waist circumference.

3) To compare the effects of low-fat vs low-carbohydrate diets on ultracentrifugation and/or electrophorese results (fasting).

4) To compare the effects of low-fat vs low-carbohydrate meals on postprandial serum triglyceride after a standardized test meal.

5) To assess the patients' appreciation, compliance and tolerability for each experimental diet.

Material and Methods

Study Population and Recruitment

- Study participants will be recruited at the Institut de recherches cliniques de Montreal (IRCM).
- The recruitment will be under the responsibility of Chantal Blais (registered dietitian) and a master student specifically assigned to this project (registered dietitian).
- N=20 MCM patients.
- Recruitment of the participants between January 2019 and January 2020.

Inclusion Criteria

1) M or F ≥ 18 years
2) Fasting triglycerides values ≥ 10 mmol/L (≥ 880 mg/dL) at week 0.

3) A prior diagnosis of MCM or the presence of both a white collar and a lactescent infranatant in the reference tube (preferably but not mandatory).

Exclusion Criteria

1) A diagnosis of familial chylomicronemia syndrome, familial hypercholesterolemia or type III hyperlipidemia.
2) An episode of accute pancreatitis in the 6 months prior the screening.
3) The use of lipid-lowering medication in the 4 weeks prior the screening such as fibrates or statins.
4) Recent changes (in the last 3 months) in other medication or supplement known to affect lipid or glucose metabolism such as steroid or oral contraceptive.
5) Any condition known to affect lipid or glucose metabolism such as uncontrolled hypothyroidism or Cushing's syndrome.
6) Major surgery in the 3 months preceding the study.
7) Significant weight change (±10 %) within 3 months prior to beginning the study.
8) Alcoholism
9) The necessity or the wish to follow a specific diet.
10) BMI $\geq$ 40 kg/m^2
11) Pregnancy
12) Consumption of dietary supplements such as omega-3, psyllium or phytosterols.
13) Any serious health condition associated with a life expectancy of $\leq$ 1 year.

Randomization

The order of the experimental periods will be assigned by sequential order according to patients' screening date (e.g.: the first randomized patient will begin by the low-fat diet, the second one by the low-carbohydrate diet, the third one by the low-fat diet, etc.).

Intervention (Diet) and Experimental Design

- Isocaloric diets (no weight loss).
    - Low-fat diet (20% of fat, 20% of protein, 60% of carbohydrate).
    - Low-carbohydrate diet (45% of fat, 20% of protein, 35% of carbohydrate).
- The energy requirements of the study participants will be estimated using Harris-Benedict's formula. The participant's caloric intake will be adjusted if a participant's weight changed by ±2 kg from the diet specific baseline value.
- The participants will have to monitor their body weight each week in order to maintain a stable body weight. If a variation of more than 2 kg occurs, the participant must call the student for readjustment of energy requirement.
- Randomized crossover design.
- Free-living subjects.
- Run-in period of at least 1 week (usual diet and physical activity level).
- 3-week experimental periods (stable physical activity level).
- 2-week wash-out period (on usual diet and physical activity level).
- 10 days menu and recipes for each experimental diets will be provided to the participants (2000 kcal). The menu will be adjusted for each participant according to their energy requirement.

Nutrients	Low-fat diet	Low-carbohydrate diet
Fat (% kcal)*	20	45
Protein (% kcal)	20	20
Carbohydrate (% kcal)**	60	35
EPA + DHA (portions) ***	2 meals of fish/week	2 meals of fish/week
Fiber (g)	20-25	20-25
Cholesterol (mg)	≤ 300	≤ 300
Alcohol (drinks of alcohol)****	0-2/week	0-2/week

Figure3. Composition of the experimental diets.

* Mostly unsaturated dietary fat from vegetal sources.

** Mostly complex carbohydrates (avoid added simple sugars and fructose-rich food).

*** Or 0 meal of fish/week in each diet for patients who do not like fish.

**** Alcohol consumption must be stable in each experimental period.

Intervention (Test Meal)

- Participants will be advised to consume the meal within 15 min.
- Blood samples will be collected at time 0, 1, 2, 4, and 6 h for measurement of blood lipids.
- The test meal will take place after a 12-hour overnight fast.
- 600-kcal test meal.
- The test meal will take place after the consumption of the matching diet for 3 weeks.

Intervention (timeline)

Week 0 (screening)

- The nurse will perform blood analysis (including complete lipid profile, ultracentrifugation, electrophorese, complete blood count, liver enzymes, glucose, HbA1c and hsCRP) and clinical data collection (such as blood pressure, BMI, waist circumference, medication, smoking habit, alcohol intake, etc.) as usual for new patients with hypertriglyceridemia referred at the IRCM lipid clinic (**Figure 1**). This will also include a blood sample for *APOE* genotype and for genetic screening for mutations in *LPL, APOC2, APOA5, LMF1* and *GPIHBP1* genes.
- The registered dietitian will meet the patients for nutritional assessment using a validated food frequency questionnaire (FFQ).
- Potentially eligible patients for the present study (referred for high triglycerides values) will be identified before the screening and met by the student. The study will be briefly explained and two copies of the informed consent form will be given to these patients (no signature).
- A reference tube of serum will be kept overnight (24h) at 4◦C for each potentially eligible patients in order to establish a preliminary diagnosis of MCM (a white collar of variable importance with a lactescent infranatant must be observed).

Week 1

- The principal investigator of the study must identify the patients with a MCM phenotype according to the information available from the screening visit.

- The student will call the patients for whom a preliminary diagnosis of MCM have been established in order to invite them to officially participate in the study. The study will be explained in details. If they agree, the patients must sign the two copies of the informed consent form and begin the run-in period at least 1 week before their next visit at the clinic.

Week 3 (visit 1- pre)

- The patients included in the study will be randomized to determine the diet for the first experimental period: low-fat or low-carbohydrate.
- The patients must bring one signed copy of the informed consent form.
- Patients will start the first experimental period after receiving the nutritional education concerning the diet (registered dietitians).
- Blood analysis, anthropometric and blood pressure measurements will be performed.

Week 4

- 1 week after the beginning of the first experimental period, the student will call the patients in order to assess the compliance and to provide support and motivation.
- Confirmation of date for the visit 2.

Week 5

- The patients must complete a 3-day dietary journal (2 week days and 1 weekend day).

Week 6 (visit 2 - post)

- The visit takes place at the end of the first 3-week experimental period.
- Blood analysis, anthropometric and blood pressure measurements will be performed.
- Confirmation of date for the visit 3.
- A 6-h test meal will be performed (representative of the first experimental diet).
- Beginning of the wash-out period.

Week 8 (visit 3 - pre)

- The patient will start the second experimental period after receiving the nutritional education concerning the diet (registered dietitians).
- Blood analysis, anthropometric and blood pressure measurements will be performed.

Week 9

- 1 week after the beginning of the second experimental period, the student will call the patients in order to assess the compliance and to provide support and motivation.
- Confirmation of date for the visit 4.

Week 10

- The patients must complete a 3-day dietary journal (2 week days and 1 weekend day).

Week 11 (visit 4 - post)

- The visit takes place at the end of the second 3-week experimental period.
- Blood analysis, anthropometric and blood pressure measurements will be performed.
- A 6-h test meal will be performed (representative of the second experimental diet).
- End of the study.

Week ≥ 16

- Medical visit.

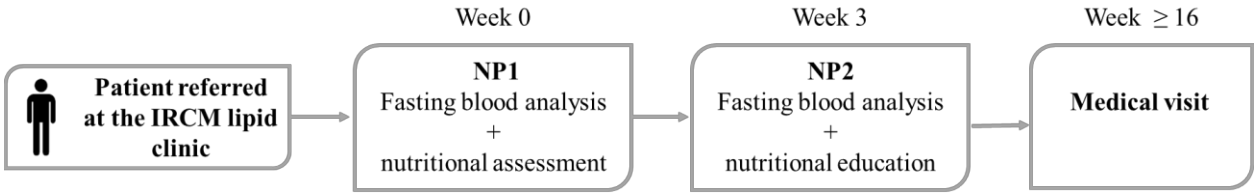

Figure 1. The usual visits algorithm for a new patient referred at the IRCM lipid clinic. NP: new patient. NP1: visit with nurses # 1. NP2: visit with nurses # 2.

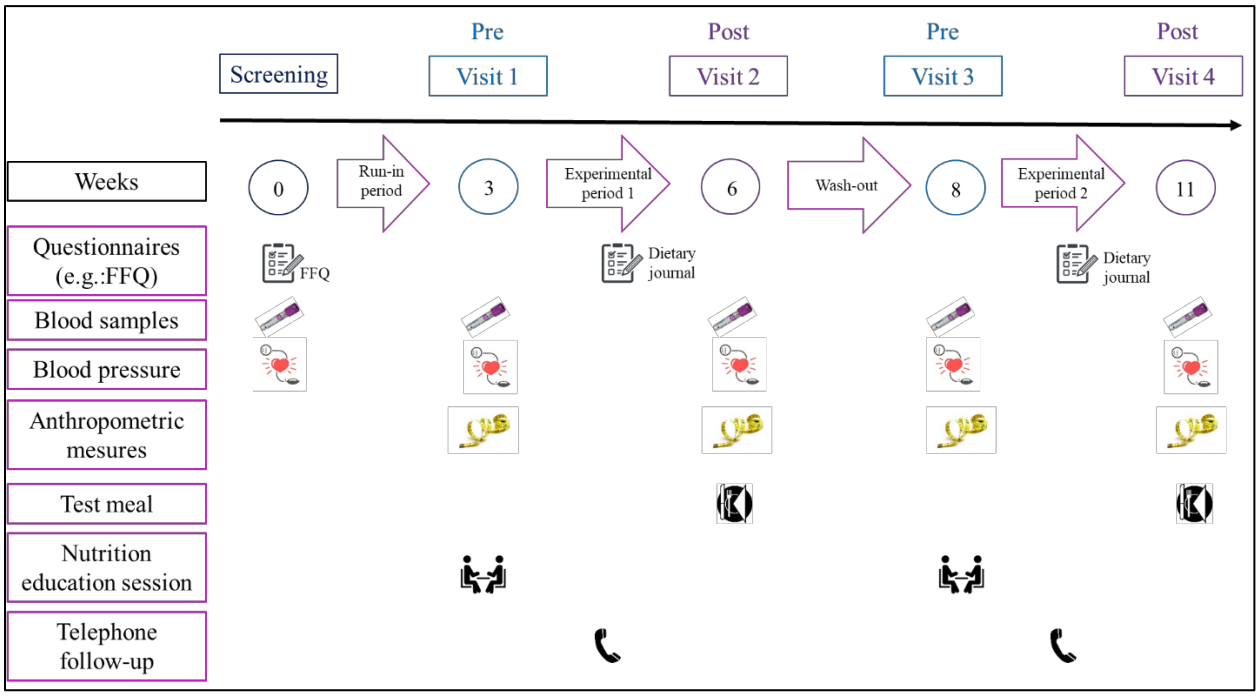

Figure 2. Study design. FFQ: food frequency questionnaire.

Blood Sampling, Processing and Storage

- One plasma sample per visit will be collected for the measurement of PCSK9 using EDTA plasma tubes (6 mL). Filled tubes should be kept on ice until centrifugation, which should be

done within 2 hours of collection. This sample will be separated by centrifugation at 4 ° C, 3000 rpm, 15 minutes. Using a transfer pipette, the plasma will be separated into 4 aliquots (at least 0.5 mL of plasma in each aliquot). Plasma samples will be kept at - 80◦C freezer until analyzed.

- All other blood samples will be sent at the ICM or CHUL laboratories for the following analysis: TG, measured LDL-C, total cholesterol, HDL-C, glucose, insulin, HbA1c, Lp(a), apoB, hs-CRP and electrophoresis/ultracentrifugation.

Data Collection

Three questionnaires will be used during the study to collect information:

- A validated food frequency questionnaire (FFQ) to assess the baseline diet of the study participants.
- A validated dietary journal to assess the compliance of the study participants to the experimental diets.
- A questionnaire to assess the patients' appreciation, compliance and tolerability for each experimental diet.

Blood pressure and anthropometric measures will be performed by the student using standardized methods.

All baseline information (week 0) will be collected in the medical file of the patient. The remaining information will be collected in a research file.

Biochemical Analysis

Total plasma PCSK9 concentration will be analyzed by the team of Dr. Nabil Seidah at the IRCM. Briefly, an in-house enzyme-linked immunosorbent assay (ELISA) using a polyclonal antibody against human PCSK9 will be used.

Statistical Analysis

Statistical analyses will be performed using SPSS statistical software (version 20.0; IBM Corp, Armonk, NY) and SAS 9.4 (SAS Institute, Cary NC). Variables with a skewed distribution will be normalized by log transformation prior the analysis. The PROC MIXED procedure for an ANOVA for crossover design with 2 periods will be used to compare the effects of the 2 dietary treatments on each outcome. Standard Bonferroni correction will be performed to reduce the chances of obtaining false positive results. Furthermore, repeated-measures analysis of variance (ANOVA) will be applied for variables measured periodically during the test meal. Patients with weight variation of more than 2kg during one of the two experimental periods will be excluded of the analysis. For all measures, $P < 0.05$ was considered as statistically significant.

Financial Compensation

- None (except for the parking)

References

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2. Scherer J, Singh VP, Pitchumoni CS, Yadav D. Issues in hypertriglyceridemic pancreatitis: an update. J Clin Gastroenterol. 2014;48(3):195-203.

3. **Tremblay** K, Méthot J, Brisson D, Gaudet D. Etiology and risk of lactescent plasma and severe hypertriglyceridemia. J Clin Lipidol. 2011;5(1):37-44.

4. Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2011;123(20):2292-333.

5. Liu GC, Coulston AM, Reaven GM. Effect of high-carbohydrate-low-fat diets on plasma glucose, insulin and lipid responses in hypertriglyceridemic humans. Metabolism. 1983;32(8):750-3.

6. Tremblay AJ, Lamarche B, Guay V, Charest A, Lemelin V, Couture P. Short-term, high-fat diet increases the expression of key intestinal genes involved in lipoprotein metabolism in healthy men. Am J Clin Nutr. 2013;98(1):32-41.

7. Jacobs B, De Angelis-Schierbaum G, Egert S, Assmann G, Kratz M. Individual serum triglyceride responses to high-fat and low-fat diets differ in men with modest and severe hypertriglyceridemia. J Nutr. 2004;134(6):1400-5.