FOOD 4 GUT
Programme d'excellence de la Region wallonne

Clinical trial protocol

Nutritional intervention aims to study the beneficial impact of prebiotics intake on health (using vegetables and/or fibers supplementation) in obese patients.

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Duration : From Mars 2015 to Mars 2019

Cliniques universitaires SAINT-LUC UCL BRUXELLES

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Summary

1. Context and issues

Worldwide, the proportion of adult people with a body mass index of 25 $kg.m^{-2}$ (corresponding to overweight) or more, increased between 1980 and 2013 from 29% to 37% for men and from 30% to 38% for women (Ng et al., 2014). Nowadays, the world Health Organization estimates the number of obese individuals of approximately 300 billion around the world. Obesity is associated with a prevalence to develop a type 2 diabetes, cardiovascular diseases and some cancers, leading to an avoidable and global concern for this "disease" from public health actors. The increasing incidence of obesity is multifactorial and results from an initial imbalance between energy intake and expenditure, influenced by many variables including genetic, cultural and environmental characteristics (Ghosh, 2014). Since the last decade, the gut microbiota appears to be an important factor involved in the development of obesity (Delzenne et al., 2011). Indeed, obese subjects are characterized by changes in both composition and activity of the gut microbiota, compared to healthy people with a normal body weight (Le Chatelier et al., 2013).

Recent works performed in the laboratory of Professor N Delzenne (UCL) suggest that the intake of non digestible carbohydrates (prebiotics) are prone to modulate the gut microbiota and could lead to a beneficial impact in the context of obesity and associated metabolic disorders (Delzenne et al., 2013 et 2011; Neyrinck et al., 2010). Preclinical studies in mice suggest a molecular mechanism targeting the gut barrier function and intestinal endocrine functions following the prebiotics supplementation. The gut microbiota of mice and humans being different, the extrapolation of results is difficult. However, a randomized clinical intervention performed in a cohort of obese women has shown that inulin-type fructans supplementation compared to a placebo (maltodextrin) significantly changed the composition of the gut microbiota, leading to an increase of some bacteria (*Faecalibacterium prausntizii, Bifidobacteria ssp., Akkermansia muciniphila*) known to play a crucial role in the inflammatory processes and the expansion of fat mass (Dewulf et al., 2013; Salazar et al., 2014). Nowadays, the beneficial impact of nutritional intervention targeting the gut microbiota in the context of obesity remains to be studied.

A clinical study performed in China suggests that the modulation of gut microbiota with a nutritional intervention associated with dietary supplement (whole grains) ameliorates the integrity of gut barrier and reduce the burden of circulating antigens, leading to an improvement of inflammatory and metabolic phenotype in obese people (Xiao et al., 2014).

In the context of public health setting for the nutritional management of obesity and its prevention, the need of innovative intervention study in human appears to be essential in Europe.

In this context, we propose to develop a novel nutritional approach based on prebiotics intake in a multi-disciplinary project "FOOD4GUT project: Programme d'Excellence de la région Wallonne". This project postulates that the intake of locally produced vegetables rich in fructan-type prebiotics may create a real public health benefit. The goal is to increase the knowledge of

interactions between the gut microbiota and the host, in the context of nutritional management of obesity and its prevention.

We hypothesize that the changes in the gut microbiota due to prebiotics supplementation, combined with dietary advices promoting the consumption of vegetables enriched in inulin-type fructans could modulate the eating behavior, body composition, inflammation and obesity-associated comorbidities.

2. Study protocol

2.1. Investigation centers :

The protocol of this long-term clinical study was developed by a collaboration between Prof. Jean-Paul Thissen (Cliniques Universitaires de Saint-Luc), Prof. Miriam Cnop (Erasme Hospital), and Prof. Nicolas Paquot (Centre Hospitalier Universitaire de Liège). In addition, they are co-investigators of this protocol and participate to the enrollment of participants in the cohort, as well as in the intervention study described in the protocol. Behavioral exploration involves the collaboration of the team of Prof. Olivier Luminet (Institute for Research in Psychological Sciences, UCLouvain) and the team of Prof. Axel Cleeremans and Prof. Olivier Klein (Faculty of Psychological Sciences and Education, Université Libre de Bruxelles) in this project. Fibroscan analysis and liver biopsies performed at the Cliniques Universitaires Saint Luc involve the collaboration of Prof. Nicolas Lanthier (Cliniques Universitaires Saint-Luc – GAEN Laboratory – UCLouvain). The gut microbiota analysis and biologic measurements in blood, faeces or urine are performed in the lab and under the supervision of Prof. Nathalie Delzenne (Metabolism and nutrition research group – UCLouvain)

2.2. Recruitment of participants:

The recruitment is wide and performed in three Belgian hospitals involved in the study: Cliniques Universitaires Saint Luc, Erasmus Hospital and Centre Hospitalier Universitaire de Liège. All patients corresponding to the following criteria will be recruited, the goal being to obtain 50 patients per hospital at the end of the study, ie 150 patients in total.

Inclusion criteria for the study are listed below:

- Body mass index (BMI) > 30 $kg.m^{-2}$
- Men and women
- Age: between 18 and 65
- Caucasian
- Obese patients who want to follow a treatment for obesity or bariatric pre-surgery
- Obese patients with at least 1 comorbidity (treated or not) :

- Mellitus pre-diabetes : fasting glucose between 100-125 mg.dL^{-1} or glycated hemoglobin (HbA1c) between 5,7 and 6,4% or glycaemia (2 hours following an oral glucose tolerance test) between 140 and 199 mg.dL^{-1};
- Diabetes mellitus: fasting glucose ≥ 126 mg.dL^{-1} or HbA1c ≥ 6,5% or glycaemia (2 hours following an oral glucose tolerance test) ≥ 200 mg.dL^{-1};
- Dyslipidemia: HDL cholesterol < 40 mg/dl for men and < 50 mg/dl for women or triglycerides > 150 mg.dL^{-1} ;
- Hypertension: blood pressure ≥ 140/90 mmHg ;
- Non-alcoholic fatty liver disease - hepatic steatosis: diagnosis using level of transaminase or gamma-GT (reference values for Saint Luc: GOT > 48, GPT > 40, γ-GT > 60 UI/l) during medical examination (standard parameters of the biomedical analysis laboratory) or using FibroScan® evaluation.

Exclusion criteria for the study are listed below:

- Serious psychiatric issues and/or use of antipsychotic (the use of antidepressant is tolerated for the study);
- Current or recent antibiotics, probiotics, prebiotics intake (<6 weeks) or others molecules able to modify the intestinal transit;
- Pregnancy in progress or planned within 6 months;
- Special dietary habits (vegans, vegetarians…)
- Special dietary treatment in progress or recent (<6 weeks) (example of high protein diet often associated with fiber supplement)
- Type I diabetes;
- High alcohol consumption (> 3 glasses/ day);
- Inconclusive acceptability test (food products proposed in the study are not accepted by the patients, tests in collaboration with IPSY and PSYULB teams)

First, we will propose to all obese participants with comorbidity and all pre-surgery bariatric patients coming for the first time at the medical examination to participate to the study. Selection of participants will be based on criteria listed above. The results will be obtained during the routine check-up performed one month before the inclusion. Eligible and interested patients will be recalled for a screening visit.

During this screening visit, patients will be informed about the experimental procedure and explanations of the protocol of the simplified clinical study, they will have to sign the informed consent form.

All documents, written by MNUT and EDIN teams, will be identical in the three hospitals and universities in charge of the study. Each center will have to designate a person in charge of this information step before the start of the study.

2.3. Nutritional intervention

The intervention study will last for 3 months (6 months maximum if bariatric surgery) and will be based on a treatment using a nutritional approach. This study is a randomized, single-blinded and multicentric placebo-controlled trial.

Although patients will be their own control, participants will be divided in two groups, one group receiving the treatment and the other receiving the placebo will be considered as the control group.

For the treatment arm, patients will be daily supplemented with inulin (Fibruline®) and will receive dietary advices in order to consume vegetables enriched in fructans (diet recommended by dieticians involved in the project, including a recipe book). For the placebo, patients will be daily supplemented with maltodextrin (C*Dry A 01318 from Cargill); patients will receive a recipe book in the context of body weight management, as well as dietary advices from dietician involved in the study.

Probiotics or prebiotics consumption (except those provided by the study) will be prohibited during the intervention.

Inulin supplementation, or placebo, will be administered as follows: 8g per day in the morning during the first week, then 8g per day for lunch and dinner to obtain 16g intake per day for the rest of the intervention study.

2.4.Test days

Some parameters will be analyzed in obese patients, at baseline and at the end of the intervention protocol (3 months). These parameters will be assessed on a test day (performed at M0 = baseline and M3 = 3 months/ end of protocol) according to the patients' availability, staff, rooms/materials. These test days will be organized as follows:

Biological analysis:

- An oral glucose tolerance test (OGTT) with a fasting blood test at -20 min, -10 min, -5 min and then at 10, 30, 60, 90, 120 min following ingestion of 75 g glucose (= time 0), to measure the degree of disturbance of glucose homeostasis: analysis of blood glucose, insulinemia and C-peptide (except if diabetic patient).
- Using the fasting blood taken after the ingestion of glucose, many biological parameters could be measured:
    1. glycated hemoglobin (HbA1c) level;
    2. lipid profile (levels of HLD-C, triglycerides, total cholesterol, and serum free fatty acids);
    3. Biomarkers of systemic inflammation (interleukin (IL-1β, IL-17a, IL-8, mcp-1 and tnf-α).

4. Measurement of liver enzymes; alanine aminotransferase (ALT) and aspartate aminotransferase (AST), (Perazzo et al., 2014), measurement of α-2-macroglobulin (Mα2), haptoglobin, Apolipoprotein-A1 (Apo-A1), gamma-glutamyl transpeptidase (GGT), total bilirubin to produce Fibrotest® (Munteanu et al., 2014 and Poynard et al. , 2014) ;

- Stool sample collection will be made at home or at the Hospital center and will be used for the following analyzes :
    1. Gut microbiota analysis (16S rDNA gene sequencing and qPCR for some specific bacteria)
    2. Transfer of fecal material into germ-free mice in the MNUT laboratory in order to develop an innovative animal model and tool for exploratory research on molecular mechanisms induced by the prebiotics consumption;
    3. To develop a an *in vitro* model of fermentation of human microbiota to study the impact of fructans enriched foods on the intestinal characteristics, in collaboration with Zootecnics team from Université de Liège.
    4. Saliva samples (for the measure of amylase activity).
- Urine samples, taken in the morning, for the metabolomics analyzes (Druart et al., 2014).

Anthropometric study :

- Measurement of height, weight, hip circumference and waist circumference for BMI calculations and waist / hip ratio;
- Measurement of blood pressure ;
- Bio-impedancemetry for body composition analysis in order to assess body fat and lean mass.

Evaluation of hepatic fibrosis and/or steatosis:

Fibroscan analysis were performed for the quantification of liver stiffness (elasticity) and controlled attenuation parameter (CAP) (Castera et al., 2014 et Tapper et al., 2014, de Ledinghen V. et al., 2014).

Evaluation of white fat mass

Evaluation of white adipose tissue mass (visceral and subcutaneous fat area) and muscle mass by abdominal CT-scan (Cnop et al. Diabetes; 2002).

Behavioral assessment

Two behavioral questionnaires will be submitted to the patients: the Questionnaire for Eating Disorder Diagnoses (Q-EDD) and the French version of the Dutch Eating Behavior Questionnaire (DEBQ). In addition to evaluate the changes on food preferences, we will

perform an assessment of cognitive flexibility, sensitivity to fear messages or individual vulnerability. These tests will be led by IPSY and PSYULB teams.

Physical activity report will also be assessed using a IPAQ questionnaire (International Physical Activity Questionnaire).

Dietary follow-up

A follow-up with a dietician will be done at M0 and M3, but also at the end of the first and second month of intervention (M1, M2). During these meeting, the dietician also performed a one-week recall questionnaire to evaluate the dietary intake at baseline and at each month of the intervention.

2.5. Medical and dietary follow-up

In each hospital, obese patients enrolled in the study will meet once a month a dietician involved in the project. In this way, we ensure that the energy intake of participants will be controlled once a month by a dietician. Side effects of treatment (such as gastrointestinal symptoms) will be followed by a daily questionnaire to fill at home, during the first week of treatment and then during the meeting with the dietician.

If fibroscan analysis suggests a severe risk of cirrhosis, patients could benefit from a liver biopsy (“standard care”) performed by Prof. Nicolas Lanthier. If bariatric surgery is needed, an hepatic biopsy will be done during the surgery. We will take advantages of these liver biopsies to evaluate the effects of prebiotics on hepatic metabolism and inflammation. During the bariatric surgery, visceral and subcutaneous adipose tissues biopsies could also be taken in order to measure inflammation in these tissues.

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