Diet-Induced Alteration of Microbiota and development of Obesity, Nonalcoholic fatty liver disease and Diabetes (DIAMOND): study protocol of a prospective study.

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Abstract

Background: Development of obesity and obesity related diseases such as type 2 diabetes mellitus and nonalcoholic fatty liver disease is associated with altered gut microbiota composition. The aim of this study is to investigate associations between dietary compounds, intestinal cell function and gut microbiota composition. We hypothesize that dietary lipid intake is associated with Paneth cell and goblet cell properties that affect gut microbiota composition.

Methods: This is a single-centre prospective study including one obese group undergoing laparoscopic roux-en-y gastric bypass and two lean control groups undergoing either laparoscopic cholecystectomy or upper gastrointestinal endoscopy (n=228). During laparoscopy, biopsies will be taken of visceral fat (omentum majus), liver, muscle tissue of the abdominal wall, and subcutaneous fat. In the obese group, a small segment of the jejunum will be collected for analysis, which will be compared with an endoscopically derived jejunal biopsy from the upper gastrointestinal endoscopy control group. Stool samples for microbiota profiling will be collected at baseline and one year after surgery.
Primary outcomes are fecal microbiota composition, mucus characteristics, and Paneth cell phenotype. Secondary outcomes include body weight, diet composition, glucose tolerance and resolution of comorbidities.

Discussion:
The DIAMOND-study will improve insight into the pathophysiology of obesity and its associated metabolic disorders. Better understanding of weight loss failure and weight regain following bariatric surgery might also behold new therapeutic opportunities for obesity and obesity-related comorbidities.

Trial registration:
Nederlands Trial Register: NTR5660

Keywords
Microbiota ; Type 2 Diabetes ; Obesity ; NAFLD ; Gastric bypass

Introduction

Obesity-associated diseases like type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD) are major public health issues worldwide, affecting more than 6% respectively 25% of the world population.[1, 2] The influence of gut bacteria on the development of obesity and metabolic syndrome is not entirely understood. Recent studies show that gut bacteria are influential in the development of obesity. For example, transplantation of gut microbiota from obese into germ-free mice was shown to cause a higher fat mass increase than transplantation of ‘lean’ microbiota. [3] Thus, altering gut bacterial composition can have a direct effect on body weight.

In addition to this, gut microbiota might play an important role in the potential treatment of T2DM. Targeting gut microbiota by antibiotic treatment improves body weight and glucose tolerance of high-fat fed mice.[4, 5] Prebiotic as well as probiotic treatment also improve glucose metabolism in high-fat induced diabetes.[6, 7] Infusion of gut microbiota from lean human donors into subjects with metabolic syndrome resulted in increased
insulin sensitivity, demonstrating the feasibility of gut microbiota modulation for improving glucose homeostasis in a clinical setting.[8]

Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic dysfunction, caused by ectopic fat accumulation in the liver. The link between development of NAFLD and gut microbiota and therefore the existence of the gut-liver-axis has been determined in previous studies. Gut microbiota transplantation from mice with NAFLD to wild-type recipients led to replication of the NAFLD phenotype, showing that NAFLD is transmissible. [9]

The factors that underlie the microbiota alterations in obesity, T2DM and NAFLD are unclear, although the genetic make-up of the host is recognized to play a significant role. Gut microbiota composition and function are strongly affected by endogenous antimicrobial proteins secreted by Paneth cells in the small intestine[10], as well as by mucus components made by intestinal goblet cells.[11] Next to these host factors, diet has a strong impact on gut microbiota. In particular, high-fat diets decrease *A. muciniphila* abundance, a mucus associated bacterium. This bacterium’s abundance was found to be inversely correlated with body mass in mice. [12] Besides this specific bacterium, diet also has an effect on the ratio of two major intestinal bacterial phyla: high-fat diets induce Firmicutes while reducing Bacteroidetes. [13–15]

The data on changes in gut microbiota composition after bariatric surgery are relatively limited. Within three months after Roux-en-Y gastric bypass, gut microbiota has been found to be more diverse with a relative increased abundance of *A. muciniphila*. [16] However, most of these clinical studies have small sample sizes and only analyse faecal samples collected at 12 months postoperatively or less. The possible impact of gut microbiota on failure to maintain weight loss after bariatric surgery is still unknown. This phenomenon, better known as weight regain, can occur in up to 25% of all patients who undergo Roux-en-Y gastric bypass surgery, and can become apparent at 12 to 24 months postoperatively.[17, 18]
The goal of this study is to investigate associations between dietary compounds, intestinal cell function and gut microbiota composition. We hypothesize that dietary lipid intake is associated with Paneth cell and goblet cell properties that affect gut microbiota composition.

Methods

Recruitment
The DIAMOND-study is registered within the Netherlands National Trial Register (NTR560). The protocol was ethically approved by the official Independent Ethics Review Board of Máxima Medical Centre (reference 15.053) in November 2015. Written informed consent will be obtained from all participants. The study will be performed in accordance with the principles of the Declaration of Helsinki as well as the guidelines of Good Clinical Practice.

Aims and objectives
The overall aim is to investigate associations between dietary compounds, intestinal cell function and gut microbiota composition. The primary objective is to determine the relationships between diet, Paneth cell characteristics, and gut microbiota composition in obesity, T2DM and NAFLD. Secondary objectives are assessment of the relationships between diet, intestinal goblet cell and mucus alterations, and gut microbiota composition, as well as changes in these parameters in association with weight loss failure and weight regain.

Study design
This study is conducted as a single centre prospective study and consists of a cross-sectional and a longitudinal part. In the cross-sectional part, differences at baseline between severely obese patients and lean subjects will be studied, focusing on the presence and severity of NAFLD, insulin resistance, type 2 diabetes and intestinal microbiota composition, Paneth cell products and mucus composition. The longitudinal part will focus on changes in microbiota composition, Paneth cell products and mucus in
the severely obese group between baseline measurement and one year after Roux-en-Y gastric bypass.

**Study population**
The study population will consist of three groups: the obese group and two lean control groups.

For the obese group, patients are screened by a multidisciplinary team and approved for surgery according to the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) guidelines. All patients with severe obesity undergoing laparoscopic Roux-en-Y gastric bypass surgery (RYGB) are considered eligible for inclusion. The surgical procedure is performed according to the circular stapling technique described by Dillemans et al.[19]

Further inclusion criteria are a Body Mass Index (BMI) between 35 and 45 kg/m² and willingness to sign the informed consent form. Patients are excluded based on the following criteria: (1) age <18 or >65; (2) presence of type 1 diabetes, substance abuse, inflammatory diseases or neoplasms; (3) chronic use of corticosteroids prescribed by a physician; (4) use of antibiotics in the last three months preceding surgery.

For the two lean control groups, all patients with a BMI between 20 and 25 kg/m² undergoing either upper gastrointestinal endoscopy or a laparoscopic cholecystectomy are eligible for inclusion. Patients are excluded based on the following criteria: (1) age <18 or >65; (2) presence of type 1 or T2DM, substance abuse, inflammatory diseases or neoplasms; (3) chronic use of corticosteroids prescribed by a physician; (4) use of antibiotics in the last three months preceding surgery or endoscopy; (5) presence of cachexia, defined as unintended weight loss (>5% in 1 month or >10% in 6 months).
Flow chart

Data collection

Characteristics
Phenotyping of obesity will be based on measurement of body weight and calculation of BMI. Presence and severity of T2DM will be assessed by analysis of haemoglobin A1c in blood as well as analysis of plasma glucose and insulin levels, both fasting and during an oral glucose tolerance test. Presence of NAFLD will be determined based on the liver biopsy according to the validated Kleiner score. Plasma levels of aspartate transaminase and alanine transaminase will be measured as markers of liver damage. Furthermore, plasma lipid spectrum, total leukocyte count and differentiation count as well as C-reactive protein will be measured.

Dietary habits
Dietary habits will be recorded using a web-based food tracker. All participants will be asked to record their diet for the duration of seven consecutive days at baseline. The obese group will repeat this at one-year follow-up.

Intestinal microbiota composition
Participants will be provided a stool sample collection kit and will be asked to sample their stool prior to surgery. The samples will be stored in the home freezer (-20°C) and transported to the hospital on the day of admission, where they will be stored in the laboratory freezer (-80°C) until analysis. Participants in the obese group will be asked to provide a second stool sample at one-year follow-up.
**Biopsies**

In the both the obese group and the lean control group, the following biopsies will be performed during the laparoscopic surgery: visceral fat (omentum majus), liver, muscle tissue of the abdominal wall and subcutaneous fat. In the obese group, a small segment of the jejunum will be collected during laparoscopic RYGB, a standard element of the procedure, and used for analysis. In the control group, the jejunal full thickness biopsy will be substituted by the endoscopically derived jejunal biopsy in the endoscopy group. Directly after sampling, all tissues will be flash frozen and stored in the laboratory freezer (-80°C) until analysis.

Stored data and materials are only identifiable to the person by an assigned subject number. As such, patient privacy is guaranteed according to the Dutch Personal Data Protection Act.

**Statistical analysis**

**Power**

Sample size is calculated based on the smallest expected difference in the main outcome parameter, i.e. intestinal mucin-2 expression. A pilot study with lean non-diabetic and obese diabetic rats revealed a difference in expression of 1.00±0.71 vs 0.65±0.78. Taking this into account and using a significance level of 0.05 and a power of 80%, each group will require 73 patients. Considering an expected loss to follow-up of 5%, 76 patients need to be included in each group, amounting to 228 patients in total.
Analysis of primary and secondary outcome parameters
A 2-tailed P-value <0.05 will be considered statistically significant. To allow comparison between groups, data will be tested for normal distribution and if appropriate, a Students t-test, Mann-Whitney U-test, Chi-square test or Fisher’s exact test will be used.

Discussion

The field of microbiota research is rapidly expanding and a plethora of links between diseases like obesity, T2DM or NAFLD and gut microbiota composition are currently unravelled. However, the majority of novel findings in this context are based on animal models and remain to be proven in humans. The DIAMOND study aims to identify associations between dietary compounds, gut microbiota composition, and Paneth cell function as well as intestinal mucus characteristics in man.

A limitation of the DIAMOND study design is the inability to prove causality. Cancelling out potential direct effects of dietary compounds on microbiota and metabolism, such as fermentation of dietary fibers to produce short-chain fatty acids [21], is unattainable. In addition, obtaining new biopsies after one year of weight loss to identify the impact of weight loss on primary and secondary outcomes is not implemented in this study because it is considered too invasive.

In conclusion, the DIAMOND study will explore whether Paneth cell function and mucus composition are associated with diet and with alterations in gut microbiota composition, and will investigate the impact of RYGB-induced weight loss on these parameters. This will not only benefit our understanding of weight loss failure and weight regain following bariatric surgery, but might also behold new therapeutic opportunities for obesity and obesity-related comorbidities.

Trial status
Recruitment since February 2016.
Declarations

Ethics approval was granted by the Máxima Medical Centre Ethics Committee (reference 15.053) in November 2015.

Acknowledgements
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Authors' contributions
This is an investigator initiated study.
MU, WL, FvD and SR designed the study. MU, WL, FvD and SR drafted the manuscript. All authors read, edited and approved the final manuscript.

This is an investigator initiated study.

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Conflicts of Interest
None declared.

Abbreviations
BMI: Body Mass Index; EWL: Excess Weight Loss; NAFLD: Nonalcoholic Fatty Liver Disease;
RYGB: Roux-en-Y gastric bypass; T2DM: Type 2 Diabetes Mellitus

References


