The role of intestinal integrity and inflammation in the pathogenesis of insulin resistance and non-alcoholic steatohepatitis in morbid obesity.

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The most important objectives of this study:1) examine the bacterial translocation as a consequence of elevated intestinal permeability in the morbid obese compared to controls, and to proove this is causes an inflammatory status predisposing IR/…

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Glucose metabolism disorders (incl diabetes mellitus)
Study type	Observational invasive

Summary

ID

NL-OMON33407

Source
ToetsingOnline

Brief title
Intestinal permeability in diabetes type 2 and fatty liver disease

Condition

- Glucose metabolism disorders (incl diabetes mellitus)
- Hepatic and hepatobiliary disorders
- Lipid metabolism disorders

Synonym
fatty liver disease, NAFLD, non-alcoholic steatohepatitis

Research involving
Human

Sponsors and support

Primary sponsor: Medisch Universitair Ziekenhuis Maastricht
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: insulin resistance, intestinal permeability, morbidly obese, non-alcoholic steatohepatitis

Outcome measures

Primary outcome

Primary study parameters:

•Intestinal permeability, by measurement of excretion of orally administered

sugars in urine after intake of ibuprofen (2x400mg)

•Plasma endotoxin levels, measured by LAL and ENDOCAB assay

•IR/Diabetes type II prevalence, measured by fasted glucose/insulin plasma

levels and HbA1c

•The extent of NAFLD/ NASH by histopathological stainings of the liver biopsies

Secondary outcome

Secundary study parameters:

•Intestinal damage

-Plasma levels IFABP, ILBP and LFABP

-Histology, gene expression and plasma/urine levels of tight-junction proteins

(claudin, ZO-1, occludin)

•Intestinal microflora

-faecal flora content

•Inflammatory parameters

-Plasma levels CRP, IL-6, TNF-α, MPO, PAI-1, adiponectin, leptin, zinc;

intracellular zinc in leukocytes

-Fat tissue inflammation: gene expression of IL-6, IL-1β, IL-8, IKKβ,

infiltration by macrofages

-Muscle inflammation & insulin sensitivity: IL-6 and IRS phosphorylation

-Liver inflammation: neutrofil infiltration, MPO content, extent of NASH (by

Brunt scoring system)

•Metabolic parameters

-total cholesterol, LDL-cholesterol, HDL-cholesterol, free fatty acids and

triglycerides

•Liver damage

-ALT, AST, LFABP

Other study parameters are general health status, Body Mass Index (BMI),

waist/hip ratio, and relevant medical history.

Furthermore changes in medication intake will be noted as a matter of course.

Study description

Background summary

The incidence of obesity, non-alcoholic fatty liver disease (NAFLD) and insulin resistance (IR)/diabetes mellitus type II (DMII) is strongly increasing. There is an important overlap between the development of obesity, NAFLD and IR/DMII. Obese patients have an increased risk of developing DMII (5-40x) and 20fold increased risk of developing NAFLD, correlated to increasing BMI. Furthermore, IR is in itself an important risk factor to develop NAFLD.
NAFLD can lead to liver inflammation, also known as non-alcoholic steatohepatitis (NASH). Eventually this process can progress to liver fibrosis and cirrhosis, with a high mortality. Currently there are no non-invasive methods to determine whether or not patients suffer from NAFLD, and there is no

treatment yet, other than prevention of weight gain. After weight reduction due to bariatric surgery, liver inflammation diminishes.

In the last decade, researchers showed that activation of the immune system is an important causal factor in the development of IR/DMII and NASH.{zie referenties in het onderzoeksprotocol: Shoelson, 2006 #10}
In mice studies, mice deficient for genes encoding the production of inflammatory cytokines are resistent to the development of obesity-induced DMII. This is illustrated by the fact that tumor necrosis factor (TNF)-α knock-out mice do not develop obesity induced IR [3].
Unfortunately, the source of inflammation in the obese population remains unclear. However, exposure to bacterial endotoxins seems one of the major players in the development of both NASH and IR/DMII. From previous research we know that obese mice and rats develop NASH relatively fast in respone to low dose exposure to bacterial endotoxins {Yang, 1997 #43}.

There is more evidence for the suggestion that living bacteria play a role in the pathogenesis of NASH, since administration of probiotics reduce liver inflammation{Li, 2003 #41}. The most likely source of bacterial endotoxins is the intestine. Strikingly, it is indeed true that mice on high fat diet have relatively high levels of endotoxin in their portal vein {Yoshimatsu, 2004 #44}. More recently, obese mice have a higher permeable intestine leading to higher portal endotoxin levels and as a consequence activation of immune cells in the liver{Brun, 2006 #8}. Another supporting finding is the fact that the luminar bacterial flora in humans influences bodyweight.{Ley, 2006 #22}
Furthermore, the microflora is and intestinal integrity varies in a mice model of obese mice with DMII versus controls, and their insulin resistance improves when they are treated with antibiotics (Cani Diabetes 2007).
The first human results show that, just like in mice, obesity coincides with a relative overgrowth of bacteria of the Firmicutes class, which is reduced after weight reduction. {Ley, 2006 #22},{Turnbaugh, 2008 #106}
However, human data are still scarce. In a study of Creely, endotxin plasma levels in type 2 diabetics was 76% higher than in matched controls{Creely, 2006 #38}. Even though it was unknown whether these patients suffered NASH, it seems presumable since the majority of the type 2 diabetics has NASH. However, the official diagnosis NASH can only be diagnosed with histological liver biopsies.
More recently, in patients with NASH, after aspirin intake, there is a higher intestinal permeability, and a higher level of endotoxins, compared to controls (Farhadi A, Gundlapalli S, Shaikh M, et al Liver Int. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis.2008 Aug;28(7):1026-33).
Moreover patients with NAFLD (preliminary NASH), have higher plasma levels of both endotoxins and inflammatory markers versus controls (Thuy J Nutr 2008).
Overall these studies are performed with small numbers of patients.

Conclusively, obesity coincides with a disturbed balance in intestinal microflora, a higher permeability and elevated plasma endotoxin levels. These

endotoxins induce inflammation and thereby contribute to the development of NASH and insulin resistance.
The treatment of morbid obese individuals with bariatric surgery (laparoscopic gastric banding, gastric bypass, etc.) leads to a significant reduction of incidence and prevalence of DMII and NAFLD/NASH. Interestingly enough, parallel to the improvement of DMII and NASH, there is a reduction of initially elevated inflammatory mediators involved in the pathogenesis of IR/DMII/NASH.
It is therefore expected that also the intesinal permeability and the endotoxin plasma levels will decrease after bariatric surgery.

Study objective

The most important objectives of this study:

1) examine the bacterial translocation as a consequence of elevated intestinal permeability in the morbid obese compared to controls, and to proove this is causes an inflammatory status predisposing IR/DMII en NAFLD

2) Investigate the improvement of intestinal barrier integrity, expected after the weight reduction due to baratric surgery, and subsequent reduction of inflammation IR and NAFLD in the morbid obese.

We herefor use markers such as intestinal damage, microflora, plasma inflammatory markers, inflammation in fat and insulin target organs (liver, muscle) and metabolic parameters.

The hypotheses we wat to further investigate are:

1.Is the intestinal permeability higher in morbid obese with NAFLD and/or DMII compared to morbid obese without NAFLD and/or DMII?

2.Is the intestinal permeability correlated with plasma endotoxin levels in the morbid obese?

3.Do plasma endotoxin levels correlate with inflammation, insulin sensitivity and NAFLD in the morbide obese?

4.Is the intraluminal microflora different in the morbid obese with NAFLD and / or DMII compared to morbid obese without NAFLD and / or DMII?

5.Does the expected weight reduction after bariatric surgery lead to a diminishment of the previously elevated intestinal permeability, endotoxin levels, inflammation, insulin resistence and NAFLD in the morbid obese?

6.Does weight reduction following bariatric surgery change the intestinal microflora in the morbid obese?

Study design

When a patient is eligable to undergo bariatric surgery, he or she is approached to participate in this research project. When the patient decides to participate and meets inclusion criteria, an appointment for further explanations and informed consent is made in outpatient clinic. Prior to surgery an appointment for the intestinal permeability test is made, where participants are asked to drink a glass of water with dissolved sugars, and five hours urine collection takes place.
Before this permeability test, the evening before and the day of the test, patients are asked to take a tablet of 400mg ibuprofen

The patient will be operated following general guidelines. Patients will be asked to collect faeces in advance to evaluate the intestinal microflora. At the day of surgery 20ml blood samples are taken routinely, when a patient participates in the research project, 6ml extra is taken to determine relevant standard measurements. The blood sampling takes place in the surgical ward where the patient awaits surgery, simultaneously with routine blood sampling.

During surgery, biopsies of visceral and subcutaneous fat, muscle and liver are taken. When the operation involves the intestine (in case of a gastric bypass or biliopancreatic diversion) a biopsy of the intestine is taken.

During the standard postoperative protocol for follow-up, related to the bariatric surgery, the patient will return to the outpatient clinic after 6, 12 and 24 weeks, and after one and two years. When patients participate in the research project, compared to otherwise, we will sample blood samples with an extra 6 ml.
After one year postoperatively, a subcutaneous fat biopsy is taken and there will be a second measurement of intestinal permeability (sugars in water, blood and urine sample after intake of ibuprofen).
Furthermore patients will be asked again to collect faecal material at 12 months post surgery.

A schematic design is illustrated in the research protocol (figure 1).

Study burden and risks

Risk of testing sugar substances
The saccharide solution consists of nutritional substances approved by the European Union. These substances have been used previously for research purposes in these amounts and no (side) effects were reported. In this study the amounts proposed are very small and therefore the risk of use is expected to be very small.

The risk of the use of NSAIDs

The non-steroid anti-inflammatory drugs (NSAIDs) administered the evening before intestinal permeability testing and the morning of the test (in a dose of 400mg each time) are commonly used drugs freely available in drugstores. Importantly, the research population, both morbid obese subjects as well as controls, are often familiar with these drugs.
The NSAID are for example prescribed for pain, artritis, headache, migraine, tooth extractions, non-bacterial infections, reumatic exacerbations, oedema and dysmenorroea.

The side effects in these doses are generally mild. Side effects are upset stomach, most prevalent are nausea, vomiting, heartburn, diarrhea, constipation, ulcus in case of chronic use. Central effect such as headache, drowsiness, unusual fatigue and ringing in the ears (tinnitus) may occur. Very seldom allergies can occur, such as skin reactions (rash, itching, swelling). However in our population and in the prescribed dosage, these side effects are most unlikely.
The expected risk is therefore minimal. To further reduce the risk evidently each patient will be thoroughly questioned about previous drug use and previous experiences.

Risk associated with interventions
Blood sampling will take place in outpatient clinic, as a service to the patients, by an experienced physician. Usually, when patients do not participate in this research projects, they have to turn to the general hospital blood sampling laboratory. However, we will make sure patients are sampled for trivial standard follow-up and additionally take 6ml extra blood for research purposes.
The blood is withdrawn from the lower arm, the procedure causes little discomfort. The risk is neglicable, it is a standard procedure in this hospital. The only important risk is when the vein is not correctly found, there can occur a blue (hematoma). This will however vanish within days.

The subcutaneous fat biopties are sampled in outpatient clinic as well, simulaneously with a blood sample, in outpatient clinic when the patient would otherwise also come to the hospital for standard postoperative follow-up.
If necessary, supervision can be present (dr. Bouvy, surgeon in the MUMC+, and dr. Greve, surgeon in the Atrium Medical Centre).
The procedure is as follows: primarily a standard needle (also used for blood sampling) is introduced at the previous operation site to prevent scarring and in order to give a local anaesthetic. Hereafter the procedure is no longer painfull for the patient (and of course this is also tested).
Maximal 100 mg subcutaneous fat tissue is excised. This is small enough to leave no cosmetic damage. Possible risks are the occurence of an hematoma or a superficial infection at the site of intervention. In advance, participants are explained what they can and cannot expect and in case of doubts or troubles are requested to contact the researcher.
If necessary, the previously mentioned surgeons can be contacted to reassure

further treatment in case of infection.
However this procedure is performed approximately 80 times in the morbid obese in the MEC 04-141 and there were no infections. 2 (!?) patients suffered an hematoma.

The biopsies taken peroperatively (liver- fat- muscle- and intestinal biopsies if possible) don't cause discomfort since they are taken by the surgeon performing the operation, and the patient is under general anaesthesia at the time of collection.
One possible complication is bleeding at the site of the biopsy after collection (only in the case of fat, muscle and liver).
However in the current study **Role of fatty tissue resident macrofages in the pathogenesis of insulin resistence in the morbid obese* (MEC 04-141) over 130 participants have been treated comparably. None of the biopsies caused bleeding.

In a similar study of 515 morbid obese where similar biopsies were taken during gastric banding procedures, there was no complication reported (35).
We do not expect any complications, but in case they might occur, biopsies are always taken under clear view and possible bleeding can be treated immedeately by the surgeon.
Given the small risk of complications and the unique oppertunity to study this material and get valuable information about the inflammatory status of the patients liver, insulin target organs and the functional integrity of the intestine, we consider the procedure of taking these biopsies justified.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Researchpopulation:
• BMI >40 or BMI>35 kg/m2 with co-morbidities
• planned for bariatric surgery
• Age 18-60 years (to prevent inclusion of patients who are unable to give informed consent and to have inclusion criteria adjusted to the criteria for surgical intervention)
Control population:
• BMI 20-25
• planned for Nissen fundoplication or cholecystectomy
• Age 18-60 years old

Exclusion criteria

For all patients:
• Age <18 or >60 years
• Diabetes Mellitus type I
• Inflammatory diseases such as auto-immune diseases (due to a higher inflammatory profile)
• Degenerative disase
• Alcohol or drug abuse
• use of corticosteroids and / or prednisolone (confounding effect on inflammatory status)

Study design

Design

Study type:	Observational invasive
Intervention model:	Other

Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	07-09-2010
Enrollment:	190
Type:	Actual

Medical products/devices used

Registration:	No

Ethics review

Approved WMO

Date:	26-08-2009
Application type:	First submission
Review commission:	METC Z: Zuyderland-Zuyd (Heerlen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL27933.096.09