Immunomodulatory effects of Inulin-type fructans in a hepatitis B vaccination study

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Other condition
Study type	Interventional

Summary

ID

NL-OMON41596

Source
ToetsingOnline

Brief title
Inulin-type fructans and immune modulation

Condition

- Other condition

Synonym
vaccination efficacy, vaccine-specific antibody response

Health condition

vaccination efficacy in healthy individuals

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: Carbohydrate Competence Center; Top Instituut Food and Nutrition

Intervention

Keyword: dietaryfiber, hepatitis B, inulin, vaccination

Outcome measures

Primary outcome

Hepatitis B vaccine-specific antibody titers:

Blood samples of 10 mL per timepoint of all subjects will be taken at day 21 and 35 of the study, for measuring antibody titers by ELISA hepatitis B antibody-specific microtiter plates.

Secondary outcome

Secondary study parameters:

1: Peripheral immune cell populations:

Blood samples (10 mL heparinized) of all subjects will be taken at day 0, 7, and 14, for analysis of changes in immune cell populations. Blood will be stimulated with a general stimulator (PMA) for stimulation of all T-helper cells, or left unstimulated. Immune cells will be stained with fluorescent antibodies, and changes in immune cell populations e.g. T-helper cells and NK-cells will be determined using flow cytometry.

2: Fecal parameters:

Fecal samples will be collected by the subjects weekly starting at day 0 until day 35. Sterile containers and small scoops for this purpose will be provided

by the principle investigators and samples will be stored by the subjects at

-20ºC and delivered to the UMCG at day 7, 14, 21, and 35. Fecal IgA

measurements will be performed using capture ELISA assays. Fecal SCFA levels

will be measured and fecal microbiota profiles will be analyzed using

HIT-chip/pyrosequencing.

Study description

Background summary

Beneficial health effects of dietary fibers have gained considerable attention by the scientific community during recent years. Fibers can be found in many products such as cereals, bread, pasta, vegetables as well as in sugar-, and fat replacers. High fiber intake is associated with lower mortality in humans suffering from circulatory, digestive, and non-cardiovascular noncancerous inflammatory diseases (Jacobs et al. 2007,Park et al. 2011,Chuang et al. 2012). These associations are similar for men and women and are observed in most countries even after careful adjustment for potentially confounding lifestyle and dietary differences (Landberg. 2012). However, all these studies have applied mixtures of fibers rather than specific types of molecules. Therefore, it is currently still unknown which type of fiber(s) are responsible for these effects. There is an urgent need for studies addressing specific fibers (Landberg. 2012) to obtain a better understanding of what mechanisms and which components underlie beneficial health effects. There are different types of dietary fibers. In this study Inulin type fructans (ITFs) and resistant starch corn (RSC) will be studied.
Inulin-type fructans (ITFs) are a specific type of dietary fiber, isolated from chicory root and sugar beet root. Upon supplementation in food, ITFs have been reported to enhance immunity in the gut and to lower frequency of infections and to alleviate symptoms of colitis (Leenen and Dieleman. 2007) and even allergy (Schouten et al. 2011). These ITFs can be found in many food products in which sugar beet and chicory has been applied. Before the beneficial effects were discovered, ITFs were already widely and safely used in various products as sugar-, and fat replacers, and applied as texturizers in *light* food products (Hidaka et al. 1991).
ITFs are marketed as so-called prebiotics, meaning that ITFs support the function and growth of microorganisms in the intestine such as gut lactobacilli (Leenen and Dieleman. 2007,Casellas et al. 2007) and bifidobacteria (Leenen and Dieleman. 2007,Lindsay et al. 2006). The beneficial effects of these fibers are thought to derive from the growth and activity of these bacteria, as well as

from the increases in their fermentation products, such as Short Chain Fatty Acids (SCFAs) (Meijer et al. 2010,Vinolo et al. 2011). These bacteria are by this mechanism considered to induce immune effects, such as increasing the number of IgA-producing plasma cells (Guilliano et al. 2001,Woof. 2002), increasing or improving phagocytosis (Nagl et al. 2002), or increasing the proportion of T lymphocytes and Natural Killer cells (Reid et al. 2003,Ouwehand et al. 2002).
However, our recent research showed that ITFs can do more, i.e. they can directly affect immune cells. Specific ITFs can bind and stimulate specific pattern recognition receptors (PRR) in humans. PRRs are the sensors of the human immune system and determine whether an immune response will occur. The best characterized family of PRRs is that of the Toll-like receptors. We found on human reporter cell lines that ITFs stimulate specific Toll-like receptors and in particular TLR2. This stimulation of TLR signaling is considered to be important, as TLR signaling in gut dendritic cells enhances antigen uptake and presentation (Veldhoen et al. 2008) and therefore stimulates immunity. The effects of ITFs were, however, dependent on the molecular weight of the ITFs applied. Low molecular weight ITFs were found to have a more regulatory effect on human leukocytes (isolated from blood) while high molecular weight ITFs activate the immune response. We especially found a difference in the IL-10/IL-12 ratio, which is considered to be regulatory when high and stimulating when low. These data not only suggest that ITFs can directly affect immune responses, but also that high and low molecular weight ITFs may have different in vivo effects. These findings made us decide to perform a pilot phenotyping study of human immune cells when the diet was supplemented with 8 grams of low molecular weight ITFs. This was done as part of an educational training in which third year life science students were trained in immunological techniques with their own blood. We found even after a short term intake of not more than 7 days and in a very small study, that the frequency of T helper 1 (Th1) cells, as well that of regulatory T cells both showed increased trends. This prompted us to verify these data in a larger study.
The other fiber supplement that will be studied in this research is RSC. From earlier experiments, which are performed in UMCG and the university of Wageningen related to Th1 or Th2 immune skewing. RSC showed to be a strong Th1-stimulating fiber (see C2 Samenvatting amendement for more details).
Resistant starch is starch that is not digested in the small intestine, and enters the large bowel. Resistant starch is a type of dietary fiber that is found in many different (plant) food products like beans, whole vegetables, whole grains and rice. There are four types of resistant starch: RS1, RS2, RS3 and RS4. Ingestion of resistance starch may be useful in management of the metabolic (glycaemic) control and reduction of appetite (Robertson 2012, Bodinham 2010, Bodinham 2014). Also a role in the reduction of inflammation has been suggested (Higgins 2013, Moreau 2003). The fermentation of resistant starch predominately takes place in the proximal colon. The fermentation of resistant starch results in the production of short chain fatty acids (SCFAs) (Cummings 1987). Until now, only little research has been performed evaluating the potential immunomodulatory capacity of resistant starch (Vos 2007). The

choice of the fiber RSC in these vaccination protocol increases the window to observe fiber mediated vaccination efficacy enhancement. Considering the data of these experiments performed in the UMCG and the university of Wageningen related to Th1 and Th2 immune skewing, the above mentioned RSC seems to be a well argumented choice to improve Th1 responses.
To be able to show more robust effects of ITF or RSC intake, we will include a vaccination study in the present protocol. Vaccination efficacy studies are often applied to demonstrate stimulation of immunity by food components (Olivares et al. 2007,Soh et al. 2010) and is therefore an accepted method to study effects of food components. As we found that ITFs stimulate TLR2 which is involved in enhancing antigen-uptake (Blander and Medzhitov. 2006,Underhill et al. 1999), and as we found a trend in increased Th1 and regulatory T cells we expect that ITFs may be effective in boosting vaccination responses. We also expect differences in efficacy with the different molecular weight as they induce different responses on human leukocytes. For the reasons described above, we also expect that RSC may boost vaccination response in a Th1-dependent manner.
A vaccination protocol that might benefit from a stimulation of the immune system is hepatitis B vaccination. This vaccination protocol is applied at the UMCG as a mandatory vaccination for third year bachelor-students of Life-sciences and Medicine. At the moment of their study, students of Life-sciences and Medicine start research training at the UMCG in which they might have to handle human material. Hepatitis B vaccination usually has a low efficacy, since most people have to undergo three shots of vaccination before they reach protective immunoglobulin titers. Especially the first shot is characterized by low efficacy. This, however, is an advantage for our study as we may be able to increase the efficacy of the first vaccination.

Study objective

The present study has 1 primary and 1 secondary objective:

1: The primary objective of the present study is to analyse the effects of supplementation with ITFs of different chain lengths and RSC on hepatitis B vaccination efficacy. The main parameter to study will be hepatitis B vaccine-induced antibody titers.

2: To study underlying and concomitant mechanisms, modification of various immune cell populations will also be evaluated. To evaluate whether the effect of ITFs and RSC is induced by changing the microbiota, changes in microbiota and levels of IgA in the feces will be analysed.

Study design

The format of the present intervention study is a double blind randomized placebo-controlled trial. Male and female subjects will be recruited from a

population of students working in the University Medical Center Groningen (UMCG), which require a hepatitis B vaccination in order to safely work with patient material during their bachelor. The study population will consist of individuals in the ages ranging from 18 to 35 years. Equal numbers of subjects will be at random allocated into the following groups: I) receiving 8g/d of low molecular weight ITFs (Frutafit®CLR), II) receiving 8g/d high molecular weight ITFs (Frutafit®TEX!), or III) receiving 8g/d of RSC, IV) receiving 8g/d of fructose, which serves as a placebo and is accepted by the scientific community as an appropriate control. All supplements will be dissolved in 50 ml of fiber-free lemonade before administration to mask any taste effects and to standardize the way of intake. In each group, the subjects will receive a vaccination against hepatitis B on day 7 of supplementation, and supplementation of fibers will continue for the following 7 days (thus 14 consecutive days in total), since several dietary intervention studies around vaccination protocols have shown that the period around the vaccination is crucial in the development of the antibody response and this period is most important when attempting to boost the response. The study subjects are asked to fill in a nutrition diary and to refrain from intake of pre-, and probiotic nutritional supplements outside of the specified study supplements.

Although our primary outcome is antibody titers following ITF or placebo intake, we will also evaluate the effects of ITF intervention on immune cell populations. Therefore, baseline blood samples will be collected from all subjects before the start of supplementation (day 0), and blood samples will be collected at day 7 (prior to vaccination), 14, 21, and 35 (on each time point 10 ml of heparinized blood will be collected per subject). Blood sampling will be performed by trained professionals from the department of Laboratory Medicine of the UMCG. To study peripheral immune cell populations, leukocytes in whole blood will be stained for relevant cell markers to distinguish different populations using flow cytometry.

T cell responses are expected to arise from day 4 post-vaccination onward. Therefore T-cell responses will be analyzed in the blood samples collected from each vaccinated subject at days 0, 7, and 14. Antibody titers will be analyzed in the blood samples collected from each vaccinated subject at days 21 and 35; this will be 14 and 28 days post-vaccination, to monitor titer amounts in time. Antibody levels in blood samples will be analyzed using antibody-specific ELISA microtiter plates.

Finally, microbiota composition and the short chain free fatty acid (SCFA) profile (which are products produced by bacteria) as well as levels of fecal IgA will be analyzed to evaluate effects of ITFs on microbiota. For these parameters, fecal samples will be collected weekly from day 0 (baseline microbiota and IgA) to day 35.

Intervention

The subjects in this study will receive dietary fiber supplements (low molecular weight ITF, high molecular weight ITF or RSC) or fructose as a placebo that are safe, food grade compounds, which do not have any negative side effects. 8g of fibers or placebo per day, dissolved in 50ml of fiber-free lemonade (per day) will be given to healthy subjects, who then drink this solution. The used concentration is in accordance to literature an effective dose to stimulate the immune system (Gibson et al. 1995,Langlands et al. 2004). An educational assignment in which students consumed this amount to study their changes in immune cell populations demonstrated that the subjects did not have difficulties consuming this amount of fibers.

Study burden and risks

Collection of fecal samples does not carry any risks for the participants. Blood sampling will be performed by trained professionals from the department of Laboratory Medicine of the UMCG and will comprise 1 tube containing a volume of 10 ml of blood per person per sampling time point, in total 50ml of blood will be drawn, spread over a time period of 35 days. Blood will be drawn from the median cubital vein on the inside of the elbow. This may cause a small hematoma (*blauwe plek*) at the site of sampling, however in general when pressure is applied to the sampling site just after taking the sample this does not occur. The hepatitis B vaccination which is included in this study takes place in a safe and approved vaccination program which has been running for several years. Since this study population would already receive this vaccination for the safety of their student career while working in the UMCG it poses no extra burden on these individuals. The availability of the vaccination does not rely in any way on participation in this study. There are no benefits for the individuals participating in this study. The benefits of this study are represented by a gain in knowledge on the immunomodulatory effects of ITFs and RSC, and in the case that supplementation boosts the production of vaccine-specific antibodies, intake can be considered beneficial during hepatitis B vaccination programs. Furthermore, knowledge can be gained on the underlying effects such as shifts in immune cell populations and IgA production. By studying microbiota and SCFA profiles, the induction of bacterial strains can be evaluated and this can generate new hypotheses on the relation between these bacteria and the observed immunological benefits.

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

Age 18-35 yr.
Healthy subjects
Male and female subjects
Caucasian subjects
Subjects enrolled to be vaccinated against Hepatitis B
Written informed consent
Dutch speaking subjects, i.e. subjects understanding spoken and written Dutch language

Exclusion criteria

Presence of acute or chronic diseases
Gastrointestinal disorders (e.g. inflammatory bowel disease, celiac disease)
Gastrointestinal surgery
Treatment with antibiotics within 6 months of the start of the study
Prior vaccination with hepatitis B
Previous hepatitis B infection
Immunodeficiencies
Use of anti-coagulant drugs

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)
Control:	Placebo
Primary purpose:	Other

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	16-09-2013
Enrollment:	48
Type:	Actual

Ethics review

Approved WMO Date:	19-08-2013
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)
Not approved Date:	12-12-2014
Application type:	Amendment
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)
Approved WMO Date:	06-07-2015
Application type:	Amendment
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

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

ID: 21512
Source: Nationaal Trial Register
Title:

In other registers

Register	ID
Other	14815 (NTR)
CCMO	NL41644.042.13
OMON	NL-OMON21512