Insights into the pathophysiology of Hashimoto thyroiditis: Assessing thyroid Reserve capacity & the role of the Gut micrObiome in thyroid hormone metaboliSm

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Primary objective: To assess and compare the difference in thyroid gland secretion capacity by measuring maximal FT4 and FT3 response upon intramuscular administration of 0.9mg Thyrogen assessed by AUC0-48hours in SCT subjects and healthy controls....

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Thyroid gland disorders
Study type	Interventional

Summary

ID

NL-OMON49953

Source
ToetsingOnline

Brief title
ARGOS

Condition

- Thyroid gland disorders

Synonym
autoimmune hypothyroidism, Hashimoto's thyroiditis

Research involving
Human

Sponsors and support

Primary sponsor: Academisch Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: autoimmune hypothyroidism, dynamice thyroid function test, microbiome, thyroid hormone metabolism

Outcome measures

Primary outcome

Primary endpoint is difference in residual thyroid function (stimulated FT4 and FT3 response upon administration of 0.9 mg Thyrogen AUC0-48h) between SCT subjects and healthy controls. The AUC values will be derived according to the trapezoidal rule. AUC mean 0 - 48 hours denotes the AUC0-48h divided by the length of the time periode (i.e. 48h) and hence gives the mean level during the time period 0-48 hours.

Secondary outcome

- Changes in residual thyroid function (stimulated FT4 and FT3 response upon administration of 0.9 mg Thyrogen AUC0-48h) in SCT subjects and healthy controls after a short course of antibiotics.

Thyroid hormone metabolism

- Evaluate differences in thyroid hormone metabolism in SCT and healthy controls, using fecal excretion of T3 and T4.
- Evaluate differences in thyroid hormone metabolism in SCT and healthy controls, using fecal excretion of T3 and T4 after thyroid stimulation test.
- Evaluate the effect of the gut microbiome on thyroid metabolism in SCT vs

healthy controls before and after ATB challenge before thyroid stimulation test.

- Evaluate the effect of the gut microbiome on thyroid metabolism in SCT vs healthy controls before and after ATB challenge after thyroid stimulation test

Other study parameters (if applicable)

Inflammation

Evaluate the effect of thyroid stimulation on the gut microbiome of SCT and healthy controls before, during and after short term antibiotics course on plasma inflammatory markers and FACS (evaluation criteria = changes in PBMC phenotype)

Gut microbiome composition

Change in relative abundance of bacteria taxa and bacterial gene richness by 16s rRNA profiling during several timepoints. Also, plasma metabolite composition will be determined. Finally intestinal transit time will be determined by Sitzmark Transit-Pellets.

Study description

Background summary

Autoimmune hypothyroidism is a T-cell mediated autoimmune disease which is characterized by chronic lymphocytic infiltration of the thyroid gland leading to progressive and irreversible destruction of thyrocytes. This disease can either be clinical evident with an absolute hormone deficiency - Hashimoto*s thyroiditis (HT) - or subclinical, which is defined as an elevated serum TSH level in combination with the presence of anti-TPO antibodies and a serum free

thyroxine (FT4) level that is within normal range. Despite sufficient serum thyroid hormone levels, subclinical hypothyroidism is associated with an increased risk of hypothyroid symptoms and cardiovascular events, particularly in subjects with a TSH level > 10 mU/L. In this respect, subclinical hypothyroidism can be seen as an early stage of HT with still sufficient thyrocytes left to maintain serum thyroid hormone levels.
Hashimoto thyroiditis is the most common endocrine autoimmune disease of which the prevalence has increased over the past years, from 0.4% to 2.9% during 10 years in the Netherlands alone. Considering this steep increase, novel preventative and/or therapeutic opportunities are greatly needed as the current treatment consists of continuous hormone treatment rather than affecting disease progression.

Need for a dynamic thyroid function test to test thyroid reserve capacity
To test the effect of such a potential new intervention in pathophysiology of autoimmune hypothyroidism (i.e. reduction of the ongoing gradual thyroid failure), a standardized outcome measure which assesses the residual thyroid capacity is needed. To date, the current modern assays for thyroid hormone levels (serum TSH, FT4 and FT3) represent a static functional test and thus do not reflect thyroid reserve. Thyroid response upon administration of recombinant human TSH (rh-TSH; Thyrogen©) could help to determine residual thyroid function. Such a dynamic function test has already been used in current clinical practice in the follow-up of patients with differentiated thyroid carcinoma. Moreover, recent trials showed that a single dose of 0.9mg Thyrogen© in healthy individuals without thyroid dysfunction leads to a temporary significant increase in serum thyroid hormone (TH) levels without severe adverse events. As far we know, yet no published clinical study exists validating a rh-TSH driven dynamic stimulation test in autoimmune hypothyroid patients. Thus, the principal aim of this study is to validate a dynamic thyroid function test to assess thyroid reserve capacity, by measuring maximal serum FT4 and FT3 response upon an single intramuscular administration of 0.9mg rh-TSH (Thyrogen©). Therefore, we will include 10 patients in the early stage of Hashimoto*s thyroiditis (subclinical autoimmune hypothyroidism) and 10 healthy volunteers as a control. We will perform this test twice to assess the reproducibility.

Gut microbiota involved in thyroid hormone metabolism
The thyroid gland produces both the prohormone thyroxine (T4) and the biologically active triidothyronine (T3). When secreted into the circulation they are either bound to proteins (albumin, thyroid binding globulin or transthyretin) or unbound (free T4 or free T3). Circulating thyroid hormones (TH) can be metabolized by a number of different pathways of which deiodination and conjugation of the phenolic hydroxyl group with either sulfate or glucuronide are the major pathways.
About 20% of daily T4 production appears in feces, predominantly via biliary excretion of glucuronide conjugates. Conjugation of iodothyronines enhances the biliary and/or urinary clearance, however, these pathways are reversible.

Specifically, after the rapid excretion of iodothyronine glucuronides (T4G and T3G) in the bile, these conjugates can be hydrolyzed by intestinal obligatory anaerobes by the bacterial enzyme *-glucuronidase. This process promotes the intestinal reabsorption of free thyroid hormone into the enterohepatic circulation were they can be reutilized. These findings suggest that glucuronidated iodothyronines may serve as an intestinal thyroid hormone reserve, which may prevent fluctuation of serum TH levels. Evidence to support this metabolic symbiosis between host and gut microbiome is demonstrated in previous studies, showing that in absence of intestinal bacteria (using fecal suspensions from germfree rats as well as from orally decontaminated rats) only very little of the glucuronidated iodothyronines were hydrolyzed, resulting in lower fecal T3 excretion when compared to fecal samples of conventional raised rats. However, these mentioned findings are mostly identified using rat models or in-vitro experiments and warrants to be validated in humans.

More recently, two papers have evaluated the gut microbiome composition in fecal samples of HT patients. Both studies found an association between alterations in the gut microbiome and HT disease compared to matched healthy controls. However, it remains unknown whether dysbiosis in those patients also leads to an alteration in the abovementioned metabolic pathways of iodothyronines and subsequent enterohepatic circulation. A better understanding of the (patho)physiological pathway of the gut microbiome involvement in thyroid hormone metabolism in autoimmune hypothyroidism is needed in search for possible effective therapeutic options. Therefore, the second aim of this study is to evaluate the effect of the gut microbiome on thyroid hormone metabolism in subclinical autoimmune hypothyroid subjects.
Moreover, using a short course of antibiotics to temporarily deplete the gut microbiota will help to discern if indeed gut microbiota are involved in the enterohepatic circulation of thyroid hormones in human (as seen by the decreased fecal T3 and T4 excretion after the antibiotic course). To do so, we will apply a methodology already published and validated for other studies, investigating the role of the microbiome in production of metabolites (e.g. TMAO, ImP)

In conclusion, an altered intestinal microbiota composition has been implicated to play an important role in (human) metabolism, as well as in autoimmune diseases. Furthermore, recent studies have shown that HT patients display changes in the pro- and anti-inflammatory phenotype of immune circulating cells as (PBMCs) as well as in thyroid tissue. It has been demonstrated that thyroid tissue of HT subjects highly expressed CD20+ B-cells, CD4+ and CD8+ T-cells and, surprisingly, FoxP3+ T-regulatory cells, whereas this was not seen in thyroid tissue of healthy subjects. Therefore, a parallel objective of this study is to assess the immunological status (T cells, B cells and cytokines in peripheral blood) of subclinical autoimmune hypothyroid subjects, using imaging mass cytometry.

Study objective

Primary objective: To assess and compare the difference in thyroid gland secretion capacity by measuring maximal FT4 and FT3 response upon intramuscular administration of 0.9mg Thyrogen assessed by AUC0-48hours in SCT subjects and healthy controls.

Secondary objectives:
Secondary objectives will be influence of gut microbiome on thyroid hormone metabolism and effect of thyroid hormone stimulation on inflammatory status.

Gut microbiome composition:
- To assess and compare changes in the gut microbiome composition between SCT subjects and healthy controls at baseline.
- In addition, microbiota composition will be determined and compared upon thyroid hormone stimulation and before and after short term antibiotics course.

Thyroid hormone metabolism:
- To assess and compare changes of fecal excretion of T4 and T3 in SCT subjects and healthy controls before and after thyroid hormone stimulation.
- In addition, we will determine changes of fecal excretion of T4 and T3 before and after short term antibiotics course.

Immunologic parameters:
- To assess and compare changes in immunological status, based on FACS on peripheral blood mononuclear cells (Th1, Th2, Th17, Treg, B cells), cytokines and markers of thyroid autoimmunity (anti-TPO antibodies) in SCT subjects as compared to healthy controls at 0h, 5h and 48h after thyroid stimulation and before and after a short term antibiotics course.

Intestinal transit time
- Evaluate the differences of intestinal transit time as assessed by radiopaque makers between SCT subjects and healthy controls.

Study design

This is a prospective, single-centre intervention study. The study duration is 1.5 month (start seven days before intervention and then lasts 32 days) during which the faecal sample collection and the dietary questionnaire can be carried out by the subjects at home. Subjects are requested to visit the AMC 7 times, including the screening visit. They will spend a total of 20,5h in the AMC (table 1 and figure 5).
The study is designed to validate a dynamic thyroid function test (TFT) and evaluate thyroid hormone metabolism after stimulation of the thyroid gland and link the expected changes in metabolism to the gut microbiome. Therefore, we

will include 10 subclinical autoimmune hypothyroid subjects and 10 healthy controls. For the dynamic TFT serum thyroid hormone levels will be drawn at timed intervals after intramuscular administration of 0.9mg Thyrogen. To test validity of this dynamic TFT, this same regimen is repeated after two weeks. In discern if gut microbiota are indeed involved in the enterohepatic circulation of thyroid hormones, the third dynamic TFT test will be preceded by a short course of an oral antibiotics cocktail to suppress the gut microbiome. The seven-day antibiotics regimen will consist of metronidazole (500 mg twice daily) plus ciprofloxacin (500 mg once daily), plus oral vancomycine (500mg four times daily). In total a volume of 175 ml blood will be drawn per subject during the complete study period of 3 months.

Intervention

3x 0.9mg Thyrogen injection intramuscular.

Study burden and risks

The proposed administration of 0.9mg Thyrogen has previously been shown to be safe in a clinical study. The recorded adverse events were related to hyperfunction of the thyroid gland (tachycardia, increased appetite, restlessness, headache and nausea) and symptoms related to possible thyroid growth (visual enlargement of the thyroid gland or tenderness). However, these symptoms and discomfort were of short duration and self-limiting, appearing between 4h and 48h after injection. Importantly, the subjects of this study will be monitored during this timeframe with the following visit 48h after administration. Furthermore, inclusion criteria were chosen to select only relatively healthy patients without prior cardiovascular disease to limit any potential risk.

The placing of the intravenous cannula in our study can be an unpleasant experience for the subjects and may result in (self-limiting) bruising.

Transit-Pellets (radiopaque markers) are used for determing the internal transit time. On day seven, an abdominal X-ray is made to count the markers (if still present in digestive tract). In total 3x 0.7mSv per x-ray amounts to 2.1mSv in a year that equals the yearly average background radiation exposure).

For the antibiotics, the most commonly reported adverse reactions are nausea and diarrhoea. Furthermore, vomiting, headache, taste disorders, allergic reactions, mycotic superinfections or rupture of the achilles tendon are rarely mentioned.

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

Healthy controls:
- Caucasian
- 35 - 70 years
- BMI 18 - 30 kg/m2
- Able to give informed consent

SCT:
- Caucasian
- 35 - 70 years
- BMI 18 - 30 kg/m2
- Able to give informed consent
- Recent diagnosis of SCT:

o TSH >= 10 mU/L, FT4 within normal range and anti-TPO positive

Exclusion criteria

For all subjects:
- Use of any medication including levothyroxine, proton pump inhibitors, antibiotics and pro-/ probiotics in the past three months
- Diagnosis or symptoms of other autoimmune disease (e.g. T1D, coeliac, rheumatoid arthritis or inflammatory bowel disease like Crohn and colitis ulcerosa)
- History of cholecystectomy
- Smoking or illicit drug use (MDMA/amphetamine/cocaine/heroin/GHB) in the past three months or use during the study period
- Pregnant or lactating women
- Previous intestinal (e.g., bowel resection/reconstruction) surgery
- Chronic illness (including a known history of heart failure, renal failure (eGFR <30 ml/min), pulmonary disease, gastrointestinal disorders, or hematologic diseases), or other inflammatory diseases

Study design

Design

Study type:	Interventional
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	07-12-2020
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO Date:	13-08-2020
Application type:	First submission
Review commission:	METC Amsterdam UMC

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	NL74358.018.20