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Thyroid axis challenges in Leiden Longevity Study

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Chapter 2

Study design and data collection TSH and T3 study

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The THYRAGE (Resetting the THYROID axis for prevention of AGE-related diseases and co-morbidities) consortium consisted of six partners in five countries that started collaborations in January 2016 with the aim of investigating the role of the thyroid axis in resetting age related diseases. As part of this consortium, at Leiden University Medical Center (LUMC) two clinical challenge studies with thyroid drugs were performed in participants from the Leiden Longevity Study (LLS) in the period from March 2016 until September 2018.

Screening of participants

A subset of participants from the LLS(1), with a preference for subjects from the Switchbox study(2), who had TSH levels higher in offspring from long-lived families than in controls, were approached for screening through databases provided by the Department of Molecular Epidemiology, LUMC.

All interested subjects were globally screened by telephone (for history of cardiac, thyroid or endocrine disease) and upon passing this screening, were invited for a medical screening at the LUMC. The medical screening consisted of patient history, standard physical examination, a 10-second ECG recording in resting state and a single blood withdrawal. The following were measured at screening from fasted blood samples obtained and analysed by the Department of Clinical Chemistry and Laboratory medicine: sodium, potassium, creatinine, gamma GT, ASAT, ALAT, HbA1c, glucose, insulin, fT4, TSH, TPO antibodies, haematology (haemoglobin, haematocrit, erythrocytes, mean cellular volume, mean corpuscular haemoglobin (concentration), reticulocytes, leukocytes, erythroblasts). The inclusion and exclusion criteria were the same for both studies, and are noted in Table 1. Upon passing the medical screening, the participants had to participate in the study days within 8 weeks in order to minimise the risk of changes in health status between screening and study days.

Table 1. Inclusion and exclusion criteria for the TSH and T3 study.

Inclusion criteria	Member of Leiden Longevity Study, with higher TSH in offspring than in control as measured at baseline of Switchbox study.
Exclusion criteria	Cardiac arrhythmias (History) of thyroid diseases TSH level > 4.0 mU/l fT4 level outside of normal range (9-24 pmol/l) Any significant chronic disease Renal, hepatic or endocrine disease Hormone therapy Difficulties inserting an intravenous line Recent participation in other research projects (within the last 3 months), participation in 2 or more projects in one year Nicotine abuse or (History of) alcohol abuse (>28 units per week) Evaluation by the physician as too frail to participate

According to Dutch rules and regulations regarding research involving human subjects, the study protocol was submitted to two authorities, namely the local medical ethics committee (in April 2016) and the National Competent Authority (in May 2016) for approval. The Declaration of No Objection (DNO) from the National Competent Authority was received in May 2016 and the approval by the local medical ethics committee was granted in July 2016. The Board of Directors granted permission in July 2016 for performing the study at the LUMC. After these approvals, the inclusions of study participants could begin.

Between September 2016 and August 2018, 83 out of 135 participants in Switchbox were approached for participation in the TSH study, out of whom 30 were included in

the study (further details in Chapter 4). Participants who did not display the necessary TSH phenotype (higher TSH in offspring from long-lived families than in controls) were not approached. All TSH study participants were approached for the T3 study, however, only 25 could be included. An additional 8 participants from the original Switchbox pool were approached to try to reach 30 inclusions, however, only 2 could be included. The T3 study inclusion was completed at 27 participants (further details in Chapter 5). The timeline and number of inclusions for the TSH study and T3 study is shown in Figure 1 and Figure 2, respectively.

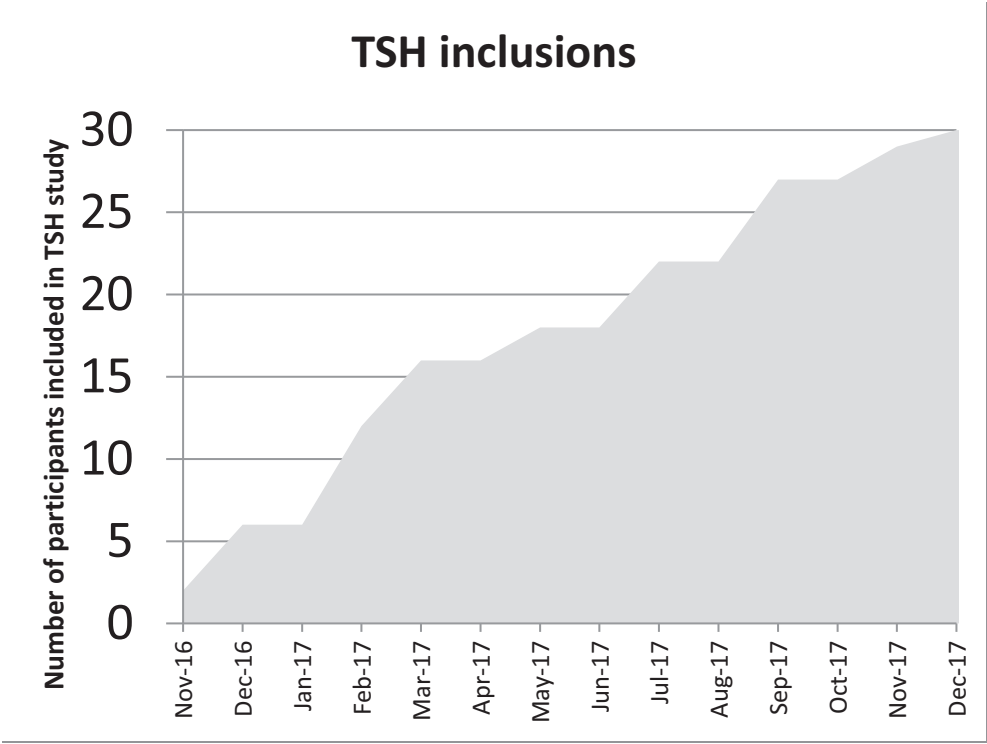


Figure 1. Number of participants and timeline of TSH study.

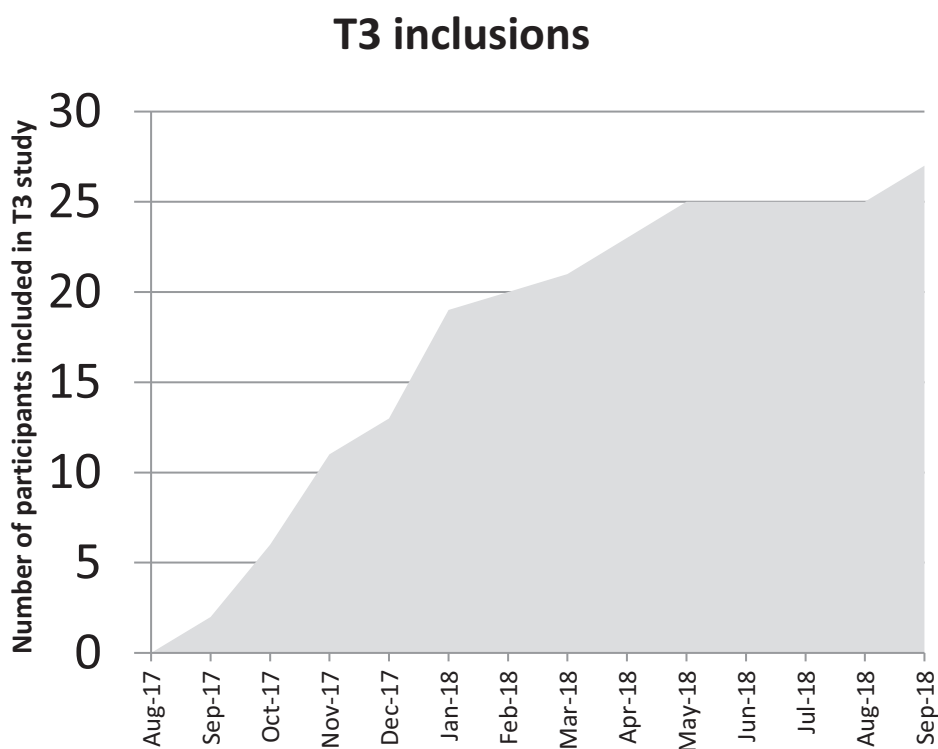


Figure 2. Number of participants and timeline of T3 study.

TSH study was always the first in order of participation. For participation in the T3 study, a minimum of three months since participation in the TSH study must have passed in order to allow adequate wash-out period of TSH study intervention and blood withdrawals on outcomes in the T3 study. Within each study, order of participation was at random, except for couples participating together. Per week, one to three subjects participated in the study, due to logistical reasons. The first TSH challenge study took place in November 2016, the last in December 2017. The first T3 challenge study took place in September 2017 and the last in September 2018.

Data collection TSH and T3 study

Blood sampling

At both studies, samples of serum, EDTA, PAX gene and whole blood samples were collected. In the TSH study, 255.5 ml of blood was withdrawn per participant, divided across 17 time points. In the T3 study, 370.0 ml of blood was withdrawn per participant, divided across 29 time points. The type of blood sample obtained, the volume of the blood and the frequency of withdrawal in the TSH challenge study are depicted in Table 2, and in the T3 study are depicted in Table 3.

Table 2. Overview of the blood sample type and frequency of sampling in the rhTSH challenge.

Measurement time	Number & type of tubes	Quantity of blood / tube (ml)	Total volume (ml)	Type of biomaterial	Measured parameters
14x day 1 + 1x day 2/3/4 (24, 48, 72h after injection)	2x serum 1x EDTA	3.5 2.0	17x 7.0 17x 2.0	Serum Plasma	Thyroid function + tissue turnover Markers tissue turnover
6x day 1 + 1x day 2/3/4 (24, 48, 72h after injection)	1x PAX gene 1x EDTA	2.5 4.0	9x 2.5 9x 4.0	RNA Plasma	Gene expression Metabolome
1x day 1 + 1x day 3 (72h after injection)	2x whole blood 1x EDTA	8.0 2.0	2x 16.0 2x 2.0	PBMCs Plasma	Immunophenotype
1x baseline + 1x day 3 (72h after injection)	1x EDTA	4.0	2x4.0	Plasma	Haematology

Table 3. Overview of the type of blood samples and frequency of blood sampling in the T3 challenge.

Measurement time	Number & type of tubes	Quantity of blood / tube (ml)	Total volume (ml)	Type of biomaterial	Measured parameters
25x day 1 + 1x day	2x serum	3.5	29x 7.0	Serum	Thyroid function + tissue turnover
2/3/4/5 (24, 48, 72, 96h after tablets)	1x EDTA	2.0	29x 2.0	Plasma	Tissue turnover
6x day 1 + 1x day 2/3/4/5 (24, 48, 72, 96h after tablets)	1x PAX gene 1x EDTA	2.5 4.0	10x 2.5 10x 4.0	RNA Plasma	Gene expression Metabolome
1x day 1 + 1x day 5 (96h after tablets)	2x whole blood 1x EDTA	8.0 2.0	2x 16.0 2x 2.0	PBMCs Plasma	Immunophenotype
1x baseline + 1x day 5 (96h after tablets)	1x EDTA	4.0	2x4.0	Plasma	Haematology

Urine sampling

Participants collected second void morning urine each morning of the study, on day 1,2,3,4 during rhTSH study and day 1,2,3,4,5 during T3.

Body composition measurements

On study day 2, participant's weight and height was measured and body composition parameters such as fat mass and lean mass were measured using a Bioelectrical Impedance Analysis meter at a fixed frequency of 50kHz (Bodystat 1500 Ltd, Isle of Man, British Isles (3)).

Continuous physiological measurements

Participants wore an Equivital monitor, which records continuous electrocardiography, accelerometry and breathing rate, and also records body temperature when used in combination with daily intake of a core body temperature capsule (Vivosense core body temperature capsule). The Equivital monitors used were Equivital EQ02 SEM from Hidalgo, UK, in combination with Equivital carry on belts, sizes 1 thru 9. On the morning of study day 1, the size of band was selected per participant, the Equivital monitor was connected to the band and participants started their log of Equivital wearing times. The participants were instructed to charge the Equivital monitor twice daily, in the morning and in the evening, until the battery light turned green. The times of not wearing the belt and charging were logged by the participants every day and checked by the study physician on each study day.

Questionnaires

Participants answered a basic questionnaire concerning age of their parents, dietary habits (the details of following a specific eating regimen) and regular exercise routine (per type of sport and weekly duration and frequency). This questionnaire was conducted by the same study physician in all participants.

Diaries

Participants kept a diary during study participation where they could log their exercise times, sleeping times, Equivital monitor charging times and any special remarks concerning meals. They received daily reminders during the study day to keep up their diary entries and were presented with the opportunity to ask any questions in case something was unclear.

Biobank sampling

All participants provided consent for storing biobank material. Samples for the biobank were withdrawn at each blood withdrawal time point. They are stored in accordance with the rules and regulations of the LUMC Biobank Ageing. The biobank samples are stored at the Department of Clinical Chemistry and Laboratory medicine (KCL) at the Leiden University Medical Center (LUMC), The Netherlands. This department is fully accredited (EN ISO 15189:2012) by the Dutch Accreditation Council.

Data management

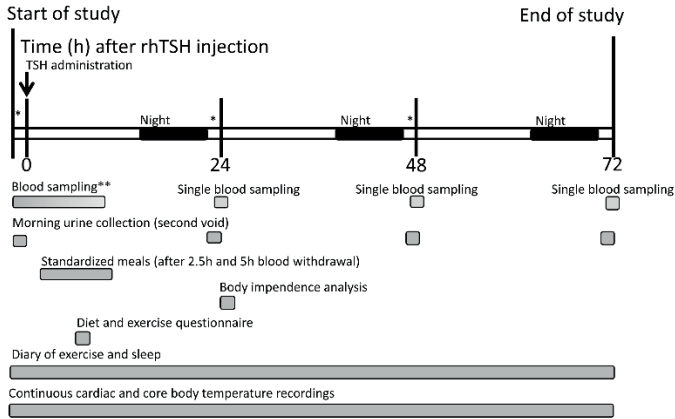
A secure and custom ProMISe database was created for this study. Information from paper CRFs was input by the study physician directly after the participants completed the study. Laboratory outcomes were imported from Excel files provided by the KCL.

Overview

A schematic representation of the study design and data collection in the TSH study and T3 study is provided in Figure 3A and B, respectively.

A

Schematic representation of study protocol TSH study

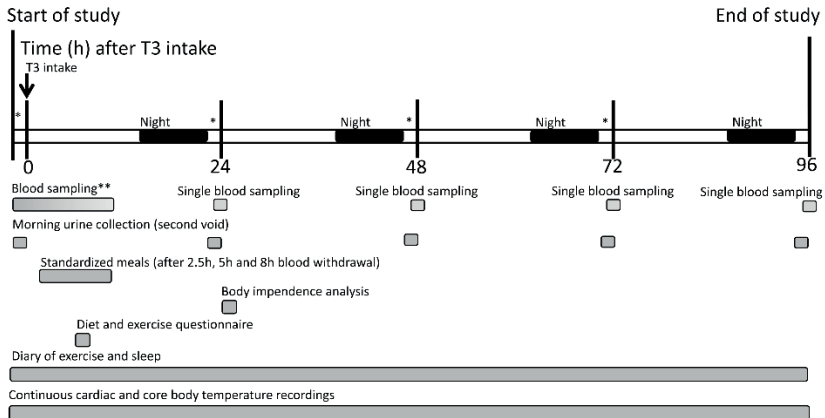


* Core body temperature new capsule intake

**Blood sampling frequency during study day 1: baseline before time 0, 0-1h every 15 min, 1-3h every 30 min, 3-8h every hour

B

Schematic representation of study protocol T3 study



* Core body temperature new capsule intake

**Blood sampling frequency during study day 1: baseline before time 0, 0-4h every 15 min, 4-7h every 30 min, 7-9h every hour

Figure 3. Schematic overview of the TSH study (A) and T3 study (B). TSH: thyroid stimulating hormone; T3: triiodothyronine.

References

1. Westendorp RG, van Heemst D, Rozing MP, Frolich M, Mooijaart SP, Blauw GJ, Beekman M, Heijmans BT, de Craen AJ, Slagboom PE, Leiden Longevity Study G. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc.* 2009;57(9):1634-1637.
2. Jansen SW, Akintola AA, Roelfsema F, van der Spoel E, Cobbaert CM, Ballieux BE, Egri P, Kvarta-Papp Z, Gereben B, Fekete C, Slagboom PE, van der Grond J, Demeneix BA, Pijl H, Westendorp RG, van Heemst D. Human longevity is characterised by high thyroid stimulating hormone secretion without altered energy metabolism. *Sci Rep.* 2015;5:11525.
3. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C, Composition of the EWG. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr.* 2004;23(5):1226-1243.

