Official Title: Association of Serum Fibroblast Growth Factor 21 (FGF-21) Levels With Resting Metabolic Rate (RMR), in Children and Adolescents With Hashimoto's Thyroiditis

NCT number: 02725879

Date: 01-10-2015

Study protocol:

Participants

Children and adolescents, aged 5–18 years, will be screened for Hashimoto's thyroiditis/ chronic autoimmune thyroiditis (AIT) at the Pediatric Endocrinology Outpatient Clinic of Papageorgiou General Hospital and AHEPA University Hospital of Thessaloniki, Greece. Diagnosis of AIT will be based on the presence of anti-thyroid autoantibodies (Anti-TPOAb and/or Anti-TgAb) and one or more of the following: clinical symptoms of thyroid dysfunction, goiter, or diffuse/irregular hypoechogenicity of the thyroid gland during ultrasound examination [1]. The first 30 patients with subclinical hypothyroidism who will receive standard levothyroxine (LT4) treatment [2], will comprise the "AIT subclinical hypothyroid group". Another 30 participants with AIT and euthyroidism at the time of enrolment will comprise the "AIT euthyroid group", whereas 30 age- and sex-matched healthy subjects will be also enrolled as "Control group" in the study.

All participants will be present with a normal body mass index (BMI) for their age and sex, were drug-naive for at least 3 months, followed no special diet, and did not present any chronic and/or acute disease or menstrual disorder. The the "AIT subclinical hypothyroid group" will be re-assessed six months after starting LT4 treatment.

Clinical and Biochemical Data

Height will be measured to the nearest millimeter with a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Limited, Crosswell Wales, U.K.). Waist, hip, and mid-upper arm circumference (MUAC) will be measured with a Seca 201 measuring tape (Hamburg, Germany), and body weight will be assessed with a Seca 711 scale (Hamburg, Germany). Body fat (BF) will be assessed by the same experienced investigator using a skinfold caliper (Harpenden Skinfold Caliper, Baty International, West Sussex, U.K.) and the equations proposed by Slaughter et al. [3]. Fat mass (FM), fat-free mass (FFM), FM index (FMI), and FFM index (FFMI) will be calculated [4,5]. Body mass index (BMI) will be calculated, and standard deviation scores (SDS) for BMI, height and skinfolds will be determined from the WHO growth charts using the LMS growth software. All subjects will undergo a complete physical examination, including posterior palpation of the thyroid gland, and will be classified according to their puberty, applying Marshall and Tanner criteria.

Resting Metabolic Rate (RMR) will be measured after a 12 h fast with a portable indirect calorimeter (FitMateTM, Cosmed, Rome, Italy) [6], using a pediatric face mask following the protocols proposed by the study conducted by Fullmer et al. [7].

Blood samples will be collected after overnight fasting, and serum levels of biochemical parameters were measured using standard methods and an ARCHITECTc 16000 clinical chemistry system (Abbott, Abbott Park, IL, USA). Concentrations of insulin,

thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxin (FT4), as well as anti-thyroid peroxidase antibody (Anti-TPOAb), and thyroglobulin antibody (Anti-TgAb) titers were measured with an ADVIA Centaur XPT Immunoassay System (Siemens Healthcare GmbH, Erlangen, Germany). Laboratory's reference range for TSH, FT4, and FT3 levels are 0.80–3.99 μIU/L, 10.55–20.72 pmol/L, and 4.21–7.57 pmol/L, respectively. The positive cut-off value of Anti-TPOAb and Anti-TgAb titers is>60 IU/mL. A thyroid gland ultrasound will be performed by the same radiologist at the beginning of the study.

Serum FGF-21 levels will be measured in patients with subclinical hypothyroidism and the control group. FGF-21 levels were determined in pg/mL using the Solid Phase Sandwich ELISA method according to the manufacturer's protocol (Quantikine® Elisa, Human FGF-21 immunoassay DF 2100, R&D Systems Europe Ltd., Abingdon Science Park, Abingdon, U.K.) with a sensitivity of 8.69 pg/mL, intra-assay CV < 4%, inter-assay CV < 5% and an assay range of 31.3–2,000 pg/mL.

In order we have indirect information regarding the dietary state of participants, all participants, with the help of their parents and/or caregivers, will complete the KIDMED questionnaire at their first visit. KIDMED questionnaire consists of 16 diet-related questions. A total score of 0–3 reflects a poor adherence to the Mediterranean diet, 4–7 an average compliance, and a score of 8–12 a suitable adherence [8,9].

[1] Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: Clinical and diagnostic criteria. Autoimmun Rev 2014;13:391–7. https://doi.org/10.1016/j.autrev.2014.01.007.

[2] Vigone MC, Capalbo D, Weber G, Salerno M. Mild Hypothyroidism in Childhood: Who, When, and How Should Be Treated? J Endocr Soc 2018;2:1024–39. https://doi.org/10.1210/js.2017-00471.

[3] Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, van Loan MD et al. Skinfold equations for estimation of body fatness in children and youths. Hum Biol 1988;60:709–23.

[4] Al Shammari E, Bano R, Suneetha E, Alshammri ARH. FFM Index, FM Index and PBF in Subjects with Normal, Overweight, and Obese BMI in Saudi Arabia Female Population. J Food Res 2015;5:40. https://doi.org/10.5539/jfr.v5n1p40.

[5] Wendel D, Weber D, Leonard MB, Magge SN, Kelly A, Stallings VA, et al. Body composition estimation using skinfolds in children with and without health conditions affecting growth and body composition. Ann Hum Biol 2017;44:108–20. https://doi.org/10.3109/03014460.2016.1168867.

[6] Nieman DC, Austin MD, Benezra L, Pearce S, McInnis T, Unick J, et al. Validation of cosmed's FitMate™ in measuring oxygen consumption and estimating resting metabolic rate. Res Sport Med 2006;14:89–96. https://doi.org/10.1080/15438620600651512.

[7] Fullmer S, Benson-Davies S, Earthman CP, Frankenfield DC, Gradwell E, Lee PSP, et al. Evidence Analysis Library Review of Best Practices for Performing Indirect Calorimetry in Healthy and Non-Critically Ill Individuals. J Acad Nutr Diet 2015;115:1417-1446.e2. https://doi.org/10.1016/j.jand.2015.04.003.

[8] Serra-Majem L, Ribas L, Ngo J, Ortega RM, García A, Pérez-Rodrigo C, et al.

Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr 2004;7:931–5. https://doi.org/10.1079/phn2004556.

[9] Štefan L, Prosoli R, Juranko D, Čule M, Milinović I, Novak D, et al. The reliability of the mediterranean diet quality index (KIDMED) questionnaire. Nutrients 2017;9. https://doi.org/10.3390/nu9040419.

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Statistical Analysis

Statistical analysis will be performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA). Continuous variables will be tested for normal distribution by the Kolmogorov–Smirnov or Shapiro–Wilk test. Data will be presented as mean ± standard deviation (SD) or medians with lower or upper quartiles. The differences between the examined groups of individuals will be investigated using the Kruskal–Wallis test and ANOVA within the GLM function with Box-Cox transformation of the response variable to improve normality. The differences between the two groups of individuals will be assessed using the Tukey post-hoc and Fisher least significant difference tests. When data are paired (e.g., before vs. after), we will account for subject ID in our model. The correlations between two continuous variables will be investigated using the Spearman rank correlation test. The level of statistical significance is set at $p < 0.05$.

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INFORMED CONSENT FORM
(STUDY OF FGF-21 IN RELATION TO THE RESTING METABOLIC RATE)

	SIGNATURE
1. I confirm that I have read and understood the newsletter for the above study and that I have the opportunity to request any further clarifications.	☐
2. I understand that participation in the research is voluntary and that I am free to leave at any time and for any reason, without affecting in the least my medical care and my legal rights.	☐
3. I understand that some information from my medical record may be examined by individuals who will be legally involved in this research. I authorize these individuals to have access to my medical record.	☐
4. I agree to take part in this study and consent to the publication of its scientific results by the researchers.	☐

Approval:

.....	.....	.....
Full name of participant	Date	Signature
.....	.....	.....
Full name of parent/caregiver	Date	Signature
.....	.....	.....
Witness	Date	Signature