Title: In vivo characterization of the non-genomic effects of T3 on endothelial and cardiovascular function

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Study protocol

Research Description

Hypothyroidism is a common condition caused by failure of the thyroid gland or as a result of thyroid surgery for benign or malignant lesions. Hypothyroidism is treated by administering levothyroxine (LT4) a synthetic inactive form of the thyroid hormone. This drug is then metabolized by the organism into its active form, T3.

Several lines of evidence indicate that LT4 alone may not be sufficient in assuring adequate tissue levels of T3 and some studies suggest that the addition of liothyronine (LT3) the synthetic form of T3 to LT4 can improve the symptoms and signs associated with hypothyroidism. LT3 has a short half life and it is usually administered in two-three doses daily. There is also evidence supporting the hypothesis that T3 exerts rapid, "non genomic" effects, particularly on the vessels. If this is the case a tight regulation of the LT3 dose to maintain the serum concentration of T3 becomes of primary importance.

To assess the non genomic effect of LT3 we will perform a proof-of-concept, double blind randomized crossover study in twenty healthy volunteers aimed to measure the acute cardiovascular and energy expenditure changes following a single equimolar dose of LT3 or LT4.

Specific aims or goals.

Aim 1: To compare the effects of a single dose of 0.7mcg/kg in liquid formulation (equivalent to a dose of 50 mcg in a 70 Kg individual) of LT3 to an equimolar 0.86 mcg/kg single dose of LT4 in liquid formulation, and to placebo on cardiovascular system. We will record the following parameters: heart rate, blood pressure, cardiac output, flow-mediated dilation.

Aim 2: To compare the effects of a single dose of 0.7mcg/kg of LT3 in liquid formulation to an equimolar single dose of LT4 in liquid formulation, and to placebo on the energy metabolism and substrate utilization. We will record the following parameters: energy expenditure by whole room indirect calorimeter, respiratory quotient, skin and core temperature.

Aim 3: To measure the changes in circulating thyroid hormone following a single dose of 0.7mcg/kg of LT3 in liquid formulation to an equimolar single dose of LT4 in liquid formulation, and to placebo. We will record at multiple time points the following parameters: free T3, free T4, TSH.

Background and significance.

Thyroid hormone (TH) affects development, growth and function on the majority of tissues of the organism and the specificity of the signaling is assured by a complex, multilevel system that assures time- and tissue-specificity of the hormonal signal relatively independent from the circulating levels(1). Among the various systems, the peripheral metabolism of pro-hormone T4, the main product of the thyroid, into the hormonally active T3 plays a crucial role in assuring the tissue-specific delivery of the hormonal signal(2). Two enzymes, type-1 (D1) and type-2 (D2) deiodinase, convert T4 into T3. Of note, D1 is thought to be responsible for a substantial fraction of circulating T3(3). Hypothyroidism is an extremely prevalent condition affecting approximately up to 10% of the adult population and is

associated with dyslipidemia, cognitive impairment, hypertension, and increased cardio-vascular morbidity and mortality(4, 5). Autoimmune thyroid disease (AITD) is the most common etiology of hypothyroidism in the US and it is characterized over a period of years by progressive loss of functional parenchyma. Since the thyrotroph is exquisitely sensitive to minimal changes in circulating TH, a compensatory increase in Thyroid Stimulating Hormone (TSH) secretion occurs while levels of TH are still well within the range of normality. This is illustrated by the classical linear-logarithmic relationship between free T4 and TSH(6). As the disease progresses, while the circulating T4 decrease to frankly pathologic levels, T3 tends to remain within normal range until extreme hypothyroidism ensues(7, 8). Similar conditions are observed in iodine insufficiency, and in experimental models of perturbation of the thyroid homeostasis(9). In aggregate, these observations suggest that multiple homeostatic mechanisms have developed to protect the circulating, and by extension the intracellular levels, of T3. Of interest, some of the hormonal actions of T3 are exerted via a rapid non-genomic pathway, which appears to play an important role in some of the cardiovascular effects of TH(10, 11). The therapy of hypothyroidism consists in administration of LT4, which in turn is converted into the active metabolite T3 in the peripheral tissues by D1 and D2. The therapeutic goal is a TSH within normal range, indicating the achievement of euthyroidism at the level of the thyrotroph on the assumption that a state of pituitary euthyroidism equates to adequate T3 concentration in all the tissue targets of the hormonal action(12). This assumption has been challenged by clinical observations(13), clinical trials(14), and mechanistic studies(15). The results of clinical studies aimed to measure the effects of TH replacement on “hard” endpoints (i.e. mortality and cardiovascular events) have been marginal or disappointing, particularly in subclinical hypothyroidism and in the elderly(16). This is surprising since the cross-sectional data provide compelling evidence of the association between hypothyroidism and indexes of cardiovascular risk. Collectively these data question the utility of TH replacement, its therapeutic goals, the target population who could benefit from the intervention, and the modality of the treatment, and indeed there is active discussion on this very topic within the thyroidologist community(17). The attempt to provide a more “physiologic” form of therapy has led to the experimentation of combinationLT3 /LT4 regimens(13, 18-29), or to the use of thyroid extracts(30). Furthermore, some observations suggest that carriers of a common polymorphism of the D2 gene (Thr92Ala) (31)may benefit from combination therapy, suggesting a pharmacogenomics component in the therapeutic response(14, 32). This information indicate that the current therapy for hypothyroidism is costly and suboptimal, and there is need for a formal characterization of the pathophysiologic state of hypothyroidism during replacement treatment.

This project challenges the established notion that LT4 generates identical biological effects on the pituitary and on the end-organ tissues, and pituitary euthyroidism (*i.e.* a normal TSH) is pharmacodynamically equivalent to production of TH from the normal thyroid gland. We will interrogate the clinical relevance of non-genomic effects of TH by assessing the short-term effects of a single-dose of LT3 on endothelial and cardiovascular function. This translational project will provide important information on the need (or lack thereof) of maintaining stable circulating levels of T3. In this clinical protocol, we chose as primary endpoints measurable and clinically relevant indices of TH action, which in turn could be used in the future as outcomes for large intervention studies. Specifically, beside measurements of circulating TH, we will assess energy expenditure(33, 34) and endothelial (35, 36)and cardiovascular function(37). The results of the proposed experiments will shed light on the interaction between the pathophysiologic state of hypothyroidism and administration of therapy, providing new

insights on the clinical rationale and utility of targeting the circulating levels of T3. Collectively, these studies will expand the knowledge base necessary to design a rationale treatment of hypothyroidism.

Study design.

Proof-of-concept, randomized double blind cross-over study in healthy volunteers. Twenty adult healthy volunteers will be invited to participate in the study.

A) Screening visit. Age >18<45 year, BMI >20<30 kg/m^2 TSH >0.5<5.0 mcIU/mL and no evidence of thyroid autoimmunity by history or anti-thyroid peroxidase (TPO) antibodies measurement. Exclusion criteria will be: pregnancy; hypothyroidism or autoimmune thyroid disease by history or presence of anti TPO antibodies; use of prescription drugs; diabetes mellitus; dyslipidemia; coronary artery disease; hypertension (systolic blood pressure > 140 mm/Hg, and/or diastolic blood pressure > 90 mm/Hg); anemia; renal insufficiency; liver disease or ALT >2.5 x the upper laboratory reference limit; psychiatric conditions; and current tobacco use. During the screening visit, the following procedures will be performed: informed consent, history and physical examination, EKG, CBC, glucose, creatinine, ALT, hCG (in females), TSH, freeT4, TPO, and genomic DNA.

B) Study visit #1-3. Study participants will be evaluated in the morning after an overnight fast during three consecutive outpatient visits, with at least a 24-hour interval between. Upon arrival to the Clinical Research Service Unit (CRSU) and abbreviated history and physical examination with recording of anthropometrics will be obtained; an i.v. cannula will be inserted in the left arm. Study volunteers will be fitted with electrodes for EKG Holter recording, and a cuff on the third finger of the right hand to record blood pressure and heart rate, cardiac output, systemic vascular resistance and endothelial function. An echocardiogram will be recorded at the beginning and at the end of each study session. Study volunteers will then enter in the whole room ("Flex room") a 4' x 8' x 7' indirect calorimeter with a temperature set at 24°C and rest on a bed. The Flex room has a window and a TV monitor, and is connected to the nursing station via an interphone system. An i.v. port is located on the door of the Flex room allowing drawing of samples without compromising the recording of energy expenditure. After 40 minutes (time 0'), study volunteers will receive either 0.7mcg/kg (equivalent to a dose of 50 mcg in a 70 Kg individual) of LT3 to an equimolar 0.86 mcg/kg single dose of LT4, and to placebo in liquid form. The doses were chosen to be within the usual therapeutic range prescribed in preparation for radioiodine ablation. Blood samples will be obtained at the following time points: 0', 60' 120' 180' 240' for total and free T3, total and free T4, TSH and 500 μl aliquots of serum TSH will be recorded at times 0' and 240' of each Study visit. Energy expenditure and respiratory quotient will be recorded throughout the duration of the stay in the Flex room. The first 20 minute of the recording will be discarded to allow for equilibration. At the end of the session the i.v. cannula will be removed, vital signs recorded and the volunteer will be discharged provided with a coupon for a meal in the hospital cafeteria. The same procedures will be repeated in the identical order and timing for the other formulations.

Justification for the sample size:

The primary analysis will be performed by two-tailed paired t-test with an 0.05 α error as a threshold for statistical significance on the assumption that LT3 administration, compared to LT4 will result in a 30% increase in FMD, corresponding to 0.75 SD. Using this experimental conditions and assumptions, a 20 volunteers will provide 88% power to detect a significant difference, while assuming a 20% drop-out rate the power will be 80%.

List the study inclusion criteria:

Healthy volunteers, both sexes

Age range 18-45 year

Body Mass Index range 20- 30 kg/m^2

Ability to provide informed consent to the participation in the study

List the study inclusion criteria:

Abnormal TSH (>0.5<5.0 mcIU/mL)

Thyroid disease or autoimmunity by history or anti-thyroid peroxidase (TPO) antibodies.

Pregnancy or lactation

Chronic use of prescription drugs

Diabetes mellitus (by history or fasting glucose >126 mg/dl)

Dyslipidemia (total cholesterol >240 mg/dl)

Hypertension (by history or screening systolic blood pressure > 140 mm/Hg, and/or diastolic blood pressure > 90 mm/Hg)

Cardiac arrhythmia (by history or screening EKG)

Anemia (Hb<13.3 g/dl M, 12.0 g/dl F)

Renal insufficiency by history or estimated creatinine clearance ≤ 60 mL/min/1.73m^2 BSA.

Liver disease or transaminases >2.5 x the upper laboratory reference limit.

Current tobacco use.

Criteria for withdrawing an individual participant from the study:

Volunteers may withdraw from the study at any time. The investigators can withdraw any volunteer at any time from the study if medically necessary.

Medical reasons for withdraw from the study:

1) Tachycardia (heart rate greater than 100/min)
2) Anxiety
3) Development of hypertension (systolic >160 mmHg, diastolic > 90 mmHg) following administration of the study drug
4) Development of hypotension (systolic <90 mmHg, diastolic < 60 mmHg) following administration of the study drug

Adverse events mitigation:

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, whichever occurs first. All AEs and SAEs documented at a previous visit/contact

and designated as ongoing, will be reviewed at subsequent visits/contacts, where the designation may remain ongoing. The investigator will ensure that the follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. SAEs that are ongoing at the time of the subjects final study visit/contact will be documented as ongoing.

DSMP.

The PI will be responsible for data and safety monitoring during the study. A physician not affiliated with the research proposal will serve as independent compliance officer and will review quarterly compliance with inclusion/exclusion criteria, gender and minority, adverse events. The results of the review will be reported to the PI and the IRB.

1. Yen PM 2001 Physiological and molecular basis of thyroid hormone action. Physiological reviews **81**:1097-1142.

2. Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A, Bianco AC 2008 Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocrine reviews **29**:898-938.

3. Saberi M, Sterling FH, Utiger RD 1975 Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. The Journal of clinical investigation **55**:218-223.

4. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC 2000 The Colorado thyroid disease prevalence study. Archives of internal medicine **160**:526-534.

5. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, Gussekloo J, Thyroid Studies C 2010 Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA : the journal of the American Medical Association **304**:1365-1374.

6. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, Gray D, Nicoloff JT 1990 Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. The Journal of clinical endocrinology and metabolism **70**:453-460.

7. Evered DC, Ormston BJ, Smith PA, Hall R, Bird T 1973 Grades of hypothyroidism. British medical journal **1**:657-662.

8. Kumar MS, Safa AM, Deodhar SD, Schumacher OP 1977 The relationship of thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) in primary thyroid failure. American journal of clinical pathology **68**:747-751.

9. Chopra IJ, Hershman JM, Hornabrook RW 1975 Serum thyroid hormone and thyrotropin levels in subjects from endemic goiter regions of New Guinea. The Journal of clinical endocrinology and metabolism **40**:326-333.

10. Hiroi Y, Kim HH, Ying H, Furuya F, Huang Z, Simoncini T, Noma K, Ueki K, Nguyen NH, Scanlan TS, Moskowitz MA, Cheng SY, Liao JK 2006 Rapid nongenomic actions of thyroid hormone. Proceedings of the National Academy of Sciences of the United States of America **103**:14104-14109.

11. Vicinanza R, Coppotelli G, Malacrino C, Nardo T, Buchetti B, Lenti L, Celi FS, Scarpa S 2013 Oxidized low-density lipoproteins impair endothelial function by inhibiting non-genomic action

of thyroid hormone-mediated nitric oxide production in human endothelial cells. Thyroid : official journal of the American Thyroid Association **23**:231-238.

12. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM, American Thyroid Association Task Force on Thyroid Hormone R 2014 Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid : official journal of the American Thyroid Association **24**:1670-1751.

13. Bunevicius R, Kazanavicius G, Zalinkevicius R, Prange AJ, Jr. 1999 Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. The New England journal of medicine **340**:424-429.

14. Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, Dayan CM 2009 Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. The Journal of clinical endocrinology and metabolism **94**:1623-1629.

15. Escobar-Morreale HF, del Rey FE, Obregon MJ, de Escobar GM 1996 Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. Endocrinology **137**:2490-2502.

16. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frolich M, Westendorp RG 2004 Thyroid status, disability and cognitive function, and survival in old age. JAMA : the journal of the American Medical Association **292**:2591-2599.

17. Biondi B, Wartofsky L 2012 Combination treatment with T4 and T3: toward personalized replacement therapy in hypothyroidism? The Journal of clinical endocrinology and metabolism **97**:2256-2271.

18. Rodriguez T, Lavis VR, Meininger JC, Kapadia AS, Stafford LF 2005 Substitution of liothyronine at a 1:5 ratio for a portion of levothyroxine: effect on fatigue, symptoms of depression, and working memory versus treatment with levothyroxine alone. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists **11**:223-233.

19. Walsh JP, Shiels L, Lim EM, Bhagat CI, Ward LC, Stuckey BG, Dhaliwal SS, Chew GT, Bhagat MC, Cussons AJ 2003 Combined thyroxine/liothyronine treatment does not improve well-being, quality of life, or cognitive function compared to thyroxine alone: a randomized controlled trial in patients with primary hypothyroidism. The Journal of clinical endocrinology and metabolism **88**:4543-4550.

20. Appelhof BC, Fliers E, Wekking EM, Schene AH, Huyser J, Tijssen JG, Endert E, van Weert HC, Wiersinga WM 2005 Combined therapy with levothyroxine and liothyronine in two ratios, compared with levothyroxine monotherapy in primary hypothyroidism: a double-blind, randomized, controlled clinical trial. The Journal of clinical endocrinology and metabolism **90**:2666-2674.

21. Escobar-Morreale HF, Botella-Carretero JI, Gomez-Bueno M, Galan JM, Barrios V, Sancho J 2005 Thyroid hormone replacement therapy in primary hypothyroidism: a randomized trial comparing L-thyroxine plus liothyronine with L-thyroxine alone. Annals of internal medicine **142**:412-424.

22. Sawka AM, Gerstein HC, Marriott MJ, MacQueen GM, Joffe RT 2003 Does a combination regimen of thyroxine (T4) and 3,5,3'-triiodothyronine improve depressive symptoms better than T4 alone in patients with hypothyroidism? Results of a double-blind, randomized, controlled trial. The Journal of clinical endocrinology and metabolism **88**:4551-4555.

23. Bunevicius R, Jakuboniene N, Jurkevicius R, Cernicat J, Lasas L, Prange AJ, Jr. 2002 Thyroxine vs thyroxine plus triiodothyronine in treatment of hypothyroidism after thyroidectomy for Graves' disease. Endocrine **18**:129-133.

24. Clyde PW, Harari AE, Getka EJ, Shakir KM 2003 Combined levothyroxine plus liothyronine compared with levothyroxine alone in primary hypothyroidism: a randomized controlled trial. JAMA : the journal of the American Medical Association **290**:2952-2958.

25. Fadeyev VV, Morgunova TB, Melnichenko GA, Dedov, II 2010 Combined therapy with L-thyroxine and L-triiodothyronine compared to L-thyroxine alone in the treatment of primary hypothyroidism. Hormones **9**:245-252.

26. Nygaard B, Jensen EW, Kvetny J, Jarlov A, Faber J 2009 Effect of combination therapy with thyroxine (T4) and 3,5,3'-triiodothyronine versus T4 monotherapy in patients with hypothyroidism, a double-blind, randomised cross-over study. European journal of endocrinology / European Federation of Endocrine Societies **161**:895-902.

27. Saravanan P, Simmons DJ, Greenwood R, Peters TJ, Dayan CM 2005 Partial substitution of thyroxine (T4) with tri-iodothyronine in patients on T4 replacement therapy: results of a large community-based randomized controlled trial. The Journal of clinical endocrinology and metabolism **90**:805-812.

28. Siegmund W, Spieker K, Weike AI, Giessmann T, Modess C, Dabers T, Kirsch G, Sanger E, Engel G, Hamm AO, Nauck M, Meng W 2004 Replacement therapy with levothyroxine plus triiodothyronine (bioavailable molar ratio 14 : 1) is not superior to thyroxine alone to improve well-being and cognitive performance in hypothyroidism. Clinical endocrinology **60**:750-757.

29. Valizadeh M, Seyyed-Majidi MR, Hajibeigloo H, Momtazi S, Musavinasab N, Hayatbakhsh MR 2009 Efficacy of combined levothyroxine and liothyronine as compared with levothyroxine monotherapy in primary hypothyroidism: a randomized controlled trial. Endocrine research **34**:80-89.

30. Hoang TD, Olsen CH, Mai VQ, Clyde PW, Shakir MK 2013 Desiccated thyroid extract compared with levothyroxine in the treatment of hypothyroidism: a randomized, double-blind, crossover study. The Journal of clinical endocrinology and metabolism **98**:1982-1990.

31. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi FS 2002 Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. Diabetes **51**:880-883.

32. Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, Trischitta V 2008 Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. The Journal of clinical endocrinology and metabolism **93**:910-913.

33. Thompson WO, Thompson PK 1928 LOW BASAL METABOLISM FOLLOWING THYROTOXICOSIS: I. Temporary Type without Myxedema, with Special Reference to the Role of Iodine Therapy. The Journal of clinical investigation **5**:441-469.

34. Thompson WO, Thompson PK 1928 LOW BASAL METABOLISM FOLLOWING THYROTOXICOSIS: II. Permanent Type without Myxedema. The Journal of clinical investigation **5**:471-501.

35. Cikim AS, Oflaz H, Ozbey N, Cikim K, Umman S, Meric M, Sencer E, Molvalilar S 2004 Evaluation of endothelial function in subclinical hypothyroidism and subclinical hyperthyroidism. Thyroid : official journal of the American Thyroid Association **14**:605-609.

36. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, International Brachial Artery Reactivity Task F 2002 Guidelines for the ultrasound assessment of endothelial-

dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology **39**:257-265.

37. Biondi B, Fazio S, Palmieri EA, Carella C, Panza N, Cittadini A, Bone F, Lombardi G, Sacca L 1999 Left ventricular diastolic dysfunction in patients with subclinical hypothyroidism. The Journal of clinical endocrinology and metabolism **84**:2064-2067.