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The Thyroid Axis in Older Individuals with Persistent Subclinical Hypothyroidism: a Mechanistic, Randomized, Double-Blind, Cross-Over Study of Levothyroxine and Liothyronine Administration

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List of Abbreviations

AE	Adverse event
AUC	Area under the curve
BMI	Body mass index
CHS	Cardiovascular Health Study
CTRC	Clinical and Translational Research Center
CVD	Cardiovascular disease
ECG	Electrocardiogram
FDA	Food and Drug Administration
FT4	Free T4
HUP	Hospital of the University of Pennsylvania
IDS	Investigational Drug Service
IRB	Institutional review board
IV	Intravenous
LFTs	Liver function tests
LT3	Liothyronine
LT4	Levothyroxine
mRNA	Messenger ribonucleic acid
NHANES	National Health and Nutrition Examination Survey
NIA	National Institute on Aging
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PHI	Protected health information
REE	Resting energy expenditure
SAE	Severe adverse event
SD	Standard deviation
SEM	Standard error of the mean
SF	Short form
T3	Triiodothyronine
T4	Thyroxine
TPO	Thyroid peroxidase
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
TSH_{max}	Maximum serum TSH concentration

Study Summary

Title	The Thyroid Axis in Older Individuals with Persistent Subclinical Hypothyroidism: a Mechanistic, Randomized, Double-Blind, Cross-Over Study of Levothyroxine (LT4) and Liothyronine (LT3) Administration
Short Title	LT4 and LT3 in Subclinical Hypothyroidism
Protocol Number	*821564*
Phase	
Methodology	Randomized, Double-Blind, Cross-Over Study
Study Duration	Three years
Study Center(s)	Single-center
Objectives	To examine the pituitary-thyroid axis in older individuals with persistent subclinical hypothyroidism utilizing dynamic testing with thyrotropin releasing hormone (TRH) stimulation under different therapeutic conditions of either LT4 or LT3. We will measure thyroid stimulating hormone (TSH) area under the curve (AUC), TSH_{max}, change in TSH bioactivity, and change in free T4 and total T3 levels. All outcomes will be assessed at baseline and after achieving target TSH levels with each thyroid preparation.
Number of Subjects	Thirty
Diagnosis and Main Inclusion Criteria	Men and women aged 70 and older with persistent subclinical hypothyroidism
Study Product, Dose, Route, Regimen	1. Intravenous TRH 200 µg at study visit 1 (baseline), study visit 2 (on first thyroid treatment), and study visit 3 (on second thyroid treatment) 2. Oral LT4 with a total starting dose of 0.7mcg/kg/day split into three daily doses and titrated to a target TSH level of 0.5 -1.5mU/L 3. Oral LT3 with a starting dose of 1/3 of the weight based LT4 dose divided into three daily doses titrated to a target TSH level of 0.5 -1.5mU/L
Duration of administration	1. Three single administrations of TRH (at baseline study visit 1, study visit 2 on the first thyroid treatment, and study visit 3 on the second thyroid treatment) 2. LT4 taken as divided doses three times daily 3. LT3 taken as divided doses three times daily 4. For LT4 and LT3, there will be medication adjustments every four weeks as needed until the TSH level is at goal.

Reference therapy	None
Statistical Methodology	Changes in TRH stimulation test parameters and other outcomes will be compared from baseline for each thyroid hormone preparation (LT4 or LT3) via paired t-tests and between treatment arms via two-tailed tests. AUC for TRH stimulated TSH response will be calculated. Nonparametric tests will be employed as needed.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and Institutional research policies and procedures.

1.1 Background

Thyroid hormone is important for multiple physiologic functions, and insufficiency and excess of thyroid hormone are each pathologic conditions. Thyroid hormone is tightly regulated through a feedback loop between the hypothalamic hormone thyrotropin releasing hormone (TRH), the pituitary hormone thyroid stimulating hormone (TSH), and the thyroid hormones thyroxine (T4) and triiodothyronine (T3) (Fig. 1, from [1]). Individuals with normal thyroid function maintain TSH levels in a narrow range, though the setpoint for thyroid hormone levels varies between individuals.

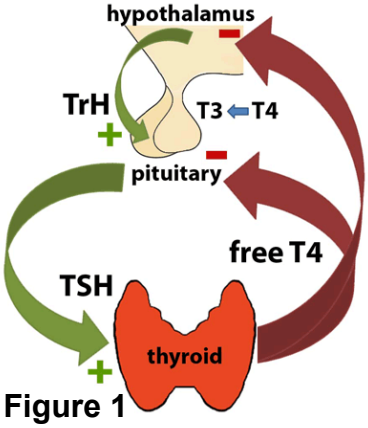

Figure 1

With increasing age, there is a progressive shift toward higher TSH levels in people who have no detectable underlying thyroid disease (Fig. 2, from [2]). This leads to a higher prevalence of subclinical hypothyroidism in older people. Subclinical hypothyroidism is defined as elevated TSH levels with normal levels of thyroid hormones. It is a common clinical diagnosis affecting 10-15% of individuals aged 65 years and older and up to 20% of those 85 years and older.[1-5] The purpose of this study is to examine mechanisms to explain the age-related increase in TSH levels and resulting subclinical hypothyroidism.

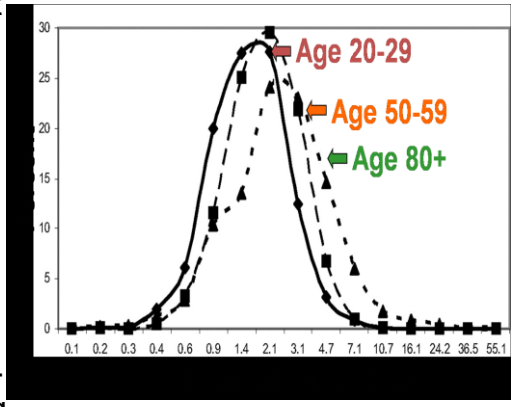

Figure 2

In addition, it is not clear if individuals with subclinical hypothyroidism benefit from replacement of thyroid hormone. Some argue that subclinical hypothyroidism should be treated as a form of mild hypothyroidism, whereas others argue that it may be adaptive. The question is whether, in older people, a TSH level just outside the reference range should be considered a pathologic finding. We hypothesize that slightly elevated TSH results from an age-related decrease in the function of TSH that leads to an adaptive shift in the pituitary-thyroid setpoint, rather than an early sign of thyroid failure. That is, the TSH that is produced by the pituitary gland may become less biologically active with older age. More TSH would then be required to keep thyroid hormone level production constant.

There is evidence to support the biologic plausibility of a decline in TSH bioactivity with age. TSH with decreased bioactivity has been detected using cell-based methodologies, and has been reported in conditions of thyroid axis dysfunction, ranging from primary and

secondary hypothyroidism to TSH-producing pituitary adenomas.[3-6] An inverse correlation between TSH bioactivity and age was also found in 11 healthy men and women aged 28-52 years.[4]

Changes in TSH structure including glycosylation patterns affect its biologic activity.[7] These changes in glycosylation could be due to alterations in TRH output from the hypothalamus, as is found with age for other hypothalamic hormones such as GHRH and GnRH. [8, 9] Unfortunately, TRH cannot be measured directly; however, TSH bioactivity can be measured before and after TRH stimulation testing to provide indirect evidence of this mechanism. We can then create perturbations to the system such as adding back thyroid hormones to understand these hormonal feedback loops. Together the TRH stimulation testing and the crossover design with T4 and T3 add back will provide insight into the dynamics of the hypothalamic-pituitary-thyroid axis in older people with subclinical hypothyroidism.

1.2 Interventional Agents

TRH, a naturally occurring hypothalamic neuropeptide in humans, is an endogenous ligand for the TRH receptor. It is composed of three amino acids and stimulates the release of TSH and prolactin from the anterior pituitary. The release of TSH then stimulates the release of thyroid hormones, T4 and T3 (Fig. 1). Synthetic TRH has been used for over 40 years in the diagnostic assessment of pituitary or hypothalamic dysfunction.

Intravenous TRH has a short half-life with a mean of approximately 5 minutes in normal subjects. Bassiri and Utiger administered 400 µg TRH to 8 healthy men and noted that plasma TRH levels rose from <0.4 ng/mL to 33 ± 7 ng/mL (mean ± SEM) 2 minutes after injection and linearly fell to 2.9 ng/mL 20 min after injection. The mean half-life of TRH was found to be 5.3 ± 0.5 min.[10]. TSH levels rise rapidly; reach a peak, and later decline after TRH administration. Anderson *et al* administered 100-800 µg TRH to 79 healthy men and found that TRH-stimulated TSH levels peaked after 30 min and fell to baseline levels in approximately 3 hours.[11]

Levothyroxine (LT4) and liothyronine (LT3) are synthetic forms of T4 and T3, which are endogenous hormones produced by follicular cells of the thyroid gland in response to stimulation by TSH from the anterior pituitary. The majority of circulating thyroid hormone in the blood is in the form of T4. T4 is then converted to T3 in target tissues, with T3 being the more physiologically active hormone. Both T4 and T3 bind to the thyroid receptor in cell nuclei and regulate DNA transcription and protein synthesis. Human studies show that thyroid hormone levels can also alter TSH bioactivity. [4, 6, 12] T4 is metabolized slowly with eighty percent of circulating T3 derived from T4 through deiodination. The half-life of LT4 is six to eight days. The half-life of LT3 is one to two days.[13-15]

1.3 Preclinical Data

Slightly elevated TSH may result from an age-related alteration in the function of TRH, analogous to the age-related alterations found in other hypothalamic hormones such as

GHRH and GnRH.[8, 9] Preclinical data show that alterations to the structure and packaging of the TSH molecule may affect its function. TSH is a glycoprotein composed of an alpha and a beta subunit that requires glycosylation of three asparagine residues in order to have maximal function at the TSH receptor.[16] When the glycosylation pattern is abnormal, TSH binding to its receptor is less efficient, resulting in decreased bioactivity. These changes in glycosylation could be due to alterations in TRH output from the hypothalamus, as suggested by animal models.[7, 17-19] Taylor et al. showed that hypothalamic as compared to primary hypothyroidism caused by lesions in the rat paraventricular nuclei alters carbohydrate structure of secreted thyrotropin. Taylor and Weintraub later showed that this alteration in TSH carbohydrate structure could be corrected by administering TRH in vivo.[19] Hypothalamic TRH output cannot be measured directly. However, TSH bioactivity can be measured before and after TRH stimulation testing to provide indirect evidence of this mechanism.

In addition to TRH, there is evidence that thyroid hormones themselves can alter the bioactivity of thyroid stimulating hormone. Thyroid hormone status has been shown to modulate glycosyltransferase mRNA levels in mouse thyrotrophs.[20] This has been postulated to result from a direct action of T3 on thyroid hormone responsive elements in the upstream regulatory region of the sialyltransferase gene. If so, TSH glycosylation could be affected by the observed age-associated decline in T3 levels.

Rodent data indicate that replacement therapy with LT4 alone does not achieve adequate levels of T3 in all tissues.[21] However, combined replacement therapy of LT3 to LT4 completely restored euthyroidism in thyroidectomized rats at much lower doses of LT4 than those needed to normalize LT3 in most tissues when L-T4 alone was used. The LT4-LT3 cross-over design will give us insight into this mechanism.

1.4 Clinical Data to Date

In men and women from the Cardiovascular Health Study with a mean age of 74 years at the time of the initial TSH, we found a gradual increase in TSH levels over a 13-year period, with an average increase of 0.28 mU/L ($p<0.01$) and no change in free T4 levels, suggesting a shift in the pituitary-thyroid setpoint. These data parallel the 0.32 mU/L TSH increase over a 13-year period published in a younger population (mean age 58 years at the second TSH), also without change in free T4 levels.[22] NHANES data also illustrate a progressive shift toward higher TSH levels with increasing age [2] and another study has shown higher TSH in people with exceptional longevity.[23]

The hypotheses that there is a decline in TSH bioactivity with age and that thyroid hormone supplementation can alter bioactivity are supported by data in humans [4, 6, 12]. Oliveira et al. evaluated in vitro serum TSH bioactivity and found a strong inverse correlation between serum TSH bioactivity and age in 11 normal controls. This study also showed a decrease in TSH bioactivity in patients with primary hypothyroidism. The bioactivity improved with levothyroxine supplementation and was positively correlated with change in T3 levels but not free T4 levels. Evaluation of TSH glycosylation patterns showed a higher proportion of sialylated TSH molecules in patients with decreased

bioactivity, which decreased after LT4 replacement.[4] Increase in sialylation of the TSH molecule was also shown in patients with central hypothyroidism due to Sheehan's syndrome [3] and chronic TRH administration in patients with central hypothyroidism has been shown to improve TSH bioactivity.[24] These data suggest that hypothalamus-pituitary-thyroid feedback loops are regulated by TSH bioactivity and thyroid hormone levels.

1.5 Dose Rationale and Risk/Benefits

TRH stimulation testing:

TRH has a short half-life and does not exhibit a sustained biological effect after a single intravenous bolus. For decades, TRH has been intravenously administered to a variety of study populations, including patients with thyroid, pituitary, and hypothalamic disorders.[10, 11, 25] In this study we will be using the standard clinical dose of 200 µg at three time points. This dose has been used in studies of dynamic pituitary testing utilizing TRH stimulation testing.[25, 26] Side effects have been reported in about 50% of patients, but side effects are generally minor and persist for only a few minutes following injection.

These side effects include[27]:

Most frequent: transient nausea, flushing, palpitations (without change in heart rate), lightheadedness, bad taste in mouth, abdominal discomfort, dry mouth, and an urge to urinate

Less frequent: anxiety, sweating, tightness in throat (without change in oxygenation or respiration), pressure in chest, tingling sensation, and drowsiness

Uncommon: change in blood pressure, seizure

The mean elevation of blood pressure reported was 16 mm of mercury. The peak blood pressure elevation occurred between one and five minutes, with a return to baseline blood pressure levels within ten minutes.[11] Participants with uncontrolled blood pressure or history of ischemic cardiac disease or stroke will be excluded.

Seizures have only been reported in those with a history of seizure disorder. Individuals with a history of seizure will be excluded.

No direct benefits are expected to result from TRH administration.

LT4 and LT3:

LT4 and LT3 have each been in therapeutic use for more than 50 years. Adverse reactions to either drug are exclusively related to excessive thyroid hormone, which can produce iatrogenic thyrotoxicosis. No serious adverse events were reported in a

published LT4/LT3 crossover study, and no symptoms of hyperthyroidism or hypothyroidism were reported.[25, 28] Participants will be asked about hyperthyroid symptoms, and a TSH level will be checked prior to the planned date if a patient reports any hyperthyroid symptoms.

Administration and monitoring of LT4 and LT3 will be modeled off of a published protocol.[28] The dosing of thyroid hormone will be based on published studies of thyroid hormone replacement in subclinical hypothyroidism. The literature supports a 1 mcg/kg/day dose for initiation of therapy.[29-34] We will tailor the thyroid hormone dose to each subject using a weight-based dosing scheme that is also based on how far the subject's TSH level is from goal. The dosing scheme is conservative. 1mcg/kg is the dose reported in the literature. The initial dose in our study will be lower than that - 0.7mcg/kg/day given that we are studying subjects 70 years and older. We will use thrice daily dosing due to the short half-life of LT3.

LT4
Each participant will start on 0.7mcg/kg/day divided into three daily doses.
The dose will be adjusted according to subject weight and how far the TSH level is from goal. There will be a medication cap such that no dose adjustment will exceed 25 mcg per day.

LT3
The starting dose of liothyronine will be 1/3 of that of levothyroxine divided into three daily doses. We will titrate the dose according to subject weight and how far the TSH level is from goal. No dose adjustment will be greater than 8.3 mcg per day.

The total daily dose of study medications will be adjusted to a TSH of 0.5-1.5 mU/L using the adjustment scheme to avoid excess thyroid hormone replacement.

At the time of crossover of study drug, participants will undergo a 4 week wash-out period from the study drug. This is to avoid over-replacement when switching from LT4 to LT3, given the longer half-life of LT4. Following Visit 2, participants will stop all study drug for 4 weeks. At the end of 4 weeks they will begin the second study drug at the equivalent dose as they were taking at Visit 2, divided into 3 equal doses. TSH level will not be checked at the end of the wash-out period. A TSH level will be checked 4 weeks after crossing over to the new study drug. All further TSH level evaluations and medication adjustments will follow the titration protocol.

No direct benefits are expected to result from LT4 and LT3 administration.

The weight based dosing scheme and examples are displayed below:

LT4 Titration Scheme:

TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Dose change (mcg/kg/day)	0.25	0.21	0.17	0.1	**GOAL: No change**	**-**0.08	**-**0.09

Example of LT4 titration in a 50, 70, & 100 kg individual

	TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Weight (kg)	**Start dose (0.7mcg/kg/day)**	**Dose Change: Total mcg change per day**						
50	35	12.5	10.5	8.5	5	**GOAL: No change**	-4	-4.5
70	49	17.5	14.7	11.9	7		-5.6	-6.3
100	70	25	21	17	10		-8	-9

LT3 Titration Scheme: LT4 Dose scheme divided by 3

TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Dose change (mcg/kg/day)	0.08	0.07	0.06	0.03	**GOAL: No change**	**-**0.027	**-**0.03

Example of LT3 titration in a 50, 70, & 100 kg individual

	TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Weight (kg)	**Start dose 1/3 LT4 Dose**	**Dose Change: Total mcg change per day**						
50	11.7	4.17	3.5	2.83	1.67	**GOAL: No change**	-1.33	-1.50
70	16.3	5.83	4.9	3.97	2.33		-1.87	-2.10
100	23.3	8.3	7	5.67	3.33		-2.67	-3.00

2 Study Objectives

To understand the function of the thyroid axis in subclinical hypothyroidism, by examining the thyroid axis in older individuals with persistent subclinical hypothyroidism under dynamic conditions.

Primary Objective:

To assess the adaptability of the thyroid axis to perturbations from TRH stimulation or thyroid hormone supplementation via assessment of TRH stimulation test parameters (TSH AUC, TSH_{max}, change in TSH bioactivity, change in free T4, change in total T3) at baseline and after achieving target TSH levels with each thyroid preparation.

Secondary Objectives:
To assess changes in between baseline and each thyroid preparation in:
1) unstimulated TSH bioactivity
2) resting energy expenditure (REE)
3) body temperature
4) weight
5) body composition
6) lipids
7) bone density
8) heart rate
9) physical activity
10) hypothyroid and hyperthyroid symptoms
11) health status and well-being assessment with SF-36
12) cognitive testing with digit symbol substitution test
13) arterial tonometry, doppler echocardiography, and arterial load assessment

3 Study Design

3.1 General Design

The study design is a randomized, double-blind, cross-over study in which we will examine the pituitary-thyroid axis in older subclinical hypothyroid individuals in response to TRH stimulation and to daily LT4 or LT3 supplementation over a minimum period of 14 consecutive weeks. Physiologic responses to LT4 and LT3 supplementation will also be examined. Thirty participants will receive a total of three TRH stimulation tests and one course each of LT4 and LT3 in randomized order.

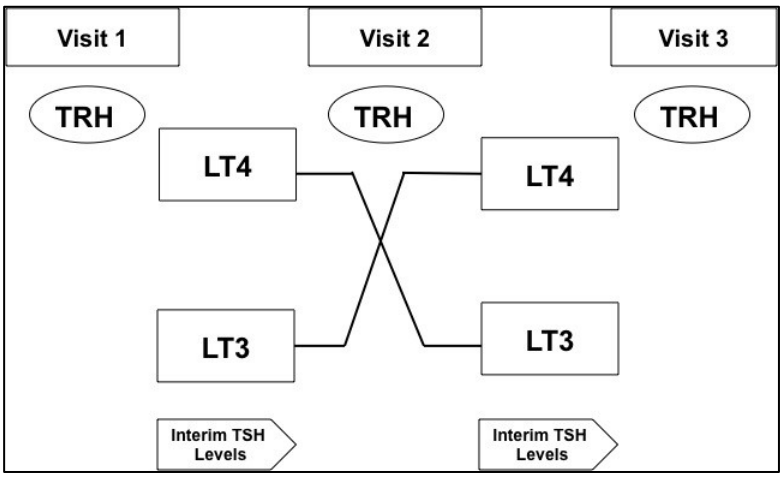

Figure 4

All study visits will be at the University of Pennsylvania's Clinical and Translational Research Center (CTRC). There will be a screening visit (Day -60 to Day -1), a baseline

study visit (Visit 1; Day 1), and a final study visit while taking their first thyroid preparation (Visit 2). Following the completion of Visit 2, participants will cross-over to the second thyroid preparation and complete Visit 3, as above.

On Day 1 (baseline study visit, Visit 1) the participants will undergo their first TRH stimulation test and receive written instruction on thyroid hormone dosing by a study team member. The participant will begin oral thyroid supplementation as divided doses three times per day for four weeks starting the morning after the baseline study visit. After four weeks, the participants will have their TSH level checked at a Penn outpatient laboratory, after which LT4 or LT3 will be titrated to a target TSH level of 0.5-1.5 mU/L by a blinded study investigator. TSH levels will continue to be checked at four-week intervals until the TSH level is at goal, after which, Visit 2 will be scheduled. At Visit 2, a TRH stimulation test will be performed and participants will cross-over to the other thyroid preparation. The sequence of TSH assessment and thyroid preparation titration will occur with this thyroid preparation. At Visit 3, a TRH stimulation test will be performed and the participant will complete the study. Physiologic endpoints will be measured at each study visit.

3.2 Primary Study Endpoints

Changes in TRH stimulation test parameters (listed below) will be measured at each study visit:

1. TSH area under the curve (AUC)
2. TSH_{max}
3. Free T4
4. Total T3
5. TSH bioactivity measured by an *in-vitro* cell-based bioactivity assay [35]

3.3 Secondary Study Endpoints

The following endpoints will be measured at each study visit:

1. Unstimulated TSH bioactivity
2. Resting energy expenditure (REE) evaluation by indirect calorimetry
3. Weight
4. Body composition including body fat (percent and total), free fat mass, and bone density measured by dual energy x-ray absorptiometry (DXA)
5. Lipid profile via laboratory measurements
6. SF-36 for health status, hypothyroid symptoms (Appendix 2)
7. Heart rate and physical activity via actigraphy
8. Digit symbol substitution test
9. Body temperature using rectal temperature measurement
10. Optional exploratory genetic testing (one study visit only)
11. arterial tonometry, Doppler echocardiography, and arterial load assessment

3.4 Primary Safety Endpoints

Participants will undergo ECG testing at the screening visit so that a baseline EKG is on file. Hyperthyroid symptoms will be assessed to monitor participant safety. Symptoms of palpitations and shortness of breath will be assessed every two weeks by telephone while

the participant is on study drug. See Appendix 1 for the hyperthyroid symptom lists for phone contacts and study visits.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

We will include men and women aged 70 and older who are able to provide informed consent and have a TSH between 4.5 and 19.9 mU/L in conjunction with a normal free thyroxine (FT4) level.

4.2 Exclusion Criteria

Laboratory Tests:
1. thyroid peroxidase (TPO) antibody positive
3. abnormal liver function tests (LFTs >3 x upper limit of normal)
4. hemoglobin <11 g/dL

Surgeries or Procedures:

1. thyroid surgery
2. pituitary surgery
3. bariatric surgery
4. Bowel resection involving the jejunum and upper Ileum
5. radioactive iodine therapy
6. radiation treatments to head or neck

Medical Conditions:
1. diagnosis of pituitary disease
2. diagnosis of amyloidosis, sarcoidosis, hemochromatosis
3. diagnosis of adrenal insufficiency
4. obesity with BMI > 35 mg/kg^2
5. history of stroke
6. chronic or ongoing angina, Class II or higher congestive heart failure, or uncontrolled hypertension with current blood pressure greater than 160/100
7. diabetes mellitus with hemoglobin A1C level greater than 8.0% in the past six months
8. celiac sprue, Crohn's disease, ulcerative colitis, Zollinger-Ellison syndrome
9. renal insufficiency with calculated glomerular filtration rate <45 cc/min
10. cognitive impairment with Mini Mental State Exam[36] <24/30
11. history of any seizures
12. unstable medical or psychological condition in the judgment of the principal investigator

Medications:

1. thyroid hormone preparations
2. antithyroid drugs
3. medications that interfere with the absorption or metabolism of thyroid hormone

4. medications that interfere with the TRH stimulation test
5. proton pump inhibitors

Please see Appendix 3 for full list of medications

Exogenous estrogen and testosterone is not an exclusion, but all participants will be required to remain on the same dose throughout the study.

4.3 Subject Recruitment and Screening

Study participants will be recruited from the general medicine, geriatrics, and endocrine clinics of the University of Pennsylvania Health System. We will use the Penn Data Store, a data warehousing and reporting entity containing patient related information extracted from five major clinical electronic systems. The Data Store can be queried by tailored request using multiple different parameters, including age, diagnosis, medication, laboratory result, and clinic over a specified time frame. Selected providers of individuals who meet our inclusion criteria and are not taking thyroid medication will be contacted by the Investigator about the study protocol and recruitment efforts in order to obtain potential participants. Penn Research Trial Advisory (electronic alerting in EPIC) will also be used for contacting potential participants. The pop-up would appear in EPIC screens of participating practice providers during patient visits. If the potential subject agrees, their information would be relayed to the study coordinator via the EPIC system. I-connect, a novel Clinical Trial search and match technology will also be utilized. A study recruitment poster will be displayed on University bulletin boards and at University physician practices. The Penn Medicine biobank will be utilized as a source of subject identification. Once identified by way of these recruitment methods, verbal contact with potential participants, utilizing incoming and outgoing telephone recruitment scripts will be followed.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

The protocol defined follow-up period per subject will range from 14-78 weeks. A subject may be withdrawn from the study prior to his or her expected completion date for the following reasons:

1. If the investigator discovers a condition(s) which indicates unacceptable risk to the subject
2. If the subject fails to adhere to the protocol requirements
3. If the subject withdraws consent from participation in the study

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Attempts will be made to obtain at least survival data on these subjects up to the end of the subject follow-up period. In the case where the subject withdraws consent from participation in the study, permission to collect survival data will be obtained first. A subject will be labeled as “Lost to Follow-Up” only after the following has been documented:

1. Failure to reach the subject by telephone on 3 separate days
2. Failure to reach the subject's next of kin on 3 separate days

5 Study Drugs

5.1 Description

TRH is an endogenous hormone that is a ligand for the TRH receptor and is involved in regulating TSH release from the pituitary. TSH stimulates release of T4, which can be converted to T3, a more active form. Both T4 and T3 regulate metabolism in humans.

TRH will be purchased from American Peptide, which will produce a pharmaceutical grade (cGMP) formulation of TRH appropriate for human use. The TRH we will use is a tri-peptide and has a molecular weight of 362.3. TRH is supplied as a white to clear crystalline and powder. Syringes containing 200 mcg of TRH will be made by the University of Pennsylvania Investigational Drug Service. TRH is intended for intravenous administration.

Synthetic forms of T4 (levothyroxine, or LT4) and T3 (liothyronine, or LT3) will be purchased and shipped to the IDS pharmacy at Penn. Each of these medications is FDA approved for treatment of conditions of hypothyroidism. LT4 has a molecular weight of 776.87 and LT3 has a molecular weight of 650.98. LT4 and LT3 will be supplied as dose-tailored capsules filled with pharmaceutical grade powder. The capsules will be provided by the IDS.

5.2 Treatment Regimen

On Day 1 (Visit 1) participants will receive an IV injection of 200 µg TRH by a CTRC nurse plus verbal and written instructions on thyroid hormone dosing and sufficient study medication for 6 weeks. On the same day, participants will receive LT4 or LT3 in randomized order. Each will be titrated to a target TSH level of 0.5-1.5mU/L. Administration of LT4 and LT3 and monitoring in this study will be performed in a similar fashion to a published protocol and based on the literature of thyroid hormone replacement in subclinical hypothyroidism.[28-34]

<u>LT4</u>
Each participant will start on 0.7mcg/kg/day divided into three daily doses.
The dose will be adjusted according to subject weight and how far the TSH level is from goal. There will be a medication cap such that no dose adjustment will exceed 25 mcg per day.

<u>LT3</u>
The starting dose of liothyronine will be 1/3 of that of levothyroxine (0.23 mcg/kg/day of LT3) divided into three daily doses. We will titrate the dose according to subject weight and how far the TSH level is from goal. No dose adjustment will be greater than 8.3 mcg per day.

The total daily dose of study medications will be adjusted to a TSH of 0.5-1.5 mU/L using the adjustment scheme to avoid excess thyroid hormone replacement.

At the time of crossover of study drug, participants will undergo a 4 week wash-out period from the study drug. This is to avoid over-replacement when switching from LT4 to LT3, given the longer half-life of LT4. Following Visit 2, participants will stop all study drug for 4 weeks. At the end of 4 weeks they will begin the second study drug at the equivalent dose as they were taking at Visit 2, divided into 3 equal doses. TSH level will not be checked at the end of the wash-out period. A TSH level will be checked 4 weeks after crossing over to the new study drug. All further TSH level evaluations and medication adjustments will follow the titration protocol.

No direct benefits are expected to result from LT4 and LT3 administration.

The weight based dosing scheme and examples are displayed below:

LT4 Titration Scheme:

TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Dose change (mcg/kg/day)	0.25	0.21	0.17	0.1	**GOAL: No change**	**-**0.08	**-**0.09

Example of LT4 titration in a 50, 70, & 100 kg individual

	TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Weight (kg)	**Start dose (0.7mcg/kg/day)**	**Dose Change: Total mcg change per day**						
50	35	12.5	10.5	8.5	5	**GOAL: No change**	-4	-4.5
70	49	17.5	14.7	11.9	7		-5.6	-6.3
100	70	25	21	17	10		-8	-9

LT3 Titration Scheme: LT4 Dose scheme divided by 3

TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Dose change (mcg/kg/day)	0.08	0.07	0.06	0.03	**GOAL:**	**-**0.027	**-**0.03

					No change		

Example of LT3 titration in a 50, 70, & 100 kg individual

	TSH (mU/L)	>4.50	3.51-4.50	2.51-3.50	1.51-2.50	0.50-1.50	0.20-0.49	<0.20
Weight (kg)	**Start dose 1/3 LT4 Dose**	**Dose Change: Total mcg change per day**						
50	11.7	4.17	3.5	2.83	1.67	**GOAL: No change**	-1.33	-1.50
70	16.3	5.83	4.9	3.97	2.33		-1.87	-2.10
100	23.3	8.3	7	5.67	3.33		-2.67	-3.00

Identical coded dose-tailored capsules containing LT4 or LT3, will be produced at Penn's Investigational Drug Service (IDS). All therapeutic adjustments will be performed every four weeks by a blinded study physician and an IDS pharmacist. Identical amounts of study medication will be provided in each of the three daily doses. Subjects will receive 1 capsule per dose, and the dose of each capsule will depend on the total daily dose requirement. New study medication will be mailed overnight to participants within 5 days of the receipt of interim lab results. Interim TSH level checks will continue every four weeks until the target TSH is achieved.
After the targetTSH level is achieved, participants will attend Visit 2. They will receive a second IV injection of 200 μh TRH. Subjects will stop taking study drug for 4 weeks after the study visit 2. During this wash-out period once every two weeks a phone contact will continue to be made by one of the study team members to inquire if the participant has experienced any adverse events. Specifically, the participant will be asked if they have any symptoms of palpitations or shortness of breath. A six week supply of crossover study drug will either be sent home with the participant from study visit 2 or it will be mailed to them. Subjects will be instructed to be beginning this study drug 4 weeks after study visit 2. TSH level will be checked 4 weeks after starting the new study drug. All study measures for the other thyroid preparation will be repeated as above and Visit 3 will be identical to Visit 2, without administration of additional thyroid medication.

5.3 Method for Assigning Subjects to Treatment Groups

The order in which LT4 and LT3 are received will be randomized. A randomization list will be created by the IDS at Penn using a web-based program. The IDS will subsequently perform randomization assignment, and all study investigators, staff, and participants with direct contact with participants will be masked to treatment assignment.

5.4 Preparation and Administration of Study Drug

TRH in powder form must be stored at -20 degrees Celsius or cooler. LT4 and LT3 must be stored at room temperature. Storage, preparation, labeling and dispensing of all study drugs (TRH, LT4, and LT3) will be done by the IDS (available at 215-349-8817 or, for after-hours emergencies, 800-670-3151) in accordance with HUP's Pharmacy Policy and

Procedures. Each drug label will include the subject's unique identification number, date dispensed, and directions for use.

One prefilled syringe containing 200 µg TRH will be administered to participants by a CTRC nurse in the CTRC at each study visit.

TRH should be administered with the patient in the supine position. It will be administered as an intravenous bolus over 1 minute. The patient should remain supine until all of the post-injection blood draws have been completed. The patient should urinate prior to the procedure as they may feel an urge to urinate after the TRH is administered.

The LT4 and LT3 will be packaged in identical coded dose-tailored capsules containing either LT4 or LT3. The participants will be given sufficient study medication for 6 weeks at the start of each treatment regimen (LT4 or LT3). Participants will obtain TSH level checks at 4-week intervals, after which they will be provided with additional study medication as needed. If a dose change is required, new study medication will be mailed overnight to participants within 5 days of review of the lab results. Unused capsules will be returned to IDS at Visits 2 and 3.

5.5 Subject Compliance Monitoring

Adherence will be assessed by direct questioning of study participants. Any evidence of noncompliance will be recorded and subjects who are significantly noncompliant with the study treatment regimen may be withdrawn from the study. Determination of noncompliance will be based on investigator judgment. To avoid unnecessary adjustments, blinded study physicians involved in any medication adjustments will be made aware by study team members of the participants' self-reported adherence to the regimen.

5.6 Prior and Concomitant Therapy

Prior and concomitant medication history will be collected for each subject. Medications that are used on a daily basis, and are used to treat medical conditions that do not meet exclusion criteria will be permitted. Excluded prescription medications include thyroid hormone preparations, antithyroid drugs, corticosteroids, medication that interferes with the TRH stimulation test, and medication that interferes with the absorption or metabolism of LT4 or LT3 (Appendix 3).

5.7 Packaging

TRH will be shipped in bulk. IDS will prepare syringes for intravenous injection.

LT4 and LT3 will be shipped in bulk and packaged by IDS in identical coded capsules containing LT4 or LT3.

5.8 Blinding of Study Drug

LT4 and LT3 will be packaged by IDS in identical coded capsules.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the drug supplies from the manufacturers, an inventory will be performed by the IDS and a drug receipt log filled out and signed by the IDS staff person accepting the shipment. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files.

5.9.2 Storage

TRH will be stored in IDS at -20 degrees Celsius or cooler. LT4 and LT3 will be stored in a dry place and protected from light at room temperature.

5.9.3 Dispensing of Study Drug

TRH obtained from the IDS pharmacy will be given to patients as an injection in the CTRC. LT4 and LT3 obtained from the IDS pharmacy will be given to patients as oral capsules to take home. Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.9.4 Return or Destruction of Study Drug

All study participants will return unused capsules of study drug to study staff at Visits 2 and 3, which will be given to IDS. At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Screening Visit (Day -60 to Day -1)

Upon arrival at the CTRC, the participant will review and sign the IRB-approved consent form. The participant will also review an optional exploratory genetics research study IRB-approved consent form and sign only if they agree to participate in the optional study. They will then be assigned a unique screening visit identification number. Participants will be asked for a thorough history detailing their past medical history, current medications, and social habits. They will perform the MMSE. The research nurse will measure weight, height, blood pressure, heart rate, and perform a 12 lead ECG. A study investigator will perform a physical examination. Subjects will have blood drawn to evaluate lab parameters for eligibility criteria. A free thyroxine (FT4) level in conjunction with a TSH level will be sent to confirm subclinical hypothyroidism. The thyroid stimulating hormone (TSH) test obtained during the screening visit is measured at least 4 weeks after the last elevated TSH. Instructions for use of the activity and heart rate monitors will be given to each participant. Participants will then be discharged to home. If participants are eligible for the study, an activity and heart rate monitor will be mailed to their home. Participants will be advised to wear the activity and heart rate monitor for 4 days) prior to the first study

visit. The heart rate band will be moistened with either water or gel per the activity monitor instructions. Participants will also be given a log to record when they place their heart rate and activity monitors on and off.

6.2 Visit 1 (Day 1)

Participants will arrive at 7:30 am after a 12-hour fast. Weight and vital signs including heart rate, rectal temperature, and blood pressure will be measured. The activity and heart rate monitors will be collected. An REE evaluation by indirect calorimetry will be completed.

An IV will be inserted into the forearm and blood will be collected at times -5 and 0, followed by injection of 200 µg TRH.[28] Additional blood will be collected at 5, 10, 15, 20, 30, 60, 120, and 180 minutes, immediately processed, and stored at -80 degrees for measures of TSH, free T4, and total T3 (all time points), prolactin, alpha subunit, reverse T3 (0, 60, and 180 minutes), and a lipid profile (time 0). Additional samples at the 0, 120, and 180 time points will be processed and saved for TSH bioactivity measurements and TSH structure evaluation. If the participant consents to the optional exploratory genetic research study, then a sample will be collected, processed and saved for genetic testing at one study visit only (time -5).

Blood pressure will be monitored before administration of TRH, and at 5, 15, 30, 60, 120, 180 minutes.

Questions about health status, quality of life, and hypothyroid symptoms will be completed. Digit symbol substitution test will be completed.

Participants will be provided with lunch and undergo a DXA scan.

Participants will then undergo arterial tonometry and Doppler echocardiography testing. Assessment of arterial load will be made from this data.

Arterial tonometry: We will use a commercially available system (SphygmoCor, AtCor Medical) that uses a high-fidelity applanation tonometer (Millar Instruments; Houston, TX). The tonometer will be used to record carotid, radial and femoral arterial pressure waveforms. Mean brachial pressure will be obtained from a radial waveform calibrated with brachial systolic and diastolic pressures, measured with a validated oscillometric device. Diastolic and mean pressures will then be used to calibrate the carotid pressure waveform. Carotid-femoral and carotid-radial pulse wave velocity (PWV) will be computed from the time delay between the waveform foot on these arterial sites and the distance between the sites (measured on the body surface), where PWV = distance/time.

Doppler echocardiography**:** will be performed using a standard protocol, which will include parasternal views and apical views of the left ventricle. It will also include pulsed-wave Doppler interrogation of flow velocities in the LV outflow tract, mitral inflow and mitral annular velocities. Flow volume will be computed by multiplying LV outflow tract flow velocity by the LV outflow tract cross-sectional area measured with 3D echocardiography.

Diastolic function will be assessed using standard parameters from mitral inflow and mitral annular tissue velocity profiles.

Assessment of arterial load will be performed via pressure-flow analyses and will include assessments of proximal aortic characteristic impedance, total arterial compliance, and reflection magnitude computed using linear wave separation analysis, using central pressure and flow waveforms measured via arterial tonometry and Doppler echocardiography, respectively.

Arterial tonometry is non-invasive and does not have any known risks.
During various procedures (arterial tonometry, echocardiography), we will use adhesive electrodes attached to the participant's skin to record the electrical signal from the heart. These may occasionally cause skin itching and irritation.

Arterial tonometry and Doppler echocardiographic examinations will only be performed by appropriately trained personnel as per our institutional standards.

Prior to the end of the visit the participant will receive verbal and written instructions on thyroid hormone dosing and provided with sufficient study medication for 6 weeks. Then they will be discharged home.

6.3 Day 2-Week 4

Participants will consume coded capsules of either LT4 or LT3 supplements as divided doses three times per day in the morning, afternoon, and evening.

6.4 Medication Titration (Q4Weeks)

Participants will have TSH testing every 4 weeks (acceptable range 4-8 weeks) at a Penn outpatient laboratory facility until TSH is at goal, and then the participant will be scheduled for Visit 2. This visit can be scheduled up to four weeks from the date of the last TSH level.

Symptoms of palpitations and shortness of breath will be ascertained at the reminder calls prior to TSH level checks. The total daily dose of study medications will be adjusted by blinded study investigators to a TSH of 0.5-1.5 mU/L. New study medication for adjustment dosing will be mailed overnight to participants within 5 days of receipt of the interim lab results. Once the TSH is at goal, an activity and heart rate monitor will be mailed to the participant to wear one week prior to the next study visit.

6.5 Visit 2

Participants will repeat all measures from Visit 1 at this study visit on their first thyroid preparation (LT4 or LT3). Subjects will stop taking study drug for 4 weeks after the study visit 2. During this wash-out period once every two weeks a phone contact will continue to be made by one of the study team members to inquire if the participant has experienced any adverse events. Specifically, the participant will be asked if they have any symptoms

of palpitations or shortness of breath. A six week supply of crossover study drug will either be sent home with the participant from study visit 2 or it will be mailed to them. Subjects will be instructed to be begin this study drug 4 weeks after study visit 2. TSH level will be checked 4 weeks after starting the new study drug, and all study procedures for medication titration and TSH checks will be repeated as above.

6.6 Visit 3

Participants will repeat all measures from Visit 2 at this final study visit on their second thyroid preparation (LT4 or LT3). The study will be complete.

6.7 Phone Contacts

Once during the first week, a phone contact will be made by one of the study team members to inquire if the participant has experienced any adverse events following LT4 or LT3 supplementation. Thereafter, once every two weeks a phone contact will be made by one of the study team members to inquire if the participant has experienced any adverse events following drug supplementation. Specifically, the participant will be asked if they have any symptoms of palpitations or shortness of breath. Additionally, one day following each TRH administration (study Visits 1, 2, and 3) a phone contact will be made by one of the study team members to inquire if the participant has experienced any adverse events following study drug injection. If the participant experiences any adverse events following the final phone contact, they will have an emergency phone number to contact, which is 954-464-8588 as noted on the front of the informed consent document and utilized throughout the entire study.

6.8 Assays

All screening blood assays will be performed at William Pepper Laboratory, and will include a CBC, TSH, FT4, TPO antibodies, and a complete metabolic panel including liver function tests to exclude hyperglycemia, renal insufficiency, or hepatic insufficiency. Total blood volume drawn at the screening visit is approximately 12 ml. This will be drawn as two gold-serum separator tubes and one lavender-top tube each containing 4 ml of blood. If a hemoglobin A1C level has not been checked on diabetic particpants in the last 6 months, a hemoglobin A1C level will be added on to the screening labs.

During the baseline study visit and study visit on each treatment arm, blood will be collected and batched for biochemical assays for TSH, free T4, total T3, prolactin, and a lipid profile, to be performed by the Pepper laboratory. Reverse T3 and alpha-subunit will be measured using available kits. TSH bioactivity will be measured under the guidance of Dr. Gershengorn's laboratory at NIDDK. A sample for genetic testing will be collected at one study visit if the participant consents to the optional genetic research study. At the completion of the subject participation portion of the study, the optional genetic specimens, de-identified, will be sent to Dr. Francesco Celi at Virginia Commonwealth University for analysis. When complete, the samples and analysis will be sent back to Penn for incorporation into the study database.
Total blood volume drawn at each study visit is approximately <90 mL. Total blood drawn at these three visits combined is approximately <270 mL.

For interim thyroid hormone titration, a TSH level will be performed every 4 weeks until TSH is at goal. These TSH levels have a turnaround time of <24 hours. Total blood drawn at each titration draw is approximately 4 mL and will not cumulatively exceed 52 mL.

7 Statistical Plan

7.1 Sample Size Determination

We plan to enroll 30 subjects with an anticipated 15% dropout rate. With an alpha of 0.05 and 80% power and 15% dropout, we will be able to detect a 0.58 standard deviation (SD) change in outcome from baseline. A 3 SD improvement in unstimulated TSH bioactivity has been reported after treatment of overt primary hypothyroidism.[4] No previous studies have measured TSH bioactivity in subclinical hypothyroidism.

7.2 Statistical Methods

All statistical analyses will be performed under consultation with Dr. Cucchiara, Ph.D., a CTRC biostatistician. Our primary goal will be to assess changes in TRH stimulation test parameters at baseline compared to each thyroid hormone supplementation (LT4 or LT3) and our secondary goal will be to compare physiologic responses to LT4 and LT3 therapy from baseline. The area under the curve (AUC) for TRH-stimulated TSH response will be calculated using the trapezoidal rule. The maximum serum TSH concentration following TRH administration (TSH_{max}) will be obtained by direct inspection of the serum concentration-time profiles. Changes in TSH bioactivity, free T4, and total T3 will be determined using the higher of the 120 or 180 minute post-TRH values. Paired t-tests will be used to examine change from baseline for each preparation, and two-tailed tests will be used to compare data between treatment arms. Nonparametric tests will be employed as needed. The primary outcomes will be the changes in TRH stimulation test parameters (AUC, TSH_{max}, Δ TSH bioactivity, Δ free T4, Δ total T3). Additional outcomes will include changes in unstimulated TSH bioactivity, REE, body temperature, weight, body composition, lipids, physical actigraphy and heart rate, cognitive testing, symptoms, arterial tonometry, and Doppler echocardiography.

7.3 Subject Population(s) for Analysis

Any subject with study data at Baseline visit or later will be subjected to study analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)

- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.

- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor by telephone within seven days of becoming aware of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and emailed or faxed

to the study sponsor within 10 days of becoming aware of the event The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and email or facsimile to:

Susan Zieman, MD, PhD, Phone: 301-496-6949, email: ziemans@nia.nih.gov

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

8.3.2 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

  Related to the research procedures (An event is “related to the research procedures” if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: “Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events” or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

Reporting Deaths: more rapid reporting requirements
Deaths that occur during the course of a research study and that are:

- Unexpected; AND
- Related to the research study; AND
- When other participants are believed to be at an increased risk of harm

Must be reported to the IRB within 3 days from the time the investigator becomes aware of the death.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety

reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
  -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

Contact information pending IND submission

8.4 Unblinding Procedures

Any intentional breaking of the blind prior to study completion, which may be necessary, for example, in the case of an SAE, will be done in conjunction with the IDS, whose responsibility it will be to notify a designated member of the research team of the individual subject's treatment assignment. Any intentional or unintentional breaking of the blind prior to study completion will be documented and reported to the PI, CTRC, and the IRB within 24 hours and the FDA within seven calendar days.

8.5 Stopping Rules

The study will be stopped if the following criteria occur:

1. The PI discovers a condition or conditions that indicate unacceptable risk to the subjects.
2. There is evidence of harm of study-related treatments or procedures to the subjects.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events. Additional medical monitoring will be performed by an external study monitor who is not an investigator in this study.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question

was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

Internal medical monitoring will be performed by Dr. Cappola, who will oversee the safety and validity of the study, according to the University of Pennsylvania Sponsor-Investigator Standard Operating Procedures. In addition, an external medical monitor who is not a study investigator will perform safety monitoring.

Safety monitoring will include careful real-time assessment and appropriate reporting of AEs and SAEs. Safety assessments will be performed by the internal medical monitor after each subject has completed the study (baseline study visit through final visit on treatment 2), and by the external medical monitor after the first, fifteenth, and thirtieth participants have completed the study. All subjects who experiences and/or discontinued due to AEs will be reviewed for key safety data. A final safety assessment will be performed within 3 months of when the last subject has completed the study.

As the Study Sponsor, the PI assumes responsibility to assure that study activities are being carried out as planned so that deficiencies can be identified and corrected. This study will in addition be monitored by a clinical research professional from the staff of Clinical Research Coordinators working for other investigators at the University of Pennsylvania who will be responsible to complete a monitoring process for this study. The designated monitor is not an active member of this study team. A CV for the monitor will be obtained and updated annually. The CV will be kept on file in the Regulatory Binder. The monitor will receive a current protocol, most recently approved Informed Consent Form, and Case Report Forms prior to each monitoring visit.

All CRFs for the first two subjects completed will be 100% source data verified. In addition, CRF’s for the 10th, 20th, and 30th subjects will be 100% source data verified upon study completion. If a greater than 10% error rate is noted during the data review, the monitor will source data verify 100% of the data on a larger sample of active subjects following the monitoring visit. The following variables will be 100% source data verified during the monitoring process:

- Informed Consent
- Inclusion/Exclusion Criteria
- Serious and Non-Serious Adverse Events
- Prohibited concomitant medications.
- Drug accountability (IDS will be responsible for drug accountability. Research staff will return all unused study drugs to IDS.

In addition, all subjects who discontinued for “lost to follow-up” or other reasons will be 100 % source data verified. All monitoring activities completed by the internal study monitor will be recorded on the OHR approved subject review checklists. Monitoring activities cited above will be documented by the monitor in a written report to the PI so that appropriate corrective actions may be implemented as indicated and the report submitted will be filed in the Regulatory Binder.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment 1 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the US National Institutes of Health.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Participants will not be paid for completing the initial screening visit, but will receive a validated parking stamp or transportation costs for this visit. Eligible participants who continue to participate in the study will receive validated parking stamps or transportation costs at each study visit plus $300 following completion of all study visits.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study. Results will be available in Pub Med Central, as per NIH guidelines.

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15 Attachments

16 APPENDIX

16.1 Appendix 1

Hyperthyroid Symptoms assessed with each telephone encounter while on study drug:

1. palpitations

2. shortness of breath

Hyperthyroid Symptoms assessed at each study visit:

1. anxiety
2. irritability
3. muscle discomfort or weakness
4. tremor
5. heat intolerance
6. palpitations
7. fatigue
8. weight loss
9. frequent bowel movements
10. shortness of breath

16.2 Appendix 2

Hypothyroid symptoms assessed at each study visit:

1. slower thinking
2. depression
3. muscle cramps
4. dry skin
5. cold intolerance
6. fatigue
7. weight gain
8. constipation

16.3 Appendix 3

Thyroid hormone preparations:
1. levothyroxine
2. liothyronine
3. desiccated thyroid extract

Antithyroid medication:
1. methimazole
2. propylthiouracil

Medication that interferes with thyroid hormone or TRH stimulation test:
1. lithium
2. amiodarone
3. Kelp tablets

4. radiographic contrast agents within 16 days of thyroid testing (if participant has had radiographic contrast agent in this time frame, we will retest at least 16 days from the day the contrast was administered
5. cholestyramine
6. colestipol
7. colesevelam
8. interferon
9. ipilimumab
10. alemtuzumab
11. pembrolizumab
12. sunitinib
13. sorafenib
14. bexarotene
15. phenytoin
16. carbamazepine
17. rifampin
18. phenobarbital
19. octreotide, somatostatin
20. dobutamine
21. dopamine antagonists such as risperidone, olanzapine, quetiapine, ziprasidone, aripiprazole, asenapine, clozapine, Iloperidone, Lurasidone, Paliperidone, chlorpromazine, fluphenazine, haloperidol, loxapine, perphenazine, pimozide, Thioridazine, thiothixexe, trifluoperazine, droperidol, prochlorperzaine, metaclopramide

Proton Pump inhibitors:
1. dexlansoprazole
2. esomeprazole
3. lansoprazole
4. omeprazole
5. pantoprazole
6. rabeprazole