Thyroid hormone to induce non-insulin mediated glucose disposal in patients with insulin receptor mutations

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**Indicates those authorized to obtained informed consent*

Protocol Overview

Protocol Title:	Thyroid hormone to induce non-insulin mediated glucose disposal in patients with insulin receptor mutations
Abbreviated Title:	T3 in insulin receptor mutations
IRB:	NIDDK/NIAMS
Research Type:	Phase II Clinical Trial
Multi-site Collaboration:	No
Intramural Collaboration:	No
Ionizing Radiation Use: (X-rays, e.g., CT; radioisotope, e.g. PET; etc.)	Research Indicated
Investigational New Drug/Device:	No
Patient Self-Referral Allowed:	Yes
List Protocol On Web:	Yes
Is tissue being collected for research purposes:	Yes

Conflict of Interest

The protocol involves no drugs/devices/products that may lead to payments and/or royalties to be paid to the investigators or the NIH.
The investigators have no equity, consultative, or other financial relationship with a non-NIH source related to this protocol which might be considered a conflict of interest**.**

Time Frame

Start Date:	12/1/2014
End Date:	11/30/2020

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Precis – Abstract

Background
Patients with mutations of the insulin receptor have extreme insulin resistance. This frequently results in diabetes in childhood that is extremely difficult to manage with conventional diabetes therapies, including insulin at doses 10-50 fold higher than usual. Poorly controlled diabetes, in turn, leads to microvascular complications (e.g. blindness) and early death. Hyperthyroidism, whether endogenous (e.g. Graves' disease) or exogenous, increases energy expenditure, activates brown adipose tissue, and enhances skeletal muscle perfusion, leading to enhanced glucose disposal. In a single patient with mutation of the insulin receptor and poorly controlled diabetes despite maximal therapy, iatrogenic mild hyperthyroidism for treatment of thyroid cancer resulted in normalization of glycemia control, suggesting that thyroid hormone treatment could have therapeutic benefit in this rare disease.

Aim
The purpose of this study is to determine if treatment with thyroid hormone will increase glucose disposal in patients with mutations of the insulin receptor, and thereby improve glycemia control. The hypotheses to be tested are:

- Thyroid hormone will increase whole-body glucose disposal in patients with insulin receptor mutations.
- This increased glucose disposal will be mediated via increased glucose uptake in BAT and muscle.
- Increases in glucose disposal will result in improved glycemia control.

Methods
This study is a non-randomized pre-post design, conducted in two sequential parts. Part 1 is a short-term (2 week) proof-of-principle study to test whether thyroid hormone will increase glucose disposal in patients with insulin receptor mutations (with or without diabetes), and the mechanisms by which increased glucose disposal occurs. Part 2 is a longer term (6 month) therapeutic study to test whether thyroid hormone will result in improved glycemia control in diabetic patients with insulin receptor mutations.

Study Objectives

Part 1 (2 week study): The primary aim of this study is to determine if triiodothyronine (T3) treatment will increase glucose disposal in patients with mutations of the insulin receptor. The null hypothesis is that T3 will have no effect on glucose disposal. The alternative hypothesis is that T3 will increase glucose disposal.

Part 2 (6 month study): The primary aim of this study is to determine if triiodothyronine (T3) treatment will improve diabetes control, measured as hemoglobin A1c, in patients with mutations of the insulin receptor. The null hypothesis is that T3 will have no effect on A1c. The alternative hypothesis is that T3 will decrease A1c.

Introduction

Insulin receptor mutations

Mutations of the insulin receptor result in extreme insulin resistance and dysglycemia in humans [1,2]. These disorders have a spectrum of clinical severity linked to the degree of residual activity of the insulin receptor [3]. Three clinical syndromes have been described: infants with Donohue syndrome (also called leprechaunism) and children with Rabson-Mendenhall syndrome (RMS) have characteristic dysmorphic features, and frequently have fasting hypoglycemia coupled with postprandial hyperglycemia [4], while patients classified as Type A insulin resistance lack dysmorphisms [5]. The majority of patients who survive beyond two years of age (RMS and Type A insulin resistance) develop persistent hyperglycemia as endogenous insulin secretion declines[5,6]. Hyperglycemia in patients with insulin receptor mutations is extremely difficult to treat [7], and patients are at risk for early morbidity and mortality from microvascular complications of diabetes [5].

High doses of concentrated insulin have been used to treat patients with insulin receptor mutations in an effort to maximize any residual insulin receptor signaling. Recombinant insulin-like growth factor-1 (IGF-1) has also been tried in small numbers of patients, and appears to be most effective in those with the mildest form of the disease [8]. In our experience, IGF-1 has not been useful in the most severely affected patients, and if its action is through residual insulin receptor activity, it is more logical and safer to use high-dose insulin. Other conventional glucose lowering therapies, such as metformin, may be modestly beneficial in patients with insulin receptor mutations, but additional glucose lowering treatments that do not require signaling through the insulin receptor are required for effective treatment of these conditions.

Brown adipose tissue

Brown adipose tissue (BAT) has recently gained attention as a potential target to increase energy expenditure in adult humans [9,10]. Whereas white adipose tissue (WAT) functions primarily as an energy storage organ, BAT burns energy for thermogenesis. It does this through the action of uncoupling protein-1 (UCP-1), which allows leakage across the mitochondrial electrochemical gradient, causing the energy generated from substrate oxidation to be released as heat rather than used to form ATP. Major activators of BAT include cold, beta-adrenergic receptor (primarily β3) stimulation, and thyroid hormone [11]. Although the primary substrate utilized by BAT is free fatty acids, BAT also takes up glucose, and BAT activation has the potential to increase glucose utilization in diabetic individuals.

Role of thyroid hormone in glucose uptake

In 2010, Monica Skarulis and colleagues reported a remarkable clinical case[12]: A 32 year old woman with homozygous mutation of the insulin receptor and poorly controlled diabetes despite up to 3000 units of insulin per day developed thyroid cancer. As part of her cancer therapy, she was treated with levothyroxine (T4) at doses of 200-225 mcg per day to suppress her thyroid stimulating hormone. Over the next 3 years, her hemoglobin A1c fell from 9.9 to 5.6%, despite discontinuing all diabetes medications and maintaining a stable body weight. As expected based on her genetic defect, her insulin resistance did not change, with negligible insulin stimulated glucose disposal during a hyperinsulinemic euglycemic clamp (500 uU/m2/min insulin infusion) both prior to and after beginning T4 treatment. PET scanning performed for cancer surveillance revealed activated BAT in the supraclavicular regions; this was no longer visible after 2 weeks of thyroid hormone withdrawal (in the hypothyroid state), and was once again visible 2 weeks following reinitiation of T4. Biopsy of a supraclavicular focus of FDG uptake showed histologic and gene expression patterns consistent with BAT, and biopsy of subcutaneous adipose tissue (a classic WAT depot) also showed gene expression patterns consistent with BAT, suggestive of

"browning" of this WAT depot. This case suggests that T4 treatment led to increased BAT activity and volume and possibly browning of WAT, which may have resulted in increased glucose uptake and the remarkable clinical improvement in glycemia observed in this patient. This case study was limited as it did not incorporate measurements of glucose uptake by metabolically active fat and skeletal muscle.

The role of thyroid hormone excess in glucose metabolism has been investigated in several studies in humans with normal insulin receptor function. In general, patients with hyperthyroidism have *increased* blood glucose in the fasting and post-prandial states [13]. This worsening of glucose metabolism appears to be due to worsening of post-receptor insulin resistance, particularly at the level of the liver [14], resulting in increased hepatic glucose production [15]. In contrast, hyperthyroid patients (compared to euthyroid controls) have a 3-fold increase in glucose uptake in BAT, and 1.9-fold increase in glucose uptake in muscle; these normalize once patients return to a euthyroid state [16].

Recently, Lahesmaa et. al., reported that BAT takes up 2.7 ± 2.3 umol/100g/min of glucose in the hyperthyroid state, and takes up 0.9 ± 0.1 umol/100g/min of glucose in the euthyroid state. They also showed that in the hyperthyroid state, skeletal muscle takes up 1.9 ± 0.6 umol/100g/min of glucose, and in the euthyroid state, takes up 1.0 ± 0.3 umol/100g/min of glucose. If we assume that an average human possesses ~50g of BAT, and ~30,000g of muscle, then BAT will take up an excess of 0.9 umol/minute of glucose in the hyperthyroid vs. the euthyroid state, and muscle will take up an excess of 270 umol/minute of glucose in the hyperthyroid vs. the euthyroid state. Thus, muscle is anticipated to be the major organ taking up excess glucose in the presence of T3 treatment.

Taken together, these data suggest that, in patients with fixed insulin resistance due to insulin receptor mutations, thyroid hormone might enhance glucose disposal without worsening insulin resistance, resulting in a net improvement in glucose homeostasis.

Subject Eligibility Assessment and Enrollment

2 week study:

Inclusion Criteria

- Mutation of the insulin receptor (either recessive or dominant negative). If mutation status is not known prior to enrollment, subjects will undergo genotyping at enrollment. In the unanticipated event that a patient does not have a mutation of the insulin receptor, he or she will not complete the study and his or her data will not be included in the analysis.
- Age 12 to 65 years old

Exclusion Criteria

- Changes in doses of diabetes medications (including metformin, insulin, sulfonylureas, thiazolidinediones, leptin, GLP-1 agonists, DPP4 inhibitors, etc.) in the preceding 10 weeks.
- Any medical condition or medication that will increase risk to the subject (e.g. ischemic or structural heart disease, congestive heart failure, uncontrolled hypertension, or arrhythmia) or that will interfere with interpretation of study data.
- Disorders that would lead to erratic gastrointestinal absorption or loss of thyroid hormone from the gut (severe diarrhea, celiac disease, use of bile acid sequestrants, excessive consumption of soybean products).
- Any form of endogenous hyperthyroidism or hypothyroidism at baseline.
- Current or recent (past 8 weeks) use of thyroid hormone or anti-thyroid drugs.

- Extreme disorders of thyroid hormone binding to thyroid binding globulin (excess or deficiency) or protein loss (nephrotic range proteinuria) that would lead to difficulties achieving a consistent thyroid hormone level for study.
- Known presence of a rare clinical disorder that leads to thyroid hormone insensitivity (known T3 receptor mutations, selenocysteine insertion sequence-binding protein 2 (SBP2) abnormalities, monocarboxylate transporter defects).
- Current use of beta blockers
- Pregnancy or breast feeding
- Any EKG abnormality that could increase risk of T3 treatment (resting sinus tachycardia (age adjusted norms), atrial fibrillation, myocardial ischemia, left or right ventricular excitation block, left ventricular hypertrophy or extrasystoles)
- Known allergy or hypersensitivity to any form of thyroid hormone
- Known adrenal insufficiency
- Dependence on oral anticoagulant medications (adults only)
- Use of tricyclic anti-depressants, as transient cardiac arrhythmias have been observed with the concomitant use of thyroid hormone.
- Use of cholestyramine.
- History of clinically significant osteoporosis per investigator judgment (e.g. previous fragility fracture)

6 month study:

Patients must meet all inclusion and exclusion criteria for the short-term study, plus have poorly controlled diabetes, defined as a hemoglobin A1c $\geq$ 7%.

Study Design and Statistical Analysis

Design

This is a prospective, non-randomized study.

Randomization

Although the optimal method to study short-term effects of thyroid hormone would be a randomized, double-blinded, placebo controlled, cross-over study, such a study design is not feasible in this population. This study design would require subjects to be hospitalized for more than 4 weeks (>2 weeks for each study period), and is unlikely to accrue sufficient subjects for successful completion in this extremely rare condition.

Outcome Measures

The primary aim of this study is to determine the effect of thyroid hormone on glucose disposal over 2 weeks and A1c over 6 months in patients with mutations of the insulin receptor. In addition to quantification of glucose disposal, we plan to study effects of thyroid hormone on energy expenditure, substrate utilization, sympathetic nervous system tone, and gene expression in adipose tissue and muscle.

- **Primary outcome:**
  - **2 week intervention:** Total body glucose disposal in the fasting state measured using isotopic tracers
  - **6 month intervention:** Hemoglobin A1c

- **Secondary outcomes:**
  - **2 week intervention:**
    - Glycemic outcomes:
      - Tissue-specific glucose disposal in the fasting state in BAT, WAT, and muscle quantified using positron emission tomography (PET) in adults only
      - Glucose area under the curve measured using 7 point daily plasma glucose measurement, continuous interstitial fluid glucose monitoring, and oral glucose tolerance testing
      - Integrated assessment of glycemia over 2 weeks using hemoglobin A1c, fructosamine, and glycated albumin
      - Quantification of fractional gluconeogenesis and glycogenolysis using isotopic tracers
    - Energy Intake/Expenditure:
      - Resting energy expenditure at thermoneutrality (measured at 25-27 °C using indirect calorimetry in the metabolic chamber)
      - Core and peripheral body temperature
      - Food intake measurement
    - Gene expression in WAT and muscle biopsies in adults only

  - **6 month intervention:**
    - Glycemic outcomes:
      - Tissue-specific glucose disposal in the fasting state in BAT, WAT, and muscle quantified using positron emission tomography (PET) in adults only
      - Total body glucose disposal in the fasting state measured using isotopic tracers
      - Quantification of fractional gluconeogenesis and glycogenolysis using isotopic tracers
      - Glucose area under the curve measured using 7 point daily plasma glucose measurement, continuous interstitial fluid glucose monitoring, and oral glucose tolerance testing
      - Total daily dose of insulin
    - Energy Expenditure:
      - Resting energy expenditure at thermoneutrality (measured using indirect calorimetry in the metabolic chamber)
      - Core and peripheral body temperature
      - Food intake measurement

Statistical Considerations

Sample Size Justification

2 week study
The primary objective of the 2 week study is to assess the impact determine the effect of thyroid hormone on total body glucose disposal over 2 weeks in patients with mutations of the insulin receptor. Sample size is determined by the change in glucose disposal.

The null hypothesis is that the glucose disposal before and after thyroid hormone treatment is same.

The alternative hypothesis is that the glucose disposal is changed after the treatment

The sample size is calculated based on previous studies in literature. A similar study by Dimitriadis et al [14] reported a difference in total body glucose uptake between the euthyroid and hyperthyroid conditions of 0.39 $mg \cdot kg^{-1} \cdot min^{-1}$ with a standard deviation of <0.27. With this mean change and SD, a sample size of only 6 subjects would give 80% power with a two-sided alpha of 0.05 for this outcome. Based on the assumption that results for this study will fall in a similar ranges as those published in the literature, we calculate the sample size dependent on the glucose disposal change and its standard deviation, which are given in the following table.

Glucose disposal change	Power=80%					Power=90%				
	SD=0.1	SD=0.2	SD=0.3	SD=0.4	SD=0.5	SD=0.1	SD=0.2	SD=0.3	SD=0.4	SD=0.5
0.2	5	10	20	34	52	5	13	26	44	68
0.3	4	6	10	16	24	4	7	13	21	32
0.4	3	5	7	10	15	3	5	9	13	19
0.5	3	4	6	8	10	3	5	7	9	13

Here we note that the sample size determined is only for studying the hypothesis about the primary response, total body glucose disposal. An additional power analysis was performed for a key secondary endpoint: the change in muscle glucose uptake on PET. Lahesmaa et al reported that the difference in muscle glucose uptake in the euthyroid versus hyperthyroid condition was 1.4 with a standard deviation of 0.6. With this mean change and SD, a sample size of only 4 subjects would give 80% power with a two-sided alpha of 0.05 for this outcome. Sample sizes for similar results are shown in the following table. For example, a sample size of 10 for this two treatment crossover study will have a probability of 80% to detect a change of glucose disposal 1.0 with standard deviation of 1.0 at a two sided 5% significance level.

Glucose disposal change	Power=80%					Power=90%				
	SD=0.4	SD=0.6	SD=0.8	SD=1.0	SD=1.2	SD=0.4	SD=0.6	SD=0.8	SD=1.0	SD=1.2
0.5	8	14	23	34	48	9	18	29	44	63
1.0	4	6	8	10	14	5	7	9	13	18
1.5	3	4	5	6	8	4	5	6	7	9

6 month study

The primary objective of the 6 month study is to assess the impact determine the effect of thyroid hormone on A1c over 6 months in patients with mutations of the insulin receptor who have poorly controlled diabetes. Recent data from our group in the same population (with a different intervention)[17] indicates the A1c change 6 month after treatment is about 2.1 with standard deviation of 1.4. If we expect similar A1c changes in the current study, then a sample size of 6 will lead to a power of 80% (at a two-sided alpha of 0.05). A sample size of 7 would allow us to detect an A1c change of 2.0 with a standard deviation of 1.5.

Based on all of the above, we will endeavor to recruit at least 10 patients with this extremely rare condition into the 2 week study, and have at least 7 progress into the 6 month study. All patients who complete the 2 week study and who are eligible for the 6 month study will (if they consent) proceed immediately from the 2 week study into the 6 month study. As not all subjects may complete the study, and the sample size calculations have some uncertainty, we will cap enrollment at 20 subjects. Currently, we follow 17 patients with insulin receptor mutations, of whom 10 have poorly controlled diabetes. In addition, we are in touch with other physicians throughout the world who follow similar patients who might be interested in participating in this study.

Statistical Methods for Data Analysis

The following analyses will be performed:

- Descriptive statistics of response variables by visit. This includes calculation of means and standard deviations of the response variables at the pre and post treatment visits.
- Paired comparison of measurements before and after the treatment for primary and secondary outcomes
- Model analysis of selected response variables

Primary Response Variable

Primary response variable for the 2 week study is change in total body glucose disposal from baseline to 2 weeks after the treatment. A paired t-test will be used to compare the glucose disposal before and after treatment. A p-value of 0.05 or less is considered significant.

The primary response variable will also be analyzed using the Linear Mixed Model (LMM). This will allow for comparison of glucose disposal at different visit adjusting for additional covariates. The potential covariates include:

- Probable covariates
    - Fasting blood glucose
    - Fasting insulin
    - T3 level
    - Lean mass
- Possible covariates
    - Age
    - Sex
    - Mutation type (dominant versus recessive)

The effect of covariates that could influence the primary response variable will be tested by including each of the covariates detailed above into a mixed model. Each model tested will only include a single covariate due to small sample size.

The primary response variable for the 6 month study is the change in A1c from baseline to 6 month after the treatment. Analyses of A1c measurements are similar to that for the primary response variable.

Secondary Response Variables

Secondary outcomes are described above (Outcome Measures) and will be analyzed in a similar manner as the primary response variables.

Study Implementation

Subjects will be admitted to the Metabolic Research Unit in the NIH Clinical Center. They will remain as inpatients throughout the 19 day duration of the short-term study. Upon admission, they will undergo a history and physical examination, and baseline labs will be obtained. Labs used to assess subject eligibility in all subjects include electrolytes, BUN, creatinine, CBC, and a pregnancy test for female patients of reproductive potential. Baseline serum 25-hydroxyvitamin D levels will be obtained in all subjects. In subjects with 25-hydroxyvitamin D levels below 20 ng/mL, vitamin D supplementation will be provided. An EKG and echocardiogram will be performed to assess eligibility. Height, weight, blood pressure, resting pulse, temperature will be obtained.

Diet

After baseline phenotyping, subjects will be placed on a metabolic diet with a fixed food quotient, consisting of 40% carbohydrates, 20% protein, and 40% fat. Subjects will be asked to consume all food at meals, and snacks (of similar macronutrient composition to meals) will be provided ad libitum. All food will be provided by the metabolic research kitchen. Uneaten food will be weighed, and daily food intake (total kcal and macronutrient content) will be calculated.

Medications

Pre-study Medications

With the exception of insulin (discussed below) subjects will continue their pre-admission medications throughout the study. This includes oral hypoglycemic agents, and other medications either related or unrelated to mutation of the insulin receptor or its complications.

Liothyronine (T3)

T3 is the active form of thyroid hormone. Our rationale for choosing liothyronine (T3) over levothyroxine (T4) for this study is for two reasons. First, due to the short duration of the initial proof of principle study (two weeks), we would prefer to use a formulation of thyroid hormone with a short half-life that can be rapidly titrated. The half-life of liothyronine is a few hours, versus levothyroxine which is one week, thus allowing us to rapidly titrate the dose. Secondly, we prefer to use a formulation of thyroid hormone that will maximally stimulate BAT without the need for conversion from T4 to T3 by type 2 deiodinase. By using liothyronine as opposed to levothyroxine, we are able to study the direct effect of T3 on BAT, and are essentially eliminating an additional step.

Replacement doses of T3 to achieve euthyroidism in hypothyroid adults are approximately 0.57 mcg/kg/day [18]. Stable plasma T3 levels can be achieved with q8 hour T3 dosing [19]. In this study, the goal will be to achieve peak plasma T3 levels between 125% and 150% of the upper limit of normal (200 ng/dL in the NIH Clinical Center laboratory). The estimated T3 dose to achieve this level of plasma T3 is 2 times the replacement dose, or 1.14 mcg/kg/day. In a 50 kg individual, this equals 57 mcg. T3 is available in dosing increments of 5 mcg; thus doses will be rounded (lower dose will be used if calculated dose is 0 to <2.5 mcg above a dose divisible by 5; higher dose will be used if dose is 2.5 to <5 mcg above a dose divisible by 5). By comparison, T3 at doses of 150 mcg per day (~2.1 mcg/kg/day, or

approximately 4 times the replacement dose) was given to healthy, euthyroid adults for 2 weeks, raising plasma T3 levels from 125 to 507 ng/dL [14]. This treatment was well tolerated, with an increase in heart rate from 65 to 78 beats per minute, and weight loss of 2 kg, and the development of "mild to moderate symptoms of enhanced appetite, nervousness, headache, or palpitations". In a similar study, T3 at doses of 75 to 100 mcg per day (1.1 to 1.4 mcg/kg/day) was given to healthy, euthyroid adults for 2 weeks, raising plasma T3 levels from 110 to 350 (range, 190-520) ng/dL, with no reported adverse events.

The dose titration scheme for T3 is as follows:

- Day 1: 1x replacement (0.57 mcg/kg/day)
- Day 2: 1.5x replacement (0.855 mcg/kg/day)
- Days 3-14: 2x replacement (1.14 mcg/kg/day)

Plasma T3 peak (2-4 hours, with a goal of 3 hours, after morning dose) and trough (just before morning dose) levels will be measured on a daily basis for the first week, and every other day thereafter. If the T3 peak or trough exceeds targets below, the peak and trough will be repeated the following day (or the same day at the investigators' discretion), and doses will be reduced if trough or peak levels exceed targets on both measurements.

- Target trough T3 = 150-250 ng/dL (25% above or below the upper limit of normal of 200 ng/dL)
- Target peak T3 = 250-300 ng/dL

In addition, if subjects meet one or more of the following clinical and biochemical criteria the dose will be reduced to avoid toxicity:

- Tachycardia, defined as heart rate consistently over 110 over a 24 hour period
- Symptomatic palpitations
- Anxiety or nervousness that are not tolerable to the patient
- Development of systolic hypertension (defined as >130 mmHg AND a ≥ 10 mmHg increase over baseline) for a 24 hour period)
- Chest pain
- Congestive heart failure
- Diarrhea that is distressing to patient
- Weight loss > 4 kg or >10% of body weight (whichever is smaller)
- Peak T3 > 350 ng/dL

After the conclusion of the 2 week T3 treatment, subjects with poorly controlled diabetes will continue long-term (6 month) management with T3, at the maximum dose tolerated during the inpatient study (up to 1.14 mcg/kg/day). Because key safety monitoring and dose titration occur during the 2 week study, subjects must participate in the 2 week study in order to proceed to the 6 month study. During the next 6 months, doses will be reduced if the following toxicity criteria are met:

- Symptoms of thyroid hormone excess that are intolerable to the subject are reported by the patient, or elicited on monthly telephone screening, including:
    - Symptomatic palpitations (sensation of racing heart)
    - Changes in sleep, mood or behavior, school/work performance
    - Tremulousness, anxiety, sweating, diarrhea, or heat intolerance.
- Signs of thyroid hormone excess are detected at the 3 month NIH follow-up visit, including:
    - Tachycardia, defined as heart rate consistently over 110 over a 24 hour period
    - Development of systolic hypertension (defined as >130 mmHg AND a ≥ 10 mmHg increase over baseline) for a 24 hour period)

- Weight loss > 4 kg or >10% of body weight (whichever is smaller), AND body mass index less than 25 kg/m^2 (adults) or less than the 50th centile (children). This will permit healthy or desirable weight loss in overweight subjects.
- Trough T3 or free T4 more than 50% above the upper limit of normal on monthly safety laboratory evaluation

Special consideration will be provided for females taking oral contraceptive pills, as levels of thyroid hormone may appear falsely elevated though the patients remains clinically euthyroid. These patients may require further increases in doses of T3 to achieve desired peak levels.

Glucose Management

*S*ubjects who take insulin or insulin secretagogues may be at a small risk of hypoglycemia after initiation of thyroid hormone due to improvements in glucose disposal. Bedside blood glucose monitoring will be performed prior to meals and at bedtime (or more frequently if clinically indicated) in diabetic subjects. Insulin doses will be reduced on an as-needed basis to minimize hypoglycemia. Decision making regarding insulin dose reduction must be individualized for each patient based on clinical expertise, as patients with insulin receptor mutations may have fasting or postprandial hypoglycemia even in the absence of exogenous insulin treatment.

Standardized Exercise

In order to maintain physical fitness during the hospitalization, sedentary subjects will engage in 30 minutes of walking on a treadmill each day (during both Periods 1 and 2) at a mild to moderate self-selected pace. In subjects who habitually exercise, the duration and intensity of exercise will be increased to be consistent with their typical exercise routine. This activity will be supervised by medical staff.

Study Procedures

Short-term study:

	Off T3				**On T3**													
Study Day	**0**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
EKG & Echocardiogram	X																		
OGTT		X																	X
Glucose & lipid turnover		X																	X
Metabolic Chamber			X														X		
DEXA (body composition)			X														X		
DEXA (bone			X																

density)																		
PET-CT (age ≥ 18y)				X													X	
Adipose biopsy (age ≥ 18y)				X														X
Muscle biopsy (age ≥ 18y)				X														X
Continuous glucose monitoring			X	X												X	X	
Temperature monitoring			X													X		
7 point plasma glucose			X	X												X	X	
Bedside glucose monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fasting research blood tests	X			X	X						X	X					X	X
Thyroid function	X				X	X	X	X	X	X	X	X		X		X		X	X
Food intake measurement	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BP & heart rate	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X															X			

Long-term study:

Study Month	**1**	**2**	**3**	**4**	**5**	**6**
Thyroid function (home labs)	X	X		X	X	
Pregnancy test (females)	X	X	X	X	X	X
Telephone contact (safety)	X	X		X	X	
NIH visit			X			X
Pill count			X			X
Fasting research blood tests			X			X
Thyroid function			X			X
Vital signs (BP, heart rate, temperature, body weight)			X			X
Metabolic Chamber						X
DEXA (body composition)						X
DEXA (bone density)						X
OGTT						X
PET-CT (age ≥ 18 years)						X
Glucose & lipid turnover						X

Bedside glucose monitoring						X
Food intake measurement						X
Echocardiogram						X

During the 6 month follow up period, safety labs will be obtained on a monthly basis, either locally near the subject's home or at NIH. Safety labs will consist of trough T3 level, free T4, TSH, and pregnancy test (females). Monthly telephone assessments will be conducted during the months that no NIH visit is scheduled. This will include review of compliance, as well as screening for symptoms of hyperthyroidism, including changes in sleep, mood or behavior, school/work performance, tremulousness, anxiety, sweating, diarrhea, heat intolerance, and patient self-measurement of pulse rate and weight.

For those subjects where it is not feasible (due to distance from the NIH or inconvenience) for them to return to the NIH for a 3 month follow-up visit, the research team will communicate with the subject's primary doctor (or endocrinologist) and see if they are willing to evaluate them. The local physicians would not be engaged in human subject research because they would be providing medical services that would typically be performed as part of routine clinical monitoring. The physician would be requested to obtain the following labs: thyroid function tests, fasting glucose, insulin, c-peptide, lipid panel, free fatty acids and high-sensitivity C-reactive protein. Research samples for storage would not be obtained as it would be difficult to ensure consistency in processing. Additionally the clinic visit would include vital signs, weight and a conversation about their medication compliance. All results would need to be faxed to the research team at the NIH.

Metabolic Testing

- DEXA scan for total body composition: Measurements of fat-free mass will be obtained and used to normalize energy expenditure measurements. A DEXA scan (iDXA, GE Healthcare, Madison WI) will be performed to determine total and regional body fat and lean soft tissue masses, bone mineral content and density. DEXA produces photons at two different energy levels, 40 and 70 KeV. The photons pass through tissues and attenuate at rates related to elemental composition. Bone mineral, with highly attenuating calcium and phosphorous, is readily distinguished from soft tissues. The different elemental profiles of fat and bone-mineral free lean components allows for the analysis of soft tissue fat content, so that bone mineral, fat, and bone mineral fat-free lean components may be resolved.

- Skin temperature: Wireless probes (iButtons, Maxim Inc., Sunnyvale,CA) will record skin temperature with data reported each minute. The positions of the skin sensors at five sites (deltoid, hand, pectoralis major, anterior thigh, and shin) will be according to the ISO standards[21].

- Metabolic chamber: Study volunteers will enter the metabolic chamber at 08:00 AM after a minimum 8-hour fast and voiding. The temperature of the chamber will be set at between 25-27 C . Study volunteers will wear standard clothing (long-sleeved hospital scrub shirt and pants, cotton t-shirt, subject's own underwear; CLO value 0.55) and will be instructed to be minimally active while staying awake in the metabolic chamber at two predefined time periods (10:30-11:00AM and 11:30AM-12:00PM). To help with the compliance and occupying the time in the chamber, each subject will be allowed to watch television (sports, movies, series, etc), play non-active video games, read, or perform deskwork. Subjects will exit the chamber at 1 pm, after a 5 hour stay. Details of the chamber have been published previously[22,23].

The metabolic chamber is a specially constructed room to assess energy expenditure. Designed as a walk-in "pull" calorimeter, it is an open circuit unit that draws conditioned room air into the chamber at the same flow rate as it is extracted into the gas analysis system.[24] The room is equipped with toilet and sink with privacy screen, treadmill, bed, desk, and computer with access to television and other forms of entertainment. Physical activity level is measured continuously through a wall mounted monitoring device (microwave sensor). Calibration tests over the past three years at temperature settings from 15.3 to 32.1C have demonstrated an accuracy of 98.4±2.5% for EE as compared to a weighed propane standard oxidized by combustion.

The relative humidity of each chamber is controlled between 30-50% for all temperatures. The overhead circulation fan unit mixes the air of the chamber uniformly. At the bed and the recliner where the subjects will be spending the 5 hour resting periods, the wind speed is less than 0.4 m/s (0.8 mile/hour), making the windchill and humidex (heat index) factors less than 0.2C. We will measure chamber wall temperature using iButtons,[25] and calculate the exact "operational temperature" as defined previously[26].

- PET-CT:
  In adult subjects, dynamic PET image data of the upper torso will be acquired by a Siemens mCT PET/CT scanner (Siemens Medical Solutions, Hoffman Estates, IL). A 5 mCi dose of [^{18}F]FDG will be injected IV at approximately 7:30 am after a 12 hour fast. Patients will be instructed to keep movement to a minimum until after the scan is completed. The temperature in the PET scanner room is maintained at normal room temperature (~20-22 C), and will be monitored and recorded. First, an attenuation CT scan of the upper torso will be performed, followed by dynamic PET image of the same area (usually requiring 2 levels, each of 22 cm) initiated at the time of F18-FDG IV injection and acquired as described below, up to about 65 min post tracer administration.

  The field of view (usually 2 levels, lower-mid chest and upper chest-neck) will include:
  - Brown adipose tissue (cervical/supraclavicular)
  - White adipose tissue (subcutaneous in the chest wall)
  - Muscle (periscapular region)
  - Heart/left ventricle (for calculation of arterial input functions, described below)

  Data acquisition will be performed alternating between the 2 levels of the torso described above, with a total of ten 30-second frames, ten 3-minute frames, and six 5-minute frames, for a total of 65 minutes from the time of FDG administration. To facilitate parametric modeling of the BAT, WAT, and muscle tracer kinetics, arterial input functions will be derived from left ventricular (LV) region of interest (ROIs) drawn on the dynamic PET images. The LV ROIs will be defined from a sum of the early 30-second PET frames, with the aid of the accompanying CT scan, being careful to minimize the quantitative bias caused by partial volume effects. Tracer time-activity curves for the regions of interest will be then obtained for the entire duration of the PET study. These will be used to calculate the fractional rate of FDG uptake (Ki).

  Regions of interest for quantification of tissue glucose uptake will be defined as follows:
  - BAT will be defined visually in on-treatment scan, when BAT is anticipated to be active in all subjects due to stimulation by T3. The BAT ROI will then be mapped to the pre-treatment scan, so that glucose uptake is quantified in the same region for both studies.
  - WAT will be defined based on CT imaging. In lean subjects, it may not be possible to define acceptable ROIs for WAT, as the small size of these depots may result in

unacceptable volume averaging effects. In subjects with adequate WAT, the same ROI will be evaluated in the pre- and post-treatment scans.

- Skeletal muscle ROIs will be visually defined based on CT imaging. The same ROI will be evaluated in the pre- and post-treatment scans.

The rate of glucose uptake will be calculated by multiplying Ki by the plasma glucose concentration of the study subject, and dividing this by the lumped constant (LC) of adipose tissue of 1.14 [27] (for BAT and WAT), or skeletal muscle of 1.2 [28].

- Glucose and Lipid Turnover: Stable isotope tracers will be used to measure glucose and lipid turnover. Endogenous glucose production will be measured using steady-state infusion of [6,6-$^2H_2$]glucose tracer. The fractional rate of gluconeogenesis will be measured using steady-state oral dosing of deuterated water (^2H_2O) [29]. The rate of lipolysis will be measured using steady-state infusion of deuterium-labeled $^2H_5$-glycerol, and the fatty acid turnover rate will be measured using steady-state infusion of [U-$^{13}C_{16}$] palmitate. Subjects will fast after 8 pm. A total of 3 grams per kg lean body mass of ^2H_2O will be given in four divided doses every two hours at 9 pm, 11 pm, 1 am, and 3 am in order to enrich the subject's body water pool to approximately 0.5% ^2H_2O. Beginning at ~5 am, a primed [6,6-$^2H_2$]glucose infusion will be given for 3 hours, after which blood samples to measure isotope enrichment will be measured over a period of 30 minutes at steady state. Two hours after the [6,6-$^2H_2$]glucose infusion begins, a primed $^2H_5$-glycerol infusion, and unprimed [U-$^{13}C_{16}$] palmitate infusion will be given for one hour, and isotope enrichment will be measured over a period of 30 minutes at steady state. Subjects will empty their bladders immediately prior to the 30 minute steady state period, and urine will be collected at the conclusion of the steady state, for calculation of urinary glucose excretion during the steady-state period.

- Oral glucose tolerance test: After a 12-hour fast preceding the test, subjects will be given 75 gram (1.75gm/kg in patients less than 40kg) oral glucose solution. Venous glucose, plasma insulin and C-peptide will be obtained from blood samples drawn at -10, 0, 30, 60, 90, 120 and 180 minutes during the oral glucose tolerance test. The area under the glucose, insulin and C-peptide will be evaluated.

- Continuous glucose monitoring: A continuous glucose monitoring device will be inserted subcutaneously to measure interstitial fluid glucose every 5 minutes. Area under the curve for glucose will be calculated using the trapezoidal method.

- 7-point plasma glucose: Plasma glucose will be measured prior to each meal, 2 hours after each meal, and at bedtime. Area under the curve for glucose will be calculated using the trapezoidal method.

- Fasting research blood tests: Fasting glucose, insulin, C-peptide, lipid panel, free fatty acids, and high-sensitivity C-reactive protein will be measured every other day. Additional blood samples will be stored for future analysis.

- Muscle biopsies: Muscle biopsies will be performed twice (once in the off-T3 period, and once in the on-T3 period) in adult subjects to study expression of molecules important in glucose homeostasis. This will be performed in adult subjects only, and subjects will have the ability to opt out of participating in the biopsies. If participants choose not to participate in the biopsies,

they can still continue with participation in the other elements of the protocol. Muscle biopsies (100-250 mg) will be obtained under local anesthesia from the vastus lateralis using a Bergstrom needle. Connective tissue will be removed. A representative portion of the tissues will be formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin using standard histological methods. The remaining tissue will be separated into aliquots of approximately 50 mg for RNA isolation and protein extraction, flash frozen in liquid nitrogen and stored at -80ºC. Analyses in muscle will include measurement of gene expression (mRNA) for glucose transporters, irisin, UCP-3, and mitochondrial glucose-3-phophate dehydrogenase. In addition, RNAseq analyses will be performed as a hypothesis-generating experiment to look for changes in gene expression profile in the off versus on-T3 periods. Subjects with low platelet counts (<100 K/uL) and/or abnormal coagulation profiles (INR>2.0), or subjects on anti-coagulants, will be excluded from the muscle biopsies.

- Adipose tissue biopsies: Subcutaneous adipose tissue and muscle biopsies will be performed twice (once in the off-T3 period, and once in the on-T3 period) in adult subjects to study expression of molecules important in glucose homeostasis. This will be performed in adult subjects only, and subjects will have the ability to opt out of participating in the biopsies. If participants choose not to participate in the biopsies, they can still continue with participation in the other elements of the protocol. Approximately one gram of adipose tissue will be removed from the abdominal or gluteal region by aspiration with a 16-gauge needle under local anesthesia (2% xylocaine). Slides will be examined visually to assess cell populations/tissue composition. The remaining tissue will be separated into aliquots of approximately 500 mg for RNA isolation and protein extraction, flash frozen in liquid nitrogen and stored at -80ºC for future studies. Analyses in fat will include measurement of gene expression (mRNA) for markers of beige/brown adipose tissue, including UCP-1, PRDM16, DIO2, etc. Subjects with low platelet counts (<100 K/uL) and/or abnormal coagulation profiles (INR>2.0), or subjects on anti-coagulants, will be excluded from the adipose tissue biopsies.

Thyroid Evaluation

- Thyroid function will be assessed as serum levels of total and free thyroxine, triiodothyroinine (T3), and thyroid stimulating hormone (TSH).
- Thyroid biomarkers: SHBG, osteocalcin, lipid NMR

Safety Testing

- EKG will be performed at baseline to rule out any rhythm abnormalities that might increase risk associated with T3 treatment.
- Echocardiogram will be performed at baseline to evaluate structural abnormalities that would increase risk associated with T3 induced heart rate increase. Examples of structural abnormalities that would exclude patients include valvular heart disease, left ventricular hypertrophy, and hypokinesis suggestive of ischemia. Repeat echocardiogram will be performed after 6 months to evaluate any changes that may have occurred with T3 treatment.
- Blood pressure and heart rate will be measured 3 times daily during the 2 week study and during each inpatient visit (at 3 and 6 months) during the 6 month study. Blood pressure (right arm) and heart rate will be measured using an automated device after subjects have been seated in quiet room for 10 minutes. Measurements will be repeated at 2 minute intervals for a total of 3 measurements, and the mean of the last 2 measurements will be recorded (excluding the first measurement).

- Body weight will be measured during inpatient visits in the morning before breakfast and after emptying the bladder. Three body weight measurements will be obtained, with the subject stepping off the scale between measurements. The mean of the 3 measurements will be recorded.
- Urine or serum pregnancy testing will be obtained on female subjects with reproductive potential after admission, and will be repeated prior to radiation exposure as needed and on a monthly basis during the 6 month follow up. Effective contraception (including abstinence, barrier methods, hormonal contraception, or IUD, will be required for female subjects with reproductive potential.)
- DEXA for bone density: Because hyperthyroidism is associated with decreased bone mineral density, DEXA for bone density at the AP spine, hip, and 1/3 radius will be performed at baseline and after 6 months of T3 in patients with poorly controlled diabetes participating in the long-term component of the study. Bone density will not be assessed after 2 weeks of T3 in patients participating in only the 2-week portion of the study, as it is extremely unlikely that changes will be seen with this short duration of hyperthyroidism.

Follow-up

Post-Study Treatment

After completion of this study, subjects may continue to receive care through NIH via protocol 76-DK-0006 (Studies of Molecular Genetics of Insulin Secretion, Insulin Action and Diabetes Mellitus) or protocol 03-DK-0257 (Effect of Leptin Therapy in the Treatment of Severe Insulin Resistance).

Post-Study Obligations

There are no anticipated post-study obligations.

Human Subject Protection

Data and Safety Monitoring Plan

The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team. Overall accrual and adverse event information will be reported to the NIDDK/NIAMS IRB annually. Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan. The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team.

Adverse Events, Protocol Deviations, and Unanticipated Problems

Adverse events, protocol deviations, unanticipated problems (UP), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded and submitted to the NIDDK/NIAMS IRB at each annual review. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Non-serious protocol deviations will be reported to the IRB (as soon as possible but not more than 14 days after the PI first learns of the event) only if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the

research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

Deaths will be reported to the Clinical Director and IRB within 7 days after the PI first learns of the event. All deaths that have occurred among study participants since the previous review will be summarized at the time of continuing review.

Rationale for Subject Selection

Subjects eligible for this study include adults and children who are 12 years or older with mutations of the insulin receptor. Children less than 12 years of age will be excluded because they are unlikely to possess the emotional maturity needed to undergo the intense testing regimen, and because young children with insulin receptor mutations are less likely to have overt diabetes or complications of diabetes. Adolescents age 12-17 will be included because overt diabetes (and its complications) commonly develops during puberty in this patient population, and they are likely to be able to comply with study procedures. Because each subject will be compared to him/herself in this within-subjects design, the effects of inter-subject heterogeneity will be less important.

For adult participants, the short-term component of this study is of greater than minimal risk, and has no prospect of direct benefit. For adult participants, the long-term component of this study is of greater than minimal risk, and with prospect of direct benefit to subjects. For minor participants, the short-term component of this study involves a minor increase over minimal risk and has no prospect of direct benefit, but is likely to yield generalizable knowledge about the subjects' disorder or condition. For minor participants, the long-term component of this study is of greater than minimal risk, and with prospect of direct benefit to subjects.

Withdrawal Criteria

- **Withdrawal of consent**. A subject wishes to withdraw from the study as stated in the informed consent (all subjects reserve the right to withdraw from the study without prejudice).
- **Adverse event**. A subject experiences an adverse event that in the investigator's opinion necessitates withdrawal from the study. For example, the investigators would withdraw a subject if he/she developed intolerable symptoms of hyperthyroidism despite T3 dose reduction.
- **Investigator decision**. An investigator feels it is in the subject's best interest to terminate participation. The detailed reasoning behind this decision will be documented.
- **Protocol violation**. Includes subject noncompliance, pregnancy, study entry criterion violation, or start of an unacceptable concomitant medication.

Risks/Benefits Analysis including Considerations of Alternatives to Participation

Benefits

There are no direct medical benefits to either minor or adult subjects from participating in the initial 2 week T3 treatment. For subjects participating in the 2 week study, study procedures represent a minor increment over minimal risk, and are likely to yield generalizable knowledge about the subject's condition. Subjects with diabetes who continue T3 for 6 months have the potential to experience clinically meaningful improvements in diabetes control, which could reduce the risk of vascular complications of diabetes.

Risks/Discomforts

- General: Some patients may find the time needed to complete the research studies an inconvenience in their routine lives.

- T3:
    - Hyperthyroid symptoms: Subjects treated with supraphysiologic doses of T3 are likely to experience some symptoms of hyperthyroidism. These include palpitations, sleep disturbance, weight loss, anxiety, mood change, tremor, sweating, heat intolerance, menstrual irregularities, and diarrhea. In most cases, these symptoms are expected to be mild. If these symptoms are not tolerable to subjects, or exceed defined toxicity criteria, the dose of T3 will be reduced. Likewise, subjects may experience increases in systolic blood pressure or heart rate. If these exceed toxicity criteria, the dose of T3 will be reduced. Because the T3 dose will be titrated to avoid toxicity during the 2 week study, the risk of meeting toxicity criteria (especially for blood pressure and heart rate) during the 6 month study is minimized. Thus, subjects will not be required to conduct home blood pressure or heart rate monitoring during the 6 month study.
    - Decreased bone mineral density: Hyperthyroidism increases bone turnover, which over the long term can result in decreased bone mineral density. This effect is not different in children versus adults. This is unlikely to result in clinically meaningful increases in fracture risk over a 6 month period. If the 6 month treatment with T3 results in clinically meaningful improvements in diabetes control, the benefits of T3 would likely outweigh the risks related to decreased bone density. Bone density changes of the 6 month treatment period will be monitored using DEXA. If dietary intake is inadequate or Vitamin D levels are low, subjects will be prescribed appropriate supplementation. Subjects participating in the short-term part of the study will undergo one DEXA for bone mineral density, and those participating in the 6 month study will have a total of two DEXA scans for bone mineral density.
    - Cardiovascular disease: Long-term hyperthyroidism may place patients at increased risk of cardiovascular events. If the 6 month treatment with T3 results in clinically meaningful improvements in diabetes control, the benefits of T3 would likely outweigh the risks related to cardiovascular disease. Our rationale for this is that patients with insulin receptor mutations likely have a decreased risk of macrovascular complications of diabetes, because the insulin receptor mutations protects them from de-novo lipogenesis in the liver, resulting in a favorable lipid phenotype (high HDL and low triglycerides), in opposition to what is seen in typical type 2 diabetes mellitus and the metabolic syndrome. We do not have good data on the actual incidence of macrovascular disease in this population, as most of these patients unfortunately don't live long enough to develop macrovascular complications. Thus, due to the favorable lipid phenotype and the severity of this life-limiting disease, we believe that the benefits of T3 treatment outweigh the risks. Patients with baseline EKG or echocardiographic abnormalities that might place them at risk for acute cardiovascular events will be excluded. Changes in cardiac function will be monitored via echocardiography after 6 months.

- Blood Sampling: Peripheral blood draws (venipuncture) performed during this study for research will not exceed 10.5 mL/kg, or 550 mL (whichever is smaller) per 8-week period for adults. For pediatric patients, blood draws will not exceed 5 mL/kg in a single day, or 9.5 mL/kg or 550 mL (whichever is smaller) per 8 week period. Patients may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

- Studies involving radiation: Adult patients will undergo up to 3 ^{18}F-FDG PET-CT scans (2 in the short-term study and 1 in the long-term study) , each using 5 mCi of 18 fluorodeoxyglucose and an attenuation CT scan of the upper torso. Adult and pediatric subjects will undergo up to 5 DEXA scans (3 in the short-term study and 2 in the long-term study), each having an effective radiation dose of 0.00003 rem. Children under age 18 will only undergo DEXA scans and will not undergo the ^{18}F-FDG PET-CT scans. The total radiation exposure for different groups of subjects is as follows:
    - Adults, short-term and long-term study: 1.83 rem (3 PET scans + 5 DEXA scans)
    - Adults, short-term study only: 1.22 rem (2 PET scans + 3 DEXA scans)
    - Minors, short-term and long-term study: 0.00015 rem (5 DEXA scans)
    - Minors, short-term study only: 0.00009 rem (3 DEXA scans)

  These are below the guideline of 5 rem per year allowed for adult research subjects or 0.5 rem per year allowed for pediatric research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. Aside from the radiation exposure, the risks/inconveniences associated with the PET scan are related to claustrophobia and intravenous injection. The discomfort of lying in a supine position for an extended period of time may exist.

- Glucose and Lipid Turnover: The stable isotopes ^{13}C is not associated with any toxicity at the doses used in these studies [30]. Deuterium at the doses given may cause temporary dizziness. This risk will be minimized by dividing the $^{2}H_2O$ dose into four aliquots, and by giving the doses at night while subjects lie in bed. If subjects need to get out of bed (e.g. to use the bathroom) the patient will first sit upright in bed for a few minutes prior to standing, and a nurse will be available to supervise and assist. Palmitate must be complexed to albumin prior to delivery, and thus carries the risks associated with use of human blood products. To minimize exposure to human albumin, uniformly labeled $^{13}C_{16}$ palmitate will be used, permitting use of the minimum possible dose of this tracer.

- Metabolic Chamber: Besides inconveniences that can reasonably be expected as a result of spending an extensive time (up to 5 hours) in the live-in room calorimeter, the serious risk to subjects' health is minimal.

- Fat biopsy: This will be performed only in adult subjects. The major risks/discomforts include pain, bruising, hematoma, infection, scarring, and localized lipodystrophy. Patients on anti-platelet agents, anti-coagulants or aspirin for cardioprotection will be excluded from both the skeletal muscle and fat biopsy. Patients will be asked to refrain from taking aspirin or NSAIDS (for analgesia) for 10 days before the biopsy. The procedure will be performed under sterile technique to minimize the chances of infection. Local anesthetic will be used to minimize pain. Ice will be applied to the site immediately after the procedure to limit bruising, swelling and tenderness. After subcutaneous tissue biopsy, patients will be monitored by nurses. Post-biopsy the incision site will be cleaned and closed with adhesive wound closures and covered with gauze and translucent dressing tape. Study participants will be instructed to report to the clinical staff any changes at the biopsy site including bleeding, secretion, erythema, pain, and signs and symptoms of infection. Study participants will be instructed to self-monitor the incision site after discharge from the Clinical Center.

- Muscle biopsy: This will be performed only in adult subjects. The major risks/discomforts include pain, bruising, hematoma, bleeding, infection, and scarring. The risk of excess bleeding is

increased if the subject takes aspirin, non-steroidal anti-inflammatory drugs (NSAIDS), anti-platelet agents or anti-coagulants. Patients on anti-platelet agents, anti-coagulants or aspirin for cardioprotection will be excluded from both the skeletal muscle and fat biopsy. Patients will be asked to refrain from taking aspirin or NSAIDS (for analgesia) for 10 days before the biopsy. The procedure will be performed under sterile technique to minimize the chances of infection. Local anesthetic will be used to minimize pain. Ice will be applied to the site immediately after the procedure to limit bruising, swelling and tenderness. After muscle biopsy, patients will be monitored by nurses. The incision site will be cleaned and closed with adhesive wound closures and covered with gauze and translucent dressing tape. Direct compression will be applied for 10 minutes. Study participants will remain on bed rest with bathroom privileges for 2-4 hours post-procedure. Study participants will be instructed to report to the clinical staff any changes at the biopsy site including bleeding, secretion, erythema, pain, and signs and symptoms of infection. Study participants will be instructed to self-monitor the incision site after discharge from the Clinical Center.

Alternatives to participation
Participation in clinical trials is completely voluntary. Refusal to participate will not affect a subject's ability to participate in other studies at NIH or elsewhere.

Financial Compensation

All subjects will receive financial compensation for their time per Clinical Center guidelines for on-site visits, and additional compensation will be provided for specific procedures based on inconvenience units, as follows:

			Short-term (2 week) study		3 month visit		6 month visit	
Procedure	**Incon-venience Units**	**Compensation**	**Count**	**Total**	**Count**	**Total**	**Count**	**Total**
Inpatient stay	n/a	40	18	720	2	80	4	160
Metabolic chamber stay	2	20	2	40			1	20
PET/CT	10	100	2	200			1	100
DEXA	1	10	2	20			1	10
Tracer studies	2	20	2	40			1	20
Adipose biopsy	5	50	2	100				
Body Sensor Measurements	1	10	2	20				
Heart Rate Monitoring	1	10	2	20				
Muscle biopsy	5	50	2	100				
OGTT	4	40	2	80			1	40
Maximum Total (adults)				**1340**		**80**		**350**
Maximum Total (children)				**860**		**80**		**250**

Consent/Assent Procedures

Written consent/assent will be obtained from each subject after detailed explanations of the planned procedures by the principal or an associated investigator. Consent for participation of minors will be obtained from two parents/guardians of minor subjects. If both parents/guardians of minor subject cannot travel to the NIH Clinical Center, consent will be obtained via phone from the parent/guardian who is unable to travel. Written assent will be obtained from all minor subjects after detailed explanations of the planned procedures by the principal or an associated investigator. The consent/assent process will take place prior to any study procedures. Subjects have the right to withdraw participation from this protocol at any time. We do not anticipate enrolling non-English speaking subjects, however, we will not exclude them either. Should someone present who is non-English speaking and qualifies for enrollment in the study, we plan to request IRB approval for the use of the short form consent process (per NIH SOP 12) to obtain study consent for this patient population.

Research Use, Storage and Disposition of Human Subjects' Samples and Data

For future reference and potential use, we will store all samples (blood or fluids) in our locked freezers for an unlimited period of time. Samples will be labeled with coded identifiers linked to patient identity only via a secured database. Research records and data with personal identifiers will be stored in our locked offices, the medical record department, and the electronic study database. This material will additionally be protected by medical record and computer access procedures. Access to records and data associated with personal information will be restricted to the Principal Investigator, Co-Investigators, study support staff, and database support staff.

Stored samples and/or data may be sent to outside collaborating laboratories, or shared with other NIH collaborating investigators, to study questions related to lipodystrophy or its complications (including, for example: glucose metabolism, diabetes, obesity, weight, appetite, steatohepatitis, and lipid metabolism). Samples may be sent to outside commercial laboratories for analysis. Samples and data sent to outside laboratories and collaborators for analysis and/or testing will contain only coded numbers, without personal identifiers. Tech Transfer agreements will be completed before the exchange of samples and/or data with outside collaborators.

Subjects may request that unused samples be removed from our freezers and returned to the subject, or be destroyed. If no such request is made, we will keep samples until they are completely used or no longer of scientific value, at which time they will be destroyed. We do not plan to destroy personal medical information or stored data. The Principal Investigator will report loss or destruction of data or samples to the IRB.

Material Transfer Agreement/Collaboration(s):

Collaboration has been established with Dr. Morey W. Haymond, a board-certified pediatric endocrinologist and Professor of Pediatrics and Medicine at Baylor College of Medicine. Our direct site contact will be Shaji Chacko, Ph.D. The purpose of the collaboration is to assist in the conduct of the clinical studies determining the rates of glucose production and gluconeogenesis in subjects with mutations of the insulin receptor. This will include the analysis of coded human subject samples and interpretation of the results.

Appendix A: Investigator Qualifications and Roles

Rebecca J. Brown, M.D., M.H.Sc. is a board-certified pediatric endocrinologist, and an Assistant Clinical Investigator of the Diabetes Endocrinology, and Obesity Branch. Dr. Brown has six years of experience conducting clinical studies in children adults with diabetes, and has a Master's degree in Clinical Research. She is the Principal Investigator of this study, and is responsible for the study design, implementation, and interpretation.

Elaine K. Cochran, M.S.N., CRNP, is a nurse practitioner in the Diabetes, Endocrinology and Obesity Branch, and is lead associate investigator of the ongoing study of leptin treatment in lipodystrophy patients. She is an expert in the administration of leptin to patients with lipodystrophy. Her role in this project will be to assist in clinical management and education of patients.

Amber Courville, PhD, RD, CSSD, is a registered dietitian working for the NIH Clinical Center. Dr. Courville's role in this project will be to assess subjects' energy needs and coordinate the provision of a controlled metabolic research diet to the subjects during the study.

Phillip Gorden, M.D. is a board-certified endocrinologist, Senior Investigator in the Diabetes, Endocrinology and Obesity Branch, and Director Emeritus of the NIDDK. He has over four decades of experience conducting clinical studies in patients with rare disorders of extreme insulin resistance, and is Principal Investigator of the ongoing study of leptin treatment in lipodystrophy patients. His role in this project will be to work with Dr. Brown on study strategy, design, and interpretation.

Megan Startzell, RN, MPH is a research nurse in the Diabetes, Endocrinology and Obesity Branch. Ms. Mattingly's role in this project will be to assist Dr. Brown with the coordination of patient visits, patient education and communication.

Corina Millo, M.D. is a board-certified radiologist specializing in nuclear medicine and Positron Emission Tomography (PET). Her role in this project will be to work with Dr. Brown in the planning, implementation, and interpretation of PET scans for quantification of tissue glucose uptake.

Monica Skarulis, M.D. is a board-certified endocrinologist, and Special Volunteer with the Diabetes, Endocrinology and Obesity Branch. She has extensive expertise in the study and management of thyroid disorders. Her role in this project is to work with Dr. Brown on study design and interpretation, and management of T3 treatment.

Robert Brychta, Ph.D. holds a doctorate in biomedical engineering and is a staff scientist for the Diabetes, Endocrinology, and Obesity Branch. He has extensive expertise in the study and measurement of human metabolism. His role in the project will be to assist Dr. Brown in the measurement, analysis, and interpretation of energy expenditure using the metabolic chamber, body composition, and body temperature.

Yevgeniya Kushchayeva, M.D. is a clinical fellow in Endocrinology with the Diabetes, Endocrinology and Obesity Branch. Her role in this project is to work with Dr. Brown on the study implementation and interpretation.

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