PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR
Karen K. Miller, MD

Growth Hormone and Intrahepatic Lipid Content in Patients with Nonalcoholic Fatty Liver Disease

NCT# 02217345

Version Date: 2/17/2022 Redacted

PROTOCOL TITLE
Growth Hormone and Intrahepatic Lipid Content in Patients with Nonalcoholic Fatty Liver Disease

FUNDING
[redacted]

VERSION DATE
02/17/22

SPECIFIC AIMS
Concisely state the objectives of the study and the hypothesis being tested.

[redacted]

[redacted]

[redacted]

SPECIFIC AIM 2
We hypothesize that GH replacement will decrease intrahepatic lipid accumulation as quantified by 1H-MRS.

Primary Endpoint: We hypothesize that low-dose GH administration to raise IGF-1 levels to the upper normal range for 6 months in patients with nonalcoholic fatty liver disease (NAFLD) will decrease liver fat as measured by 1H-magnetic resonance spectroscopy (1H-MRS).

Secondary Endpoints: 1) We will investigate mechanisms responsible for the decrease in liver fat due to GH administration. We hypothesize that the decrease in liver fat will be mediated or accompanied by:

- Decreased inflammatory markers, including TNF-alpha receptors, IL-6 and hsCRP
- Increased adipokines, including adiponectin
- Improved measures of insulin resistance
- Improvement in liver fibrosis as measured by the NAFLD fibrosis and ELF scores
- Improved body composition, including a decrease in abdominal adiposity and lipid deposits in muscle and liver with a corresponding increase in muscle mass

[redacted]

[redacted]

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Nonalcoholic fatty liver disease (NAFLD), fatty infiltration of the liver in the absence of alcohol use, is an increasingly recognized complication of obesity, with prevalence estimates of about 30% of individuals in the United States. A subset of these will develop progressive disease in the form of nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and end stage liver disease. NASH cirrhosis is expected to be the most common indication for liver transplantation by the year 2020, and there are no standard of care treatments beyond diet, exercise and lifestyle modification for weight loss. The pathogenesis of NAFLD is only partially understood. Animal studies have demonstrated that growth hormone (GH) deficiency contributes to the development of NAFLD, and conversely, that administration of GH can reverse this hepatic steatosis. Human data also support an important rule for relative GH deficiency in the development of liver steatosis. GH secretion, an important regulator of abdominal lipolysis, is reduced in obesity, and GH administration in obesity reduces abdominal and visceral adiposity. Importantly, in a study from our group, low GH secretion was associated with liver fat accumulation, as quantified by proton magnetic resonance spectroscopy (1H-MRS), in obese individuals. This association remained significant after controlling for amount of visceral adipose tissue, suggesting that low endogenous GH may put individuals with abdominal obesity at higher risk for developing NAFLD. In a second important

study from our group, GH administration reduced intrahepatic lipid content in obese men after controlling for weight increase, which occurred in both the GH and placebo groups. Of importance, the study was not designed or adequately powered to detect changes in intrahepatic lipids. This is because intrahepatic lipid content was not a primary endpoint of the study and hence, significant intrahepatic lipid content was not required for participation in the study and only a minority of study subjects would have qualified as having NAFLD. Despite these limitations, we detected a decrease in intrahepatic lipid content. Therefore, our pilot data are promising with regard to an effect of GH replacement to reduce liver lipid accumulation in patients with NAFLD, a highly prevalent disease with significant associated morbidity and mortality. Additionally, multiple hormones including adipokines, such as leptin and adiponectin, as well as inflammatory markers, such as Il-6 and TNF-α, and insulin resistance, have been suggested to play a role in the pathogenesis of NAFLD, and we plan to explore these potential mediators of liver fat in our secondary endpoints.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

Eligibility for Aim 2 (prospective administration of growth hormone):

Inclusion criteria:

1. Ages 18 – 65 yr
2. NAFLD defined as demonstration of hepatic steatosis by imaging or biopsy in absence of significant alcohol consumption (>2 drinks daily for women, >3 drinks daily for men). If liver imaging or biopsy has not been performed clinically, liver ultrasound will be performed as part of the screening visit.
3. IGF-I within or below the third quartile for age and gender

Exclusion criteria:

1. Serum creatinine > 2 times the upper limit of normal
2. History of cancer, except for non-melanoma skin cancers
3. Use of systemic estrogen products (including hormonal contraceptives).
4. Active carpel tunnel syndrome
5. Diabetes mellitus, defined as a hemoglobin A1C >6.5 or use of any medications prescribed to treat hyperglycemia is exclusionary for Aim 2. The exception is metformin, but only when hemoglobin A1c has been less than or equal to 6.0 on two consecutive occasions, and subject's weight has been stable for at least 6 months.
6. Any use of pioglitazone
7. Contraindications to MR imaging.
8. Pregnancy or desire to become pregnant. Participants of reproductive age must agree to use contraception.
9. Breastfeeding
10. Aspartate and aminotransferase levels 10 times ULN
11. Other forms of active chronic liver disease
12. Decompensated or unstable cardiovascular disease (for example, heart failure, unstable coronary disease, uncontrolled arrhythmias) at the discretion of the study physician.
13. Severe decompensated liver disease, including cirrhosis.
14. Alcohol ingestion greater than two drinks per day in women and three drinks per day in men.
15. Known pituitary or hypothalamic disease affecting the growth hormone axis
16. Regular use of drugs causing hepatic steatosis in the past 12 months (oral steroids, methotrexate, tamoxifen)

[redacted]

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Study procedures:

Subjects will be recruited through the [redacted], through referring gastroenterologists and through advertisements. Eligibility will be determined at a screening visit. No procedure will be performed before informed consent is obtained.

Study visits

Screening visit for [redacted] Aim 2: ([redacted] Aim 2, n= 150)

1. A history and physical will be performed.
2. Height and weight will be measured, and BMI will be calculated.
3. TSH, creatinine, liver panel, IGF-1 and cbc will be performed.
4. A hemoglobin A1C will be performed to assess for diabetes and insulin resistance (not exclusionary for cross-sectional study).
5. A pregnancy test will be performed in all premenopausal women.
6. A liver US be performed in study subjects who have not had a liver biopsy or liver imaging to establish steatosis as part of clinical care.
7. Hepatitis C testing will be performed in study subjects who have not had this testing as part of clinical care.
8. Individuals will be queried regarding alcohol use as well as regarding a history of other chronic liver disease
9. Histology slides from prior clinically-indicated liver biopsies will be requested if/when available. Record requests will be initiated for slides to be obtained outside of [redacted]. Slides will be reviewed by a study hepatopathologist at [redacted] for a central pathology read. Slides will be reviewed for grading of NASH activity score and other NAFLD/NASH-related pathology assessments. All histology slides will be returned after review.

[redacted]

[redacted] ***Aim 2:*** [redacted]

Aim 2, n=65 subjects (50 completers), approximately 50% women)

The baseline visit will occur within 12 weeks of the screening visit. The following assessments will be made at the baseline visit:

1. Interview to determine whether any changes in medical history since the screening visit has occurred.
2. BMI will be calculated and waist-hip-ratio measured.
3. In the fasting state, a blood sample will be obtained to determine glucose, insulin,

GH, gonadal steroid levels and other hormone levels (including ghrelin, testosterone, free testosterone, estradiol and SHBG), adipokines, inflammatory markers, and ELF score liver function tests.

4. A 2-hour 75-gm oral glucose tolerance test for glucose and insulin (Aim 1 Only) and a 2-hour GHRH-arginine GH stimulation test will be performed
5. MRI: 1H-MRS of the right and left hepatic lobe will be performed to quantify hepatic lipid content and other fat depots. [redacted] sequences will also be performed for noninvasive analysis of hepatic inflammation and fibrosis.
6. A single slice CT of each of the following areas will be performed (for a total of three single slice CT scans): the abdomen at the level of L4, the liver, and the mid-thigh to quantify abdominal (visceral and subcutaneous) fat, liver fat, thigh fat and thigh muscle area.
7. Dual energy x-ray absorptiometry (DXA) scan will be performed to determine body composition of the whole body and assessment of bone density.
8. The Paffenbarger Physical Activity questionnaire, NHANES Dietary Screener Questionnaire, and the SF-36 health survey questionnaire will be administered.
9. Resting energy expenditure (REE) using indirect calorimetry, bioelectrical impediance analysis (BIA) to estimate body composition and total body water assessment will be performed.

   A nutritionist will perform an assessment of resting energy expenditure. The subject will be lying on a bed in a private room for 30 minutes. Then, a clear plastic canopy will be fitted over the subject's head and upper chest to collect the air he or she exhales for 30 additional minutes. The entire procedure takes approximately 60 minutes to complete.

   The bioelectrical impedance analysis uses a small electric current to measure the body's electrical resistance. The data can be used to provide an accurate measure of body composition and total body water.

10. [redacted] measure liver stiffness via transient elastography (ultrasound). [redacted] will be repeated at the 6-month visit (for a total of 2 [redacted] per subject).

[redacted]

[redacted] **Subjects completing Aim 2 proceed to randomization and follow up visits as noted below.**

Randomization for Aim 2: Study subjects in Aim 2 only will be randomized to receive GH injections or to the placebo (control) group. Randomization will be stratified by sex.

Starting Doses for Aim 2: GH or placebo will be initiated via a subcutaneous injection in Aim 2 subjects only at a dose of 0.2 mg in men and 0.3 mg daily in women in patients randomized to the GH arm.

One, 2 and 3-month follow-up visits for Aim 2 Only:

1. Interim medical history and side effects to study drug will be assessed.

2. Physical exam will be performed.
3. An IGF-1 level will be measured, and dose adjustments made to target the upper quartile of the normal, age-appropriate IGF-1 range.
4. Pregnancy tests will be performed at both visits in premenopausal women.
5. Fasting glucose will be measured at the 1 and 3-month visit and HbA1c will be performed at the 3-month visit as safety labs. Both fasting glucose and HbA1c are performed at study completion at 6 months.
6. Liver function tests will be performed at screening, 1 month, 3 months and 6 months as safety labs.

Remote Visits: Subjects who do not live locally will have the option to perform three follow up visits (1 month, 2 month, and 3 month visits) remotely. These will be performed with remote labs (via Quest Diagnostics Locations) and medical history via phone call with a study physician or nurse practitioner. For these remote visits, the study doctor or nurse will speak to the subject over the phone to obtain a medical history, and at those visits a physical exam would not be performed. The remote visit will be converted to an in-person visit if there is any concern based on updated history or symptoms at the discretion of the visit medical provider. For these remote visits, the study team will FedEx study medication to the subject in a manner approved by the research pharmacy as needed for resupply. The subject would bring back unused study medication and empty vials to the study team at the next in person visit to maintain standard study drug accountability logs. Additionally, for off-site visits, a serum pregnancy test will be performed in all women of childbearing age and status.

Six-month follow-up visit for Aim 2 Only: The 6-month visit will be performed during a +/- 2 week interval around the indicated return time to allow for potential scheduling conflicts. At this visit, the identical procedures described under Baseline Study Visit will be repeated with the exception the DXA for bone density and GHRH-arginine stimulation test, which are only completed at the baseline visit. Six month laboratory assessment includes a fasting blood sample obtained to determine glucose, insulin, GH, IGF-1, CBC, gonadal steroid levels and other hormone levels (including ghrelin, testosterone, free testosterone, estradiol and SHBG), adipokines, inflammatory markers, ELF score, lipids, triglycerides, and liver function tests. Subjects will be asked to collect an at-home stool sample using a provided kit and bring it with them to their final study visit. Then, study drug will be discontinued. This is the study end.

Note regarding pregnancy tests: Due to COVID-19, subjects will be allowed to perform a pregnancy test at home for any remote visit and on the day of any in-person visit in order to minimize potential exposures. The subject will share this result with the study team for documentation.

1 Month Post-Study Follow Up Call: A study provider will attempt to contact subjects four weeks after study completion to document subject status at that time, which will be approximately one month after study drug discontinuation.

[redacted]

[redacted]

Study Endpoints

[redacted]

Specific Aim 2:
Primary and secondary endpoints for Aim 2:

The primary study endpoint will be intrahepatic lipid content.

Secondary endpoints will include: 1) liver function tests, 2) adipokines: leptin and adiponectin, 3) inflammatory markers: hsCRP, IL-6 and TNF alpha receptors, 4) measures of insulin resistance, 5) the NAFLD Fibrosis Score 6) abdominal fat, 7) ELF score 8) Liver stiffness [redacted] and 9) liver inflammation and fibrosis [redacted].

[redacted]

Methods:
Intrahepatic lipid accumulation: We will perform proton MR spectroscopy (1H-MRS) of the right and left hepatic lobe to determine intrahepatic lipids (IHL) using a 3Tesla MRI device (Siemens Trio, Siemens Medical Systems, Erlangen, Germany) as we have previously described[18]. Advantages of higher magnetic field strength are an increase in signal-to-noise ratio resulting in improved spatial resolution and increased chemical shift dispersion, potentially improving assessment of other fat resonances. MRI sequences are also performed for assessment of other fat depots and [redacted] analysis within the same session.

Adipokines: Adiponectin (total, high molecular weight, medium HMW forms) will be measured by enzyme-linked immunosorbent assay [redacted]. Leptin will be measured by ELISA [redacted]. Coefficients of variation (CVs) are less than 10% for all assays.

Inflammatory markers: hsCRP will be measured by latex particle-enhanced immunoturbidmetric assay on a Hitachi 911 analyzer [redacted]. IL-6 will be measured using an immunoassay [redacted]. TNF-alpha receptor subtypes (both 1 and 2) will be measured by ELISA [redacted]. CVs are less than 10% for all assays.

Liver function tests: A standard liver function test panel consisting of ALT, AST, GGT, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, ferritin and albumin will be measured using a standard clinical platform.

Lipids and Lipoproteins: A fasting lipid profile, including total cholesterol, LDL, HDL and triglycerides, will be determined. OxPL levels will be performed in conjunction with the laboratory of [redacted] has pioneered the use of OxPL in CVD.[19-21] OxPL markers will be measured in three ways; 1. OxPL/ApoB: amount of oxidized phosphorylcholine on LDL, 2. ApoB IC: measurement of immune complexes of IgG or IgM to ApoB, and 3. Malondialdehyde (MDA:LDL) autoantibodies.[19,20] Comprehensive lipid profiling will be performed in conjunction with [redacted].[22] Ion mobility analysis is a validated method using gas-phase differential electrophoretic macromolecular mobility to distinguish lipid subfractions.[23]

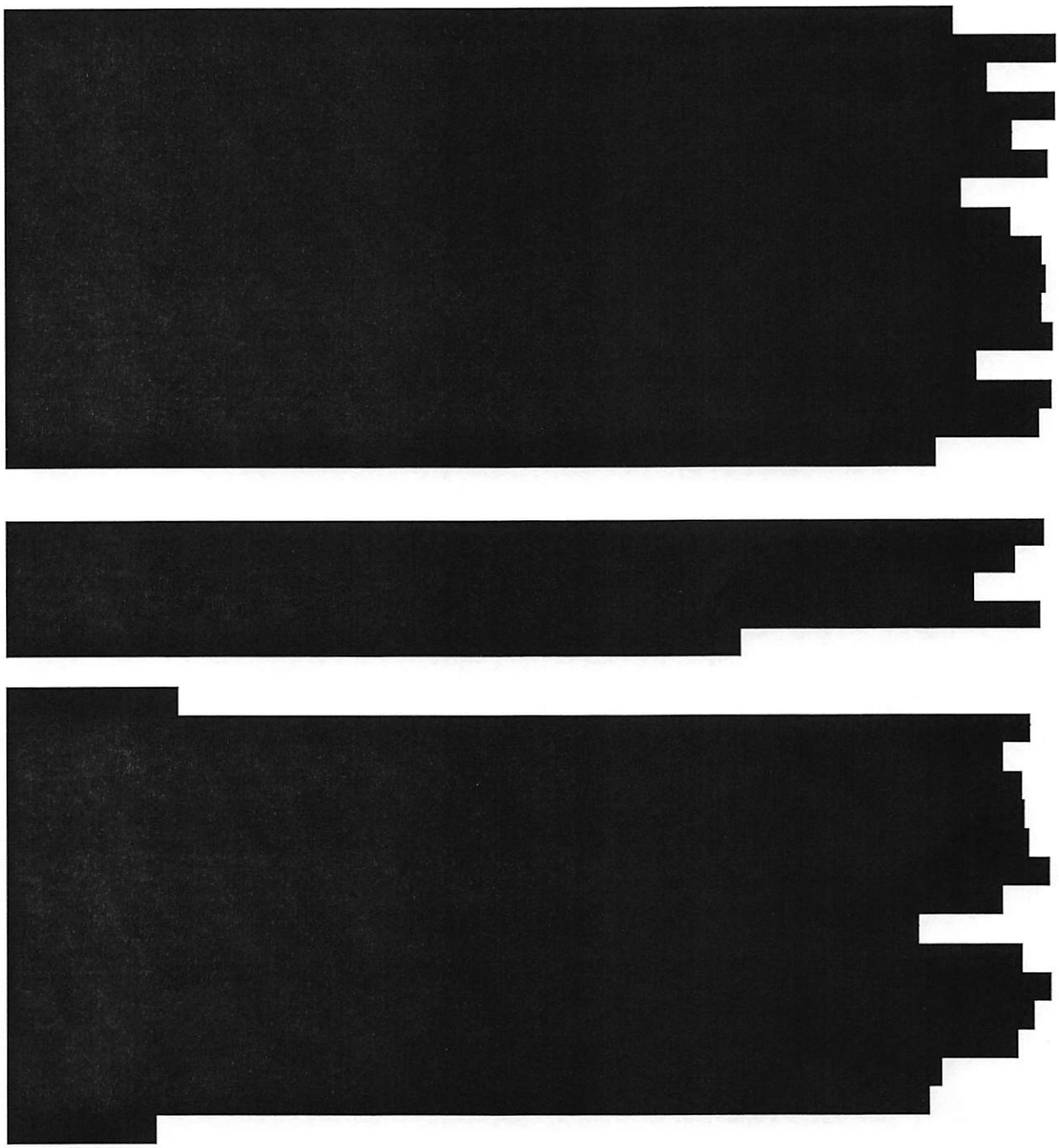
[redacted]

The NAFLD fibrosis score: This score predicts the probability of fibrosis in patients with NAFLD using a score that uses the patient's age, body mass index, blood glucose levels, aminotransferase levels, platelet count, and albumin. A high NAFLD fibrosis score of >0.676 is associated with a probability of advanced fibrosis (F3-F4) of 82 percent (sensitivity 43 percent, specificity 96 percent), and a score of <-1.455 is associated with a negative predictive value of 88 percent (sensitivity 77 percent, specificity 71 percent) based on prior studies.[24,25]

[redacted]

[redacted] Elastography [redacted] is a rapid non invasive technique that measures liver stiffness by transmission of mild amplitude and low frequency (50Hz) through the intercostal space using a vibrator at the skin surface. The vibration induces a shear wave velocity through

the hepatic tissue, which is directly related to tissue stiffness and is expressed in kilopascals (kPa). Transient Elastography is an inexpensive, reproducible, painless, rapid <10 minutes and easy to perform tool. It has been proven in many studies to correlate well with degree or severity of fibrosis and or cirrhosis.



For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

Treatment of NAFLD is extremely limited at this point in time. Patients are recommended to make lifestyle adjustments with a goal of weight loss, such as maintaining a healthy diet and

exercise program, which would not be affected by this study. This is the only standard of care at this time. Various other therapies for NAFLD have been tested, however, lack of robust evidence for improvement of NAFLD has limited their incorporation into clinical care. Currently NAFLD patients are monitored in the Fatty Liver Clinic and are referred for liver transplant if they develop decompensated cirrhosis, however, we will not be recruiting this end stage population. Therefore, the GH therapy in this trial would be one additional therapeutic option that could be offered to patients with NAFLD who inherently have extremely limited treatment options.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

A number of procedures will be instituted to protect against potential risk involved in this protocol. The potential effects of growth hormone on a fetus are not known and therefore precautions against administration to pregnant patients will be instituted. All patients will have pregnancy tests on admission prior to receiving radiation or study medication. Subjects will also have serial pregnancy tests – one at every study visit – and study participation will be discontinued if a subject becomes pregnant. Due to COVID-19, subjects will be allowed to perform a pregnancy test at home for any remote visit and on the day of any in-person visit in order to minimize potential exposures. The subject will share this result with the study team for documentation.

A portion of this research study involves exposure to radiation from up to six CT scans (three single slice scans per session with sessions at baseline and six months) and up to five DXA scans (3 bone sites plus body composition studied at baseline and only body composition studied at the 6 month visit).

Subjects completing Aim 2 (prospective) of the study will be exposed to a total of 0.60 mSV of radiation from completing each of the studies listed above twice (once at baseline and once at 6 months). This amount of radiation is about three months of radiation from natural background sources. Any subject who completes Aim 1 and subsequently completes Aim 2 will not have additional radiation exposure beyond what a subject completing only Aim 2 would receive (a total of 0.60 mSv).

GH will be administered to subjects in Specific Aim 2 only. Side effects of GH administration could include mild swelling or arthralgias, which occur in a minority of patients and resolve after the first few days of therapy in the vast majority of patients. In those study subjects in whom such side effects do not resolve, we will reduce the dose. Additionally, any study subject with a fasting glucose in the diabetic range at the 3-month visit will be discontinued from the study, and

fasting glucose will be retested 3 months later. We will ask permission from all study subjects to communicate with a healthcare provider about any abnormal clinically relevant test results.

[redacted]

A physician will be available at all times during the study by pager to answer any questions a patient might have. The physician will arrange to immediately see every patient with a concern. All efforts will be made to protect the confidentiality rights of the study subjects who will be referred to by code numbers only. Confidentiality of the patients will always be of paramount importance to study investigators. No data on patients will be shared with persons other than those directly involved in the study, except at the documented request of the patient. Samples that are sent to laboratories outside of [redacted] will be labeled with a non-identifying numeric code.

All adverse events will be reported to the IRB in a timely manner according to the guidelines provided by Partner's Human Subjects Research Committee.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Safety assessments for subjects in Aim 2:

1. IGF-I levels:
    a. Must have IGF-I within or below the lowest-3 quartiles for age and gender
    b. Measurements at screening, baseline, 1 month, 2 months, 3 months and 6 months
    c. Dose decrease for IGF-I levels above the upper limit of normal
2. Glucose: Fasting glucose at screen as well as 1, 3 and 6 month visits.
3. Pregnancy testing at all visits
4. HbA1c: screening, 3 month and 6 months
5. Liver function tests: screening, 1 month, 3 month and 6 months

Subjects will be discontinued from Aim 2 of the study if they develop

1. Diabetes mellitus: fasting glucose $\geq$ 126 mg/dl or HbA1c $\geq$ 6.5%
2. Positive pregnancy test
3. Severe / intolerable side effects of GH
4. Development of malignancy
5. Initiation of the following medications: OCPs and pioglitazone
6. Elevated AST/ALT >10 x the upper limit of normal OR if there is a >25% increase from baseline, whichever is greater.

[redacted]

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Because IGF-I levels, an integrated measure of GH secretion, will be raised to within the normal range appropriate for age and gender and not above, we expect the risks of GH administration to subjects in Specific Aim 2 to be minimal. Our experience in administering GH, at the doses proposed in this protocol, to healthy men and women with low GH levels (not related to hypopituitarism), we should expect minimal if any side effects. Such side effects could include headaches, mild swelling or arthralgias, which occur in a minority of patients and resolve after the first few days of therapy in the vast majority of patients. In those study subjects in whom such side effects do not resolve, we will reduce the dose. If mild side effects occur and do not resolve, the study drug will be discontinued until the side effects resolve. After resolution of side effects, the study drug will be restarted at the lower dose that did not cause any side effects.

The net effect of GH on insulin resistance appears to be neutral, despite two opposing effects of GH on insulin resistance – direct negative effects and indirect positive effects mediated through dramatic changes in body composition. Most studies have shown an acute worsening of insulin resistance followed by chronic improvement or lack of change. The most similar paradigm available in the literature is a study by Johannsson *et al.* in which normal obese men were randomized to receive GH or placebo for 9 months. Acutely insulin sensitivity, as measured by the gold standard hyperinsulinemic, euglycemic clamp deteriorated slightly, and this was followed with an improvement compared with baseline at 9 months.

Subjects will undergo low dose CTs of the thigh and abdomen at one visit for subjects in Aim 1 and at two visits for subjects in Aim 2. [redacted]. The combined radiation from all procedures for the entire study for subjects in Aim 2 is approximately 0.60 mSv – this amount of radiation is equal to approximately 3 months of natural background radiation [redacted]. Blood drawing may result in bruising or infection at the venipuncture site.

MRS and MRI will be performed using FDA approved devices and pulse sequences. There are no known foreseeable risks associated with exposure to MRI, provided there are no metallic implants (i.e., vascular clamps or pacemakers). All potential subjects will be screened for the presence of such prior to the exam. Some subjects do report some claustrophobia during the scan. If a patient expresses any discomfort during the scan, the procedure will be aborted and not repeated without his/her full consent. Subjects will be required to lie in a magnet for about 1 hr. The 3.0T MR device is FDA-approved for clinical use. No serious or lasting side effects associated with the use of MRI have been reported. Rarely, subjects report sensations such as vertigo and a metallic taste when exposed to magnetic fields. Minor theoretical hazards arise from rapid gradient switching and RF transmission. All parameters used for conventional and spectroscopic imaging fall within FDA limits for specific absorption rates (SAR) of RF

transmission and rapid gradient switching (dB/dt). Experiments properly conducted in compliance with FDA, OSHA and standard safety practices of the [redacted] pose no significant risk to research subjects. MR examinations will be performed by a Radiological Technologist certified by the Commonwealth of Massachusetts. Any adverse events will be reported immediately to the [redacted].