Study protocol with embedded statistical analysis plan

Augmenting Growth Hormone to Ameliorate Nonalcoholic Fatty Liver Disease in Adolescents

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Augmenting Growth Hormone to Ameliorate Nonalcoholic Fatty Liver Disease in Young Adults

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I. Background and Significance

Summary

Non-alcoholic fatty liver disease (NAFLD) is a significant health problem in obese adolescents, and there are currently no recommended pharmacological treatments for this condition. We and others have reported significant reductions in growth hormone (GH) in obese adolescents (1-3), and, as described below, reduced GH is highly likely to contribute to the pathogenesis of hepatic lipid accumulation. In an adult population with HIV-associated visceral obesity, we have demonstrated that augmentation of GH significantly reduces liver fat (4). The effect of augmenting GH to reduce liver fat in young adults has never been tested. We propose a randomized clinical trial to test the effects of physiologic replacement with recombinant human GH (rhGH), Norditropin, to reduce liver fat in obese young adults with NAFLD (defined as ≥5% hepatic lipid on ^{1}H-magnetic resonance spectroscopy) who have relative reductions in GH secretion.

Epidemiology and Health Consequences of Pediatric NAFLD

NAFLD, which encompasses both steatosis and steatohepatitis, is the leading cause of liver disease in adolescents, with an estimated prevalence of approximately 10% (5, 6). Although it may occur in individuals of normal weight, NAFLD is largely a co-morbidity of obesity, found most commonly in the setting of increased visceral adiposity and insulin resistance. Over the past 3 decades, in conjunction with rising obesity, the prevalence of NAFLD in adolescents has more than doubled (5) such that the current prevalence among obese adolescents is estimated at approximately 40% (6). Given the potential adverse consequences of NAFLD with respect to future liver health, this figure is alarming. More than 20% of adolescents with fatty liver show signs of non-alcoholic steatohepatitis (NASH), which is characterized by inflammation, hepatocellular injury and fibrosis (6-10). In many, NASH will progress to advanced fibrosis and cirrhosis. An early report described fibrosis-cirrhosis in 75% of children presenting with NASH (11). In a large autopsy study, 9% of children with NASH already had advanced fibrosis or cirrhosis (6). Further, a longitudinal study showed a significantly increased standardized mortality ratio of 13.6 (95% CI 3.8-34.8) in children with NAFLD, with two of the 66 members of the cohort dying and two requiring liver transplantation for decompensated cirrhosis over 20 year follow-up (12). Histological findings of NAFLD in children differ from those in adults (9, 13, 14), suggesting unique pathophysiological mechanisms in the pediatric age group. Further, a recent study suggests that severely obese adolescents with NAFLD may have more severe liver damage compared to adults matched for BMI, gender, race, and features of metabolic syndrome (15). **Due to FDA requirement, the current study is limited to those ≥18 years of age, with the intention of pursuing further studies in the pediatric age group if data from this pilot study demonstrate benefit.**

In addition to conveying risk of progressive liver disease, NAFLD is strongly associated with dyslipidemia (16, 17), insulin resistance (IR) (18, 19), and cardiovascular disease (20-24). Although causal pathways have not been definitively established, evidence suggests a bidirectional relationship between NAFLD and both dyslipidemia and IR, whereby NAFLD contributes to dyslipidemia and IR, both of which in turn may drive further accumulation of hepatic lipid (16-19, 25). Additionally, large studies in adults show associations between NAFLD and CVD events, independent of traditional risk factors and metabolic syndrome components, suggesting that NAFLD may also play an etiologic role in CVD (23, 24, 26). Thus treatment strategies for NAFLD in young adults are critical both to prevent liver disease and to ameliorate cardiometabolic risk in adulthood.

Lack of Effective Therapies for NAFLD

Effective treatment options for NAFLD in young adults are limited. Although lifestyle modification appears to be effective (27-30), exercise and weight loss are difficult to sustain. To date, no highly effective

pharmacologic treatment options exist to reduce liver fat in obese adolescents. Vitamin E has modest benefit in adults with NAFLD (31) and showed initial benefit in the pediatric population (32). In the TONIC trial – a large, randomized, controlled study of Vitamin E or metformin for NAFLD in the pediatric age group – Vitamin E resulted in histological improvement of liver disease but showed no benefit over placebo to consistently reduce ALT (33). Similarly, metformin has shown benefit in some studies (34, 35), but also proved no better than placebo in the TONIC trial with respect to ALT reduction (33). Other treatment strategies, including ursodeoxycholic acid (36), cysteamine (37), probiotics (38), and polyunsaturated fatty acids (39), have been investigated, but to date there is no definitive evidence of efficacy. Thus no pharmacologic agents are currently standard of care for pediatric NAFLD. The 2012 Practice Guideline for diagnosis and management of NAFLD endorses lifestyle modification as the only recommended treatment in pediatrics, mentioning possible benefit of Vitamin E but concluding that confirmatory studies are necessary before its use can be recommended in clinical practice (40).

Treatment strategies for NAFLD have thus far focused on ameliorating oxidative stress (e.g., with Vitamin E) or reducing insulin resistance (e.g., with metformin). Whereas both oxidative stress and insulin resistance play a significant role in NAFLD and steatohepatitis, we argue that a treatment strategy targeting hepatic lipid accumulation itself should also be considered. **In this respect, as described below, Norditropin (rhGH), will be explored as a potential treatment to reduce liver fat in obese young adults with NAFLD.**

Reduced GH contributes directly to NAFLD

NAFLD is defined by macrovesicular accumulation of triglyceride (TG) in the cytoplasm of at least 5% of hepatocytes. Investigation with stable isotope tracers has quantified three major sources of hepatic TG: (i) uptake of circulating free fatty acid (FFA), responsible for about 59% of hepatic TG; (ii) hepatic *de novo* lipogenesis (DNL) whereby palmitate is synthesized from glucose, particularly during conditions of carbohydrate excess, responsible for about 25% of hepatic TG; and (iii) dietary fat, responsible for about 15% of hepatic TG (41, 42). We hypothesize that relative growth hormone (GH) deficiency of obesity contributes to the pathogenesis of NAFLD through two mechanisms.

(i) Relative reductions in GH are associated with increased visceral adipose tissue (VAT) (3, 43), which causes increased free fatty acid flux through the liver. VAT quantity is strongly associated with NAFLD and NASH in adults (44, 45), and lipolysis of VAT but not subcutaneous adipose tissue (SAT) is significantly associated with increased liver fat (46, 47). Further, FFA derived from VAT are a primary contributor to increased hepatic very low density lipoprotein (VLDL) production in individuals with NAFLD (48).

(ii) GH plays a critical role in the regulation of hepatic de novo lipogenesis (DNL), and increased hepatic DNL is strongly associated with NAFLD. The rate of DNL is more than twice as high in individuals with high liver fat compared to controls (49). GH administration suppresses hepatic DNL in animal models (50-54), and the same effect has been demonstrated in human models (55).

Studies of GH deficient individuals demonstrate the importance of GH in preventing net accumulation of liver fat. Patients with pituitary GH deficiency have a higher prevalence of NAFLD and NASH than the general population, and replacement of GH reduces transaminases and improves histological findings (56-60). Further, a study of individuals with childhood onset GH deficiency in whom treatment was stopped at attainment of final height demonstrated a high incidence of NAFLD, which developed as soon as 1 year after discontinuation of therapy and at a mean BMI of 26kg/m^2, arguing against obesity per se as an etiologic mediator and suggesting instead a causal role of GH deficiency (61). Just as individuals with GH deficiency have a higher prevalence of NAFLD, individuals with NAFLD appear to have reductions in GH, as demonstrated by lower concentrations of insulin-like growth factor 1 (IGF-1) (62). Further, among individuals with NAFLD, IGF-1 levels are inversely associated with degree of steatosis and fibrosis (63-65), and hepatic liver IGF-1 mRNA expression is a negative predictor of histological severity of NAFLD (63). *In vitro* and animal models support these findings and provide mechanistic insight. Transgenic mice that overexpress GH have decreased liver fat, whereas liver-specific GH-receptor (GHR) knock-out mice have severe hepatic steatosis

(66). In the liver-specific GHR knock-out, hepatic lipogenesis and hepatic triglyceride output are significantly increased, and adenovirus-mediated rescue of GHR expression reverses these changes (66). Another model of GH deficiency, the spontaneous dwarf rat (SDR), also demonstrates hepatic steatosis and fibrosis that can be reversed by treatment with GH (67). Taken together, both human and animal models of GH deficiency demonstrate the critical role of GH in hepatic lipid metabolism and illustrate the consequences of GH deficiency with respect to increased hepatic lipid accumulation.

II. Specific Aims

1. **In a randomized study of obese young adults with ≥5% liver fat by MRS (NAFLD) and reduced GH, administration of physiologic doses of Norditropin (rhGH) over 24 weeks will reduce liver fat as compared to no treatment.**
   a. Compared to no treatment, Norditropin will reduce hepatic lipid as measured by ^{1}H-MRS
   b. Compared to no treatment, Norditropin will significantly reduce markers of hepatocellular injury and steatohepatitis, including transaminases, gamma glutamyl transferase (GGT), and cytokeratin -18 (CK18).

2. **Physiologic replacement of GH with Norditropin will be neutral to glucose homeostasis and will improve other measures of cardiometabolic risk.**
   a. Fasting and 2-hour glucose, HOMA-IR, and Matsuda index will not be significantly different from baseline after 24 weeks of Norditropin treatment
   b. Norditropin will result in a reduction in VAT (measured by MRI) and trunk fat (measured by DXA) compared to no treatment
   c. Norditropin will result in an increase in adiponectin and a reduction in CRP compared to no treatment

III. Subject Selection

24 obese young adults (M and F) with liver fat ≥5% on ^{1}H-MRS and relative reduction in GH but without a known clinical diagnosis of growth hormone deficiency will be recruited for the RCT. We anticipate screening up to 150 subjects to identify 24 who meet enrollment criteria. Relative reduction in GH will be assessed by IGF-1 z-score, with subjects whose IGF-1 z-scores are ≤ 0 eligible for the study. Many previous studies have defined relative GH deficiency as an IGF-1 z-score ≤0 (68, 69).

Inclusion criteria:

1. Males and Females ages 18-29yo
2. BMI ≥95th percentile and/or ≥30kg/m^2
3. Hepatic fat ≥5% by ^{1}H-MRS
4. IGF-1 standard deviation score (SDS) ≤0

Exclusion criteria:

1. Alcohol consumption of >14 drinks per week (F) or >21 drinks per week (M) (40)
2. Use of insulin or oral anti-diabetic medications, or HbA1c >7% or fasting glucose ≥126mg/dL
3. Use of corticosteroid, gonadal steroids, or methotrexate ≤ 3 months prior to baseline visit
4. Known diagnosis of alpha-1 antitrypsin deficiency, Wilson's disease, hemochromatosis, or autoimmune hepatitis
5. HgB < 11.0 g/dL or wt < 50kg
6. AST or ALT >2.5x upper limit of normal (ULN), total bilirubin > ULN, positive Hep B sAg, or Hep C Ab
7. Routine MRI exclusion criteria (including weight >450 lbs)
8. Use of weight-loss medications or previous weight loss surgery
9. Pregnant or breastfeeding, or, for sexually-active females, unwillingness to use an appropriate form of contraception during the study
10. Use of oral combined estrogen/progesterone contraceptives or depot progesterone formulations unless stable use for 1 year prior to baseline visit. Hormone-containing IUDs are permitted.

11. Known cirrhosis or clinical evidence of cirrhosis or portal hypertension on imaging or exam
12. Use of GH or GHRH within the past 1 year
13. Change in lipid lowering or anti-hypertensive medications within 3 months of screening
14. Change in vitamin E or ursodiol <6 months before screen; subjects on stable doses of Vitamin E and/or Ursodiol for ≥6 months will be eligible.
15. History of malignancy or active malignancy
16. History of hypopituitarism, head irradiation or any other condition or chronic illness known to affect the GH axis

IV. Subject Enrollment

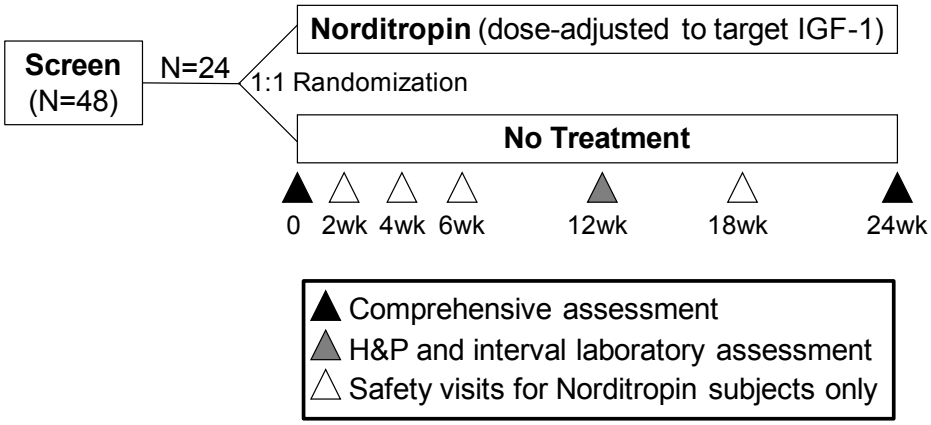
Methods of Recruitment

Subjects will be recruited from the greater Boston area through advertisements for obese young adults and through MGH ambulatory care practices including pediatric and adult gastroenterology and obesity clinics. Websites such as Facebook, craigslist, and Partners sponsored clinical-trial posting sites will also be utilized, as will other tools made available by the MGH and Partners. Obese subjects with known diagnoses of NAFLD and/or known chronic elevations in AST or ALT will be sought to optimize the likelihood of that subjects who undergo the screening visit will qualify for the study. In addition, as approximately 40% of obese young adults in the general population will have NAFLD, obese individuals without previous evaluation for NAFLD may be screened.

Methods of Enrollment

Subjects who learn of the study through their physician or through advertisement and are interested will be asked to initiate contact with the study staff. Patients who respond to website advertising may elect to fill out a survey regarding eligibility that will ask them to provide contact information if they are willing to be contacted. Alternatively, if a patient's physician obtains the patient's permission for study staff contact, we may contact potential participants who have been identified as suitable by local providers. For patients seen clinically by the PI or other physician investigators involved in the study, the following procedures will be followed to ensure that subjects do not feel obligated to participate because of the involvement of their regular physician: The provider may mention the study to the patient and provide a flyer or consent form for the subject to take home. If this occurs, the provider will not talk further about the study unless the patient initiates the conversation by expressing interest. Alternatively, the patient's provider may mention the study and either ask the patient's permission for a different study provider to contact him/her, or provide contact information of a different study provider whom the patient can contact if interested.

Preliminary eligibility will be determined based on study staff interviews of interested subjects over the phone. Eligible subjects will then be scheduled for a screening visit.



Informed Consent

Written informed consent will be obtained by a licensed physician investigator prior to screening evaluation and testing. Subjects will be informed that they may withdraw from participation in the study at any point.

V. Study Procedures

The study will be conducted over 2 years, with subject recruitment complete after 18 months. Each eligible subject will participate in the study for 24 weeks. Following the screening visit, eligible subjects will return to the Massachusetts General Hospital Clinical Research Center (CRC) for as shown in the schema. Subjects in the Norditropin group will have 7 additional visits, and subjects in the No Treatment group will have 3 additional visits. Subjects who discontinue treatment for any reason will be asked to continue to return for study visits per their original randomization assignment.

For all female subjects, urine pregnancy tests will be performed at all visits, and a positive pregnancy test will result in immediate discontinuation. At baseline, 12 weeks, and 24 weeks, subjects will be counseled regarding lifestyle modification (see Methods). Subjects will continue to receive care from their regular medical providers, including hepatologists, throughout the study. Any subject not under the care of a hepatologist who is found at screen to have ≥5% liver fat and significantly elevated AST or ALT will be offered referral to the hepatology clinic.

Screen: **(1)** Informed consent; **(2)** Detailed H&P, including medications and alcohol habits; **(3)** blood sampling for IGF-1, CBC, HbA1c, fasting glucose, AST, ALT, total bilirubin, Hepatitis B sAg, Hepatitis C antibody; **(4)** ^{1}H-MRS/MRI of abdomen in fasting state for quantification of liver fat. (This scan will also be used to assess VAT and SAT for those eligible subjects participating in the baseline visit.)

Randomization and Study Drug: After the screening visit, eligible patients who wish to participate will be randomized in a 1:1 ratio to Norditropin vs. no treatment. The study will not be blinded and placebo will not be used to avoid daily injections without benefit for subjects randomized to no treatment. Randomization will be stratified by gender and Vitamin E use. The starting dose of Norditropin will be 0.5 mg daily, which is a recommended dose for restarting growth hormone in young adults with GHD during the transition from adolescence to adulthood (70). IGF-1 will be checked at 2, 4, 6, 12, and 18 weeks, and will be reviewed by the PI. Active dose adjustments will be performed per algorithm as follows. A 20% dose increase will be performed for any Norditropin subject with IGF-1 SDS ≤ 0. Conversely, a 20% decrease in dose will be performed for any Norditropin subject with IGF-1 SDS >2.

Norditropin Dosing Algorithm (starting dose 0.5 mg)		
IGF-1 SDS ≤ 0	**0 < IGF-1 SDS ≤ 2**	**IGF-1 SDS > 2**
20% dose increase	maintain dose	20% dose reduction*
*Subjects with persistent IGF-1 SDS > 2 after 2 dose reductions will be discontinued from the study.		

Baseline Visit:

Patients will arrive after an 8-hour fast. For females, urine pregnancy test will be performed prior to the start of any procedures. When possible, women with regular menses will be studied within 10 days of the onset of the menstrual cycle.

1. Within one week prior to the baseline visit, subjects will come in the fasting state for a clonidine/arginine stimulation test to assess GH secretion (see methods).
2. Interval history and physical exam
3. Indirect calorimetry in the fasting state for the first 10 randomized subjects only
4. Fasting blood sample for glucose, insulin, IGF-1, AST, ALT, GGT, lipid panel, CRP, adiponectin, CK18
5. Standard 75g oral glucose tolerance test
6. DXA for quantification of total and regional fat and lean mass, VAT and SAT
7. Anthropometrics including height, weight, waist and hip circumferences, neck, mid-arm, and mid-thigh circumferences
8. Analysis of 24-hour food recall for macro- and micronutrient composition
9. Assessment of physical activity and lifestyle habits (including alcohol consumption) using questions from the 2013 Standard High School Youth Risk Behavior Survey (YRBS)
10. Instruction on reconstituting and administering Norditropin for subjects randomized to rhGH, with first injection prior to discharge

Safety Visits (2, 4, 6, and 18 weeks, Norditropin subjects only): **(1)** Interval H&P and safety assessment; **(2)** IGF-1, fasting glucose **(3)** Collection of used study drug and dispensation of new supply.

12 Week Visit: **(1)** Interval H&P and safety assessment; **(2)** IGF-1, fasting glucose **(3)** Collection of used study drug and dispensation of new supply for Norditropin subjects; **(4)** AST and ALT.

24 Week Visit: Subjects receiving treatment will be instructed to take their final Norditropin injection the night before the visit. Girls with regular menses will be studied within 10 days of the onset of the menstrual cycle. This visit will be identical to baseline with the addition of ^{1}H-MRS/MRI in the fasting state prior to OGTT. (clonidine/arginine stimulation test will *not* be repeated.) Indirect calorimetry will be performed only for the first 10 randomized subjects.

VI. Methods

^{1}H Magnetic Resonance Spectroscopy(MRS) & MRI: **Liver ^{1}H-MRS** will be performed using a 1.5 Tesla (Siemens Trio; Siemens Medical Systems) MRI system. After an overnight fast, each subject will undergo ^{1}H-MRS supervised by a radiologist who will review voxel placement and quality of spectra before patient is discharged. Subjects will be supine and feet first in the magnet bore. A body matrix phased array coil will be positioned over the abdomen and a tri-plane gradient echo localizer pulse sequence [repetition time (TR), 15 msec; echo time (TE), 5 msec; slice thickness, 3 mm] will be obtained to localize the liver. A breath-hold True FISP sequence (TR, 3.8 msec; TE, 1.9 ms; slice thickness, 10 mm; imaging time, 12 seconds) will be obtained for detailed assessment of hepatic anatomy. A 20 × 20 × 20 mm voxel (8 mL) will be placed in the right hepatic lobe, avoiding vessels or artifacts, followed by automated shimming. The voxel placement will be registered by screen captures for consistency in follow-up studies. Single breath-hold single-voxel ^{1}H-MRS data will be acquired in mid-expiration using point-resolved single voxel spectroscopy (PRESS) pulse sequence without water suppression: TR, 1,500 msec; TE, 30 msec; 8 averages; 1024 data points; and receiver bandwidth, 2000 Hz; acquisition time: 18 seconds. This will be repeated with a voxel placement in the left hepatic lobe. Subsequently, liver ^{1}H-MRS sequence with 2D PACE motion correction will be performed, triggered on the quiet and expiration phase of respiratory cycle with a pre-defined acceptance window during free breathing. A navigator image will be centered at the liver/diaphragm interface and a voxel measuring 20 × 20 × 20 mm (8 mL) will be placed in the right (followed by left) hepatic lobe in a similar location as breath-hold study. A PRESS pulse sequence using TR, 1,500 msec; TE, 30 msec; 64 averages; and receiver bandwidth, 2000 Hz will be obtained. In our experience, reproducibility of both breath-hold and respiratory-gated 1H-MRS is high. Correlation analysis of data before and after repositioning is r= 0.99, p= 0.0003 for breath-hold and r= 0.93, p= 0.007 for respiratory-gated 1H-MRS. Bland Altman analysis showed mean difference between same-day scans of 0.29% (95% CI: −1.46 and 2.05%) for breath-hold and −0.54% (95% CI: −2.53 and 1.45%) for respiratory gated 1H-MRS. The concordance correlation coefficient for breath-hold 1H-MRS is 0.97 and for respiratory-gated 1H-MRS, 0.91 (71). Fitting of all ^{1}H-MRS data will be performed using LCModel (72). Data will be transferred from the MR scanner to a Linux workstation and metabolite quantification using eddy current correction and water scaling. A customized fitting algorithm for liver analysis provides estimates for all lipid signals (0.9, 1.3, and 2.3 ppm) scaled to unsuppressed water peak (4.7 ppm). Proton density fat fraction will be calculated from integral lipid and water peak areas as previously described (73, 74). **Volume of abdominal fat depots** (VAT and SAT) will be obtained in the same imaging session using an 8-channel phased-array torso coil covering the xyphoid process to the symphisis pubis. A modified 3-dimensional Dixon chemical-shift 6-multiecho pulse sequence will be utilized automatically generating inline water-only and fat-only images, allowing differentiation of VAT/SAT from abdominal wall muscles, bowel and solid organs. Parameters include coronal slice/lab orientation, TR 9-13 ms, TE/echo-spacing 1.2-1.4/1.0-1.2, flip angle, 3^{o}, slice thickness 3mm, 150 slices. In addition, a T1 water-suppressed axial single slice at the level of L4 will be obtained for measurement of VAT/SAT areas. VAT/SAT areas and volume will be measured by semi-automated tracing with manual adjustments (Aquarius 3D Workstation, TeraRecon Inc, San Mateo, CA).

75 g Oral Glucose Tolerance Test will be performed after a 12-hour fast with sampling for insulin and glucose at 0 and 120 min.

Whole Body DXA will be done to determine whole body and regional fat including DXA assessed VAT and SAT (75, 76).

Clonidine/Arginine stimulation test will be performed after 12h fast according to standardized protocol. At time 0, subjects will take clonidine 150mcg/m^2 (max 250mcg) PO x 1 and will receive intravenous administration of arginine hydrochloride (0.5g/kg with max dose of 30g; 30g/300 cc infusion) over 30 minutes. GH sampling will be done at baseline and 30, 60, 90 and 120 minutes.

Nutrition and Activity: 24-hour food recall will be performed and analyzed by registered dietitians at the Clinical Research Center to assess micronutrient and macronutrient intake. Questions from the CDC 2013 Standard High School Youth Risk Behavior Survey (ftp://ftp.cdc.gov/pub/data/yrbs/2013/2013_hs_questionnaire.pdf) will be used to assess physical activity (Q80-84), eating habits (Q71-79), drinking habits (Q41-45), and recent dieting (Q66-70).

Labs will be run using standard validated assays.

Study Drug: For those randomized to treatment, Norditropin (rhGH) will be started at a dose of 0.5mg daily. The GH dosing strategy (see above) is designed to achieve IGF-1 levels between 0-2 SDS. This strategy represents a modification of the dosing strategy in a previous pilot study, which began with 0.4mg daily at baseline, increased to 0.6mg at week 1 and 0.8mg GH daily by week 2, and then included subsequent dose adjustments at 1 and 3 months using the same algorithm proposed above. This strategy resulted in a mean IGF-1 SDS of 0 in the treatment group, and thus proved somewhat too conservative. The recommended dose for restarting GH during the transition period in young adults with GH deficiency is 0.2-0.5mg/day (70). Based on this recommendation and the experience from our previous study, we will start with a baseline dose of 0.5mg and include the opportunity for further adjustments at 2, 4, 6, 12, and 18 weeks. At the Principal Investigator's discretion, subjects receiving Norditropin whose IGF-1 z-score is rising near 2 may be asked to come for an additional blood draw for IGF-1 between 12-24 weeks, to ensure that levels remain below a z-score of 2. Subjects will be instructed to administer Norditropin at night before bedtime. GH was safe and well-tolerated in the previous pilot study. Safety will be closely monitored. Although no dosing reductions were required in the pilot study and we do not anticipate this circumstance, subjects with IGF-1 SDS >2 will undergo a 20% dose reduction. If a subject undergoes 2 dose reductions and continues to have IGF-1 SDS >2, s/he will be discontinued from the study. Subjects with fasting glucose ≥126mg/dL will have the value confirmed on a repeat draw and, if hyperglycemia is confirmed, will be discontinued from the study. Subjects reporting significant adverse effects including significant arthralgia, myalgia, or edema will also be discontinued. These did not occur in the pilot study and are not anticipated to occur with the physiologic dosing strategy that we will utilize.

Compliance will be assessed using a daily study drug diary as well as vial count of returned used and unused study drug.

Lifestyle Counseling: Subjects will receive lifestyle counseling from study MDs or nutritionists at the baseline, 12 week, and 24 week visits, with recommendations and resources provided based on the 2010 Dietary Guidelines for Americans (http://www.cnpp.usda.gov/sites/default/files/dietary_guidelines_for_americans/PolicyDoc.pdf) and the 2008 Physical Activity Guidelines for Americans (http://health.gov/paguidelines/guidelines/summary.aspx). Emphasis will be placed on physical activity (150+ minutes weekly); reduction of saturated fats, total fats, and sugary beverages; and increased consumption of fruits and whole grains.

VII. Biostatistical Analysis

Statistical Consideration – Data Interpretation: The primary endpoint will be change in hepatic fat as measured by ^{1}H-MRS after 24 weeks of randomized treatment. Secondary endpoints will include changes in ALT, AST, GGT, VAT, CRP, adiponectin, IGF-1, lipids, fasting and 2-hour glucose and insulin, HbA1c, and respiratory quotient measured by indirect calorimetry. Permuted block randomization will be performed, stratified by gender and use of Vitamin E.

Analysis will be intention-to-treat, using all available data from all subjects. Wilk-Shapiro test will be used to assess for normality of distribution of all variables, and variables that are not normally distributed will be appropriately transformed. Baseline variables will be compared between treatment groups, and any baseline variable that is statistically different between treatment groups will be adjusted for in subsequent analysis. Changes in variables measured only at baseline and 6 months, including the primary endpoint of liver fat, will be initially assessed using a two-sample t-test comparing mean changes between treatment groups. A critical alpha level of 0.05 will be used as the threshold for significance, and all statistical tests will be two-tailed. Subsequently, adjusted analyses will be performed including as covariates any baseline characteristics that differ between treatment groups and any differential changes in nutritional intake or physical activity between treatment groups over the study duration. For variables measured at ≥3 timepoints throughout the study (e.g., IGF-1, AST and ALT), we will analyze the longitudinal data using general linear mixed effects modeling, testing for time x treatment group interaction. The primary analysis will be an intention to treat analysis using all available data. We will consider secondary multiple imputation based analyses if necessary.

A supplementary analysis will be performed assessing stimulated growth hormone levels in response to Arginine testing. Using peak GH as the independent variable, we will assess the hypothesis that peak GH will be negatively associated with liver fat quantity.

Sample Size and Power Calculations: The study is designed to yield pilot data, with the primary endpoint being change in hepatic fat fraction. As we do not have preliminary data on this measure in an adolescent population, we used a published standard deviation (SD) of 3.1%. A sample size of 24 subjects, with up to 20% discontinuation rate, yielding 19 evaluable patients, has 80% power to detect a 4.2 percentage point treatment effect on liver fat (e.g., a reduction from 7% liver fat to 2.8% liver fat), which is the minimum difference that would be clinically relevant, as absolute reductions of ≥ 4-5% liver fat have been considered clinically relevant in previous publications (77, 78). For secondary variables of interest, the study is has 80% power to detect a treatment difference of approximately 1.4 SD.

VIII. Risks and Discomforts

Radiation: Subjects will have 2 DXA scans over 6 months, for a total radiation risk from both scans of 2 microSieverts. This radiation exposure is minimal and does not pose excessive risk to subjects. Female subjects must have a negative pregnancy test before DXA scanning.

Blood Drawing: Blood sampling volumes will be as follows: screen 27cc, baseline and 24 week visit 99cc, safety visits 5-8cc each (5 cc at 2 and 4 weeks, 8cc at 6, 12, 18 weeks). The total blood drawing will be 286cc over 6 months. The maximum blood drawn during any 8 week period (screen+baseline+2wk+4wk) will be no more than 140cc. Body weight ≥50kg and Hgb ≥ 11g/dL will be required to participate, which will ensure that the blood volume sampled is within guidelines and does not pose significant risk to subjects.

Blood drawing may cause discomfort, pain, and bruising.

MRI/MR spectroscopy: MRI and MR spectroscopy will be performed using FDA approved devices and pulse sequences, and there are no known foreseeable risks associated with exposure to MRI. Subjects will be carefully screened for the presence of metallic implants (e.g., vascular clamps) prior to MR scanning. Some

subjects report claustrophobia during MRI scans, and if any patient experiences discomfort during the scan, the procedure will be aborted and not repeated without the subject's consent.

Oral Glucose Tolerance Test: Oral glucose tolerance test has no significant risks. Some subjects may experience nausea or lightheadedness during the procedure, and supportive nursing care will be provided.

Clonidine/Arginine Stimulation:
Administration of *arginine* for diagnostic purposes can, on rare occasions, cause nausea, lightheadedness, numbness, and tingling. *Clonidine* for diagnostic purposes may cause sleepiness and a mild decrease in blood pressure. The stimulation test will be performed on the Clinical Research Center by nursing staff who are highly experienced in conducting this test. Supportive care will be provided if needed.

Other procedures: There are no appreciable risks of the following procedures: indirect calorimetry and questionnaire administration. If subjects express or demonstrate discomfort during any research procedure, they will be reassured that the research is optional and they may choose to quit any procedure at any time.

Growth Hormone (rhGH, Norditropin):

Norditropin will be administered to subjects via subcutaneous injection. The dosing strategy has been formulated based on extensive clinical experience of Drs. Stanley (PI) and Misra (Co-I) as well as prior research experience administering rhGH in this population. To be eligible for the research, subjects must have IGF-1 SDS ≤0, demonstrating relative reductions in GH secretion and ensuring that the dosing strategy will result in physiologic increases in IGF-1. The starting dose of rhGH will be 0.5mg daily. IGF-1 will be checked at 2, 4, 6, 12, and 18 weeks, and dose adjustments will be made as described above. The goal IGF-1 is between 0-2 SDS, and frequent IGF-1 assessment and dose adjustment will ensure physiologic dosing, such that any potential side effects are minimized.

Possible side effects of rhGH include headache, edema, myalgia, arthralgia, and hyperglycemia. Subjects will be asked to contact study physicians right away with any potential side effects, and subjects who experience concerning side effects will be advised to discontinue the medication. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns. Fasting blood sugar will be checked at 2, 4, 6, and 12 weeks, and any subject with a fasting blood sugar ≥126mg/dL that is confirmed on recheck will be discontinued from the study, with appropriate follow-up to ensure return to euglycemia. RhGH, particularly at physiologic dosing, is incredibly well-tolerated in the pediatric population, and there were no significant GH-related side effects in our pilot study of obese adolescents. When side effects, including hyperglycemia, do occur with rhGH, these resolve with discontinuation of treatment unless there is an underlying, non-GH-related, etiology.

As stated elsewhere, a pregnancy test will be performed at each visit for females able to become pregnant, and subjects will be informed of the study-related risks of pregnancy and the need not to become pregnant during the study. If a subject is found to be pregnant, she will be asked to discontinue the study drug immediately, and the event will be reported to Novo Nordisk as below. She will be referred for continued primary care and OBGYN care.

Subjects will be carefully instructed regarding reconstitution, safe injection technique, and appropriate storage of the study drug and disposal of sharps. A study investigator will be on call 24/7 for any questions or concerns.

Persistent elevations in blood sugar after discontinuing growth hormone are not expected. However, if any subject is discontinued for fasting glucose ≥126mg/dL, confirmed on repeat draw (see below), we will arrange for a repeat fasting glucose approximately 2-4 weeks after stopping growth hormone. We will arrange this to be performed either at MGH through the study or locally with the patient's primary care doctor. If this is still elevated, the patient will be referred to his/her primary care doctor for continued follow-up and, if desired, we will also arrange for follow-up with endocrinology at MGH.

Individual and Study Stopping Rules
Individuals will be discontinued from the study if

- s/he undergoes 2 dose reductions and continues to have IGF-1 SDS >2
- s/he has fasting glucose ≥126mg/dL confirmed on a repeat draw
- s/he reports significant adverse effects including significant arthralgia, myalgia, or edema

The study will be stopped if

- More than 2 individuals in the GH group have persistent fasting glucose ≥126mg/dL

The study will be halted and the dosing algorithm reconsidered (and lowered) if

- More than 2 individuals in the GH group have IGF-1 SDS >2 after 2 dose reductions

Alternative Treatments

There are no FDA-approved highly effective treatments for NAFLD in young adults, and research into novel treatments is urgently needed. Vitamin E, ursodiol, and metformin are sometimes used for NAFLD, and vitamin E has been demonstrated to improve liver histology in individuals with NASH. Subjects who wish to participate and have been receiving Vitamin E or ursodiol at a stable dose for ≥6 months will be eligible for the study with continued use of these medications (under the ongoing care of their hepatologist) during the study. Metformin use is not allowed as it will confound assessment of glucose homeostasis. We will not ask any subject to discontinue any medication in order to participate in the study.

As we are not performing liver biopsy during the current study, the study procedures will not result in new diagnosis of NASH (which requires histology). However, subjects without known NAFLD who demonstrate ≥5% liver fat on MRS and who have elevated ALT or AST are likely to have NASH, and, if not already under the care of a hepatologist, these subjects will be offered a clinical referral to pediatric hepatology. Subjects who are found to have labs suggesting significant liver disease (AST or ALT > 2.5x upper limit of normal (ULN) or total bilirubin > ULN) will be encouraged to seek clinical care from a hepatologist and will not be eligible for the study. AST and ALT will be carefully monitored (0, 6, 12, 18, and 24 weeks).

VIII. Potential Benefits

Participants will receive information about their nutritional and metabolic health. In addition, subjects randomized to Norditropin may experience benefits from this treatment including decrease in fat mass and possibly decreases in liver fat and systemic inflammation. All subjects will receive lifestyle counseling and information about healthy nutrition and physical activity. Further, the information obtained from this study will inform the potential use of rhGH and possibly other strategies to treat NAFLD in adolescents.

The risks of the study are minimized by close communication between study investigators and subjects, careful monitoring of IGF-1 levels, and frequent monitoring for adverse events. The study may provide benefit to individual patients, as above, and is also expected to provide information that may lead to the development of new treatment strategies for NAFLD, which is a significant public health problem. Therefore, benefits are felt to outweigh the risks.

IX. Adverse Event, Unanticipated Problem, and Deviation Definitions

Adverse Event (AE)

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)

An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A Serious Adverse Event is an AE that results in one or more of the following outcomes:
- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Serious Adverse Drug Reaction (SADR)
An SADR is defined as a serious adverse event that has a reasonable suspected causal relationship to the study drug. For the purposes of reporting during this protocol, any serious adverse event occuring in a subject randomized to study drug that is judged by the investigator to be *possibly related, probably related, or definitely related* (see below) to study drug will be considered in this category.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Package Insert for norditropin or is not listed at the specificity or severity that has been observed.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is
1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as
1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Pregnancy will be reported as described below if it occurs in any subject receiving norditropin or if it occurs in a subject's partner whose fetus or baby develops an adverse event considered to be related to norditropin.

Relatedness will be determined as follows:
Definitely Related
- reasonable temporal relationship

- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related
- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related
- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related
- does not have a reasonable temporal relationship
  OR
- good evidence for a more likely alternative etiology

Not Related
- does not have a temporal relationship
  OR
- definitely due to an alternative etiology

X. Reporting Requirements

Dr. Stanley (PI) will be responsible for the reporting of adverse events, unanticipated problems, protocol deviations, and other necessary events as follows:

Reporting to Novo Nordisk

Serious Adverse Drug Reactions and pregnancies will be reported to Novo Nordisk as soon as possible and no later than 7 days following investigator awareness of the event. Dr. Stanly will assist Novo Nordisk with follow-up of any reports as necessary.

Reporting to IRB (Partners Human Research Committee)

The following will be reported within 7 calendar days of investigator awareness:

- Internal adverse events that are unexpected, and related or possibly related to the research and that indicate there are new or increased risks to subjects
- Deviation from the approved research protocol or plan without IRB approval in order to eliminate apparent immediate hazard to subjects or harm to others
- Deviation from the approved research protocol or plan that placed subjects or others at an increased risk of harm regardless of whether there was actual harm to subjects or others
- Breach of confidentiality or violation of HIPAA (e.g., lost or stolen laptop)
- Medication, procedural or laboratory error (e.g., errors in drug administration or dosing, surgical or other procedure, or testing of samples or test results) regardless of whether subjects experienced any harm
- Interim analysis, safety monitoring report, publication in a peer-reviewed journal, or other finding that indicates that there are new or increased risks to subjects or others or that subjects are less likely to receive any direct benefits from the research
- Change in FDA labeling (e.g., black box warning), withdrawal from market, manufacturer alert from Novo Nordisk, or recall of an FDA-approved drug, device, or biologic used in the research

- Complaint by/on behalf of a research subject that indicates that the rights, welfare, or safety of the subject have been adversely affected or that cannot be resolved by the investigator
- Incarceration of a research subject during participation in research that is not approved for involvement of prisoners as subjects
- Noncompliance with applicable regulations or requirements or determinations of the IRB identified by the research team or others (e.g., FDA Form 483 or Warning Letter) that indicates that the rights, welfare, or safety of subjects have been adversely affected
- Suspension or termination of the research, in whole or in part, based on information that indicates that the research places subjects at an increased risk of harm than previously known or recognized (e.g., FDA clinical hold)
- Suspension or disqualification of an investigator by FDA, Novo Nordisk, or others
- Scientific misconduct
- Any other problem that indicates that the research places subjects or others at an increased risk of harm or otherwise adversely affect the rights, welfare or safety of subjects or others.

The following items will be reported to the MGH IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

XI. Monitoring and Quality Assurance

Full IRB approval will be obtained by the Partners Human Research Committee before any interactions with subjects take place for the purposes of this protocol. The protocol will be conducted adhering to the standards of Good Clinical Practice.

Written, informed consent will be obtained prior to any procedures being performed on a subject for the purposes of this protocol. This consent will be obtained by a licensed medical provider (MD or NP).

The principal investigator (Stanley) will monitor all data collected for the studies. Data will be stored securely, with access restricted to co-investigators and study staff. Binders with subject information will be labeled with a coded enrollment number to protect confidentiality. Electronic databases will be locked and password-protected, with access available only to study staff. Data will not be saved on the hard drive of any laptop or desktop computers or on any removable data storage devices such as flash drives or CDs.

The risks to subjects will be minimized by using procedures which are consistent with sound research design. Subjects will be carefully screened to minimize potential risk of study procedures and treatments. A pregnancy test will be performed at each visit for female subjects of childbearing potential, and patients who become pregnant will be asked to discontinue participation in the study. Most importantly, the safety of subjects will be monitored closely by the principal investigator, co-investigators and DSMB, and subjects will be discontinued from the study if the risk to the subject is felt to be excessive.

Data and Safety Monitoring Plan
An independent data and safety monitoring board will be established to review safety data and adverse events. The DSMB will include a statistician, a gastroenterologist, and a pediatric endocrinologist, and will meet every 6 months. The studies will also be monitored continuously by the principal investigator (Stanley).

Subjects will be instructed to report any adverse events immediately. A physician or nurse practitioner will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

Clinical Trials Registration
The trial will be registered on clinicaltrials.gov, with Dr. Stanley serving as the responsible party for registration and reporting of results.

XII. Dissemination of Results

Upon completion of the protocol, an abstract summarizing the primary results will be submitted to an appropriate national meeting (e.g., Pediatric Academic Societies or ENDO), and a manuscript comprehensively reporting the results will be submitted to an appropriate journal (e.g., Journal of Clinical Endocrinology and Metabolism). Preparation and submission of the primary manuscript reporting results is anticipated to be complete within 4 months of the last study visit for the last patient.

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