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Project Number 11-CH-0004
 IND/IDE Number 111,512 (Niacin)
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Recommended by: _____, Branch Chief, PDEGEN, NICHD
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Subject: Protocol 11-CH-0004:
 Free fatty acids, body weight, and growth hormone secretion in children

Identifying Words: Child, Body Fat, Free Fatty Acids, Adipose, Growth Hormone

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*May obtain consent and assent from participants

Estimated Duration of Study: 3 years

Subjects of Study:

	Number	Gender	Age Range
Obese children (dose-establishing study 1)	10	M, F	7-14.99 y at study entry
Obese children (dose-establishing study 2)	5	M, F	7-14.99 y at study entry
Obese children (main study)	33	M, F	7-14.99 y at study entry
Short, healthy children (main study)	33	M, F	7-14.99 y at study entry
Screened but not enrolled in pilot studies	10	M, F	7-14.99 y at study entry

Project uses ionizing radiation Yes (for main study only)

Radiation use is ___ medically indicated X indicated for research

Project involves use of Durable Power of Attorney: No

Off-Site Project: No

Multi-Institutional Project Yes

Précis:

Obese children and adults display lower spontaneous and stimulated growth hormone (GH) secretion. It is presumed that dysregulation of some of the factors normally involved in controlling GH secretion underlies the hyposomatotropinemia of obesity, given that GH production usually normalizes after weight loss. Free fatty acids (FFA) are one factor thought to be involved in regulation of GH secretion. Niacin is a nicotinic acid derivative that inhibits lipolysis and lowers circulating FFA concentrations. Nicotinic acid derivatives have been used in several adult studies examining GH secretion. Specifically in obese adults, inhibition of lipolysis has been found to increase spontaneous and stimulated GH production, presumably due to direct effects of FFA on hypothalamic GH-regulating neurons. Thus far no pediatric studies have examined the effects of niacin on GH secretion, and there is only one small pediatric study of normal weight prepubertal children growing at the 5th-10th percentile in height has tested the effects of lipolytic inhibition by acipimox (a related medication also derived from nicotinic acid) on GH secretion. There are no data in obese children demonstrating the effects of inhibition of lipolysis on GH secretion.

We propose to investigate one of the mechanisms through which high adiposity alters GH secretion in children by testing the effects of inhibiting lipolysis. First we will conduct dose-establishing studies to determine the appropriate dose of niacin needed to suppress FFA concentrations in children. We will then conduct the main study, designed as a pilot, randomized, double-blind placebo-controlled trial of niacin administration, to assess its effects on stimulated GH secretion. We hypothesize that in overweight children niacin will lead to a fall in free fatty acid concentrations and consequently a rise in stimulated GH secretion. We further hypothesize that the overweight subjects will demonstrate stimulated GH secretion profiles with niacin similar to those of control subjects who receive placebo. We expect this pilot study may help improve how diagnostic testing is carried out for growth hormone deficiency in children.

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Introduction:

Assessment of GH secretion is an important component of the evaluation of the short child.¹ The tests designed for this purpose, however, have been found to have relatively low specificity such that more than 40% of all children treated for isolated GH deficiency during childhood may be found to produce normal quantities of GH when retested as adults.^{2,3} Among the many confounding factors that may impact the assessment of GH secretory capacity is obesity. A recent retrospective study of patients presenting for short stature evaluation at an endocrine clinic and undergoing GH stimulation testing with arginine, clonidine, L-dopa (L-3,4-dihydroxyphenylalanine), and/or propranolol revealed a negative association between body mass index (BMI) standard deviation score (SDS) and peak GH, particularly in prepubertal children.⁴ This study highlights the difficulty of interpreting GH deficiency testing in overweight and obese children. Given the decline in GH secretion with increasing weight, the standard cutoffs for GH deficiency may not apply to patients with higher BMIs. In particular, patients with predisposition to both GH deficiency and obesity, such as children with Prader Willi Syndrome, or children with a history of brain tumor and resulting hypothalamic obesity, may be difficult to diagnose accurately. More appropriate normative data for GH concentrations in these patients have not been established, nor have high specificity diagnostic tests for overweight subjects been found. This study aims to further elucidate one mechanism behind diminished GH production and to pilot test a modified protocol for assessing GH deficiency in obesity.

Growth hormone production

GH secretion is a complex process regulated by multiple feedback mechanisms. Growth hormone-releasing hormone (GHRH) and somatostatin, both of which are secreted from the hypothalamus, are the main regulators of pituitary production of GH. Several other factors are believed to influence secretion of these hypothalamic hormones as well as directly affect the

somatotrophs including, but not limited to, GH itself, insulin-like growth factor 1 (IGF-1), ghrelin, leptin, glucose, and FFA.⁵

Growth hormone stimulation testing

GH is secreted in short pulsatile bursts, mostly during nighttime sleep. The concentrations of GH in the serum are highly variable without predictable times of nadirs or peaks; thus, a random GH measurement is not helpful for assessing GH deficiency. Instead GH secretion is typically assessed through stimulation testing in order to measure peak GH production. Appendix A shows the most common medications used to induce GH secretion, including data on dosing and mechanism of action. Normative data have been established for various types of stimulation testing to appropriately diagnose GH deficiency. Because of the low specificity for any one GH stimulation test, this testing usually involves administration of two separate stimuli for GH release with measurements of GH concentrations every 15 to 30 minutes thereafter for 1-3 hours. For children and adolescents, a peak value following administration of a provocative stimulus of 7 nanograms/milliliter or higher is typically considered diagnostic of sufficient GH production.

Growth hormone production in obesity

GH secretion in obese patients was first noted to be different from normal controls by Roth, et al in a 1963 publication.⁶ While studying the effects of exercise, hypoglycemia, and glucose loading on GH concentrations, the authors noted lower responses in their obese patients, leading them to conclude, “Marked obesity is associated with, and possibly conditioned by, a deficient secretory response of pituitary GH to certain physiologic stimuli...”⁶ Many subsequent studies have shown lower GH concentrations in obese individuals, both during spontaneous⁷⁻¹³ and stimulated^{7,13-24} measurements. In particular, GH concentrations show negative correlation with BMI, percent overweight, amount of fat mass, and waist circumference.^{10,20,25-27} Additionally obese patients do not demonstrate a substantial decrease in GH secretion in response to a glucose load as demonstrated in normal controls.^{9,14,20,24,28,29} It is presumed that dysregulation of some of the factors

normally involved in GH secretory regulation underlies the hyposomatotropinemia of obesity, given that GH production usually normalizes after weight loss,^{12,30-39} with peak GH response to pharmacologic stimuli correlating with degree of weight loss.^{27,38,40,41} Similarly, when exercise is used as a stimulation for GH secretion, obese patients do not respond as robustly as normal controls.⁴²

Results comparable to those observed in obese adults have also been reported in obese children. Peak GH concentration after stimulation testing is lower in obese children compared to controls in studies using arginine and L-dopa,⁴³ clonidine,⁴⁴ and dexamethasone⁴⁵ as stimuli. As with adults, obese children show a blunted degree of GH secretion during exercise,^{46,47} and do not respond to a glucose load.⁴⁸ A study of 24-hour GH secretion in boys demonstrated an inverse correlation between BMI SDS and daily GH production rate, as well as mass and amplitude of each burst of GH.⁴⁹ Similarly a study comparing lean and obese children showed the lean female subjects had greater GH secretion during periods of sleep and higher and more frequent GH peaks during the day compared to their obese counterparts; the trend was similar in lean versus obese males but without achieving statistical significance.⁵⁰ Similar studies of sleep-associated GH release show fewer and smaller peaks of secretion in obese children compared to lean.^{51,52} Weight loss reverses these trends with 24-hour GH secretion among formerly obese prepubertal children whose BMI returned to the normal range mirroring that of a control population.⁵³ Even short term calorie restriction showed a 60% improvement in average GH secretion.⁵⁴

Free fatty acids in obesity

Fatty acids are absorbed from the intestine after the breakdown of dietary lipids. They combine with glycerol to form triglycerides, which are stored in adipose tissue. Triglycerides in the adipose cells can then be broken down again to liberate free fatty acids into the circulation. Serum free fatty acids concentrations correlate positively with body weight.⁵⁵ The rate of free fatty acid turnover is also faster in obese patients, suggesting increased mobilization of these compounds from

the adipose tissue.⁵⁶⁻⁵⁸ Measurements of free fatty acids in obese children have been less frequently performed but overall show elevations compared to normal weight controls.^{50,59} Some studies have found increases in polyunsaturated fatty acids, monounsaturated fatty acids, and total saturated fatty acids.^{60,61}

Effects of lipids on growth hormone secretion in animals

In animal models, infusion of fatty acids leads to blunting of GH secretion. This effect has been demonstrated in vitro with porcine pituitary cells where linoleic acid and oleic acid suppressed the GH response to GHRH.⁶² In vivo, rats given lipid infusions (with demonstrated rises in free fatty acids levels) showed diminished GH secretion after GHRH treatment, suggesting that free fatty acids lead to inhibition of the GH axis.^{63,64} The ability of the isolated porcine pituitaries to be inhibited by a lipid-rich environment suggests that at least to some degree free fatty acids may exert their effect directly on pituitary somatotrophs. This theory is supported by one in vivo animal study in which the free fatty acid inhibition was unable to be reversed by the addition of an antisomatostatin antibody, suggesting the FFA do not induce somatostatin release.⁶⁴ Another in vivo study with similar methodology did see reversal of the effect with antisomatostatin, which may be due to different timing of the antisomatostatin antibody in relation to the GHRH administration.⁶³ Humans also demonstrate decreased growth hormone response when FFA concentrations are elevated. Lipid infusion lowers basal GH levels in anorexic women and lowers the responsiveness to GHRH stimulation in normal weight and anorexic women.⁶⁵

This evidence (that FFA inhibit GH secretion) suggests a possible explanation for the decreased GH levels in obese individuals. As discussed previously, overweight children and adults have higher circulating free fatty acid concentrations than do normal weight individuals. Thus, these higher free fatty acid levels may inhibit GH secretion and thus be responsible for the observed hyposomatotropinemia of obesity.

Effects of inhibiting lipolysis on growth hormone secretion

Niacin is a nicotinic acid derivative that inhibits lipolysis and lowers circulating FFA concentrations. This medication has been used in several human studies of GH secretion. Most of these studies have examined healthy volunteers and demonstrated increased spontaneous,⁶⁶⁻⁶⁹ stimulated,⁷⁰ and post-exercise⁷¹⁻⁷³ GH production, presumably by preventing the suppressive effects of free fatty acids on GH secretion. When niacin was administered to obese adults, the inhibition of lipolysis led to increased spontaneous GH secretion in one study,⁷⁴ although no effects were seen in a second study.⁷⁵ A third study did see increased spontaneous GH secretion in diabetic subjects.⁷⁶ Acipimox, a similar medication also derived from nicotinic acid, has shown consistent suppression of FFA concentrations leading to increased spontaneous^{77,78} and stimulated⁷⁹⁻⁸⁵ GH production in obese adults. There are no data in obese children demonstrating the effects of inhibition of lipolysis on GH secretion. Thus far only one small pediatric study of normal weight prepubertal children growing at the 5-10th percentile in height has tested the effects of lipolytic inhibition by acipimox on GH stimulation tests and found non-significant differences in peak GH concentrations between acipimox and placebo arms after L-dopa infusion.⁸⁶ We propose to examine the effects of inhibition of lipolysis on GH secretion in overweight children. Physiology in children is often different from that in adults, and no nicotinic acid-derived medications have been tested in overweight or obese children as an augmentor of growth hormone release. Thus, we believe the question of the effects of niacin on overweight children warrants further investigation and will add significantly to scientific knowledge. We have chosen to use niacin to suppress lipolysis in this study because we have been unable to obtain acipimox, and niacin is FDA approved while acipimox is not. We also hope that this study will pilot an alternative and possibly more sensitive test of growth hormone deficiency in overweight children, who often fail current growth hormone stimulation tests despite probable growth hormone sufficiency.

Niacin (Nicotinic acid), administered alone, is approved by the FDA for the treatment of dyslipidemia in adults. Niacin has been shown in adults to decrease total and LDL-cholesterol by

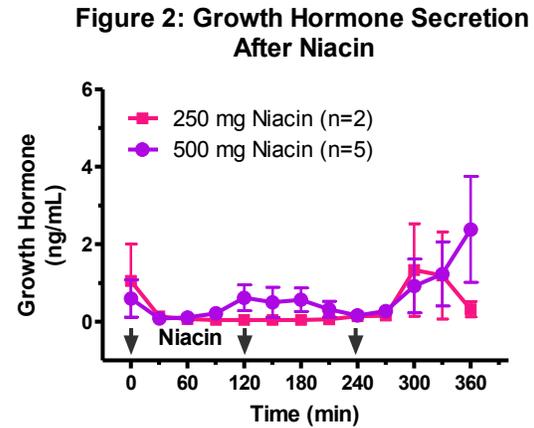
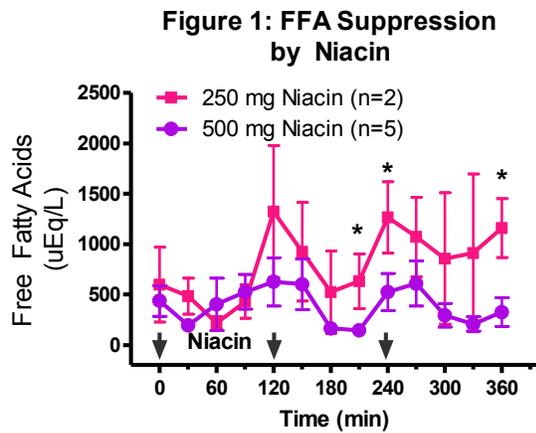
10-20%, decrease triglycerides by 30-70%, and raise HDL-cholesterol by 20-35%. Multiple placebo-controlled studies document the ability of niacin or extended-release niacin, when administered without a statin and, at least in a few studies, with a statin, to reduce the progression of atherosclerosis and lower the incidence of cardiovascular events in patients with established vascular disease.⁸⁷⁻⁹¹ Since statin medications are at least as effective in controlling total and LDL-cholesterol and do not have the gastrointestinal consequences or the skin flushing of niacin, statins have supplanted niacin as first-line treatment. However, since statin medications also generally reduce HDL-cholesterol, there has been continued interest in niacin for its potential to increase HDL-cholesterol. Recently, two large coronary outcomes trials (AIM-HIGH^{92,93} and HPS2-THRIVE^{94,95}) have demonstrated that extended-release niacin offered no benefits beyond statin therapy alone in reducing cardiovascular events in patients with established cardiovascular disease. The second study^{94,95} added an additional medication to niacin, laropiprant, a type 1 prostaglandin D2 receptor antagonist, to increase adherence by decreasing the flushing associated with niacin. These studies suggest there are no long-term unique benefits to niacin treatment, but do not aid understanding of the use of niacin for improving diagnostic growth hormone testing.

Niacin and growth hormone: Overview of proposed pilot study

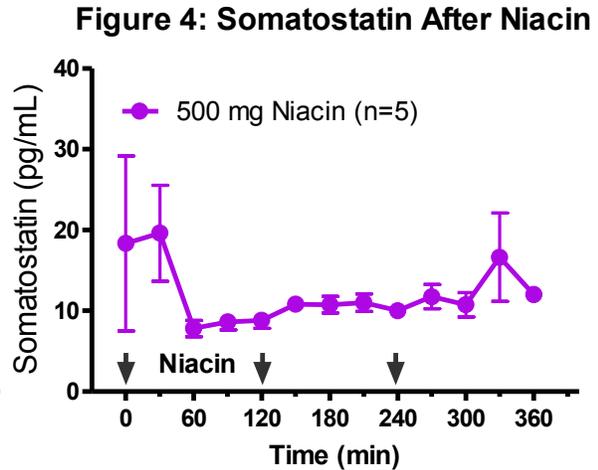
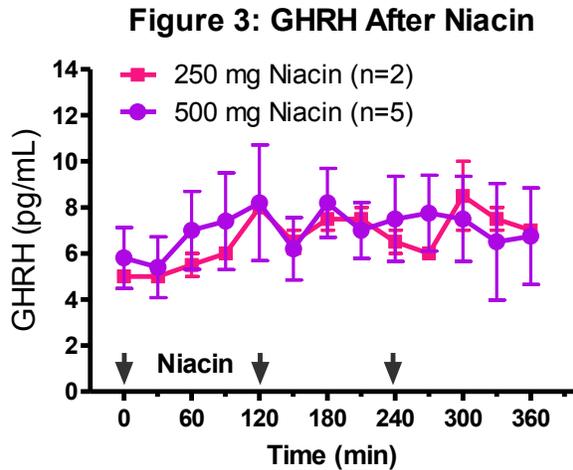
We propose to carry out a randomized, double blind, placebo controlled pilot study to evaluate the effect of niacin administration on stimulated GH secretion in children who are overweight (BMI > 95th percentile for age). We will compare these results to a control group of otherwise healthy children who are growing below the 5th percentile for height and are undergoing a short stature evaluation that results in a recommendation for growth hormone stimulation testing. We will not accept children who are clearly not growth hormone deficient and therefore do not need growth hormone testing. This control group is important to include because there are many children who falsely fail growth hormone stimulation testing. For example, Marin et al found that the percentage of normal children with normal height and growth velocity who failed to attain a GH

level greater than 7 micrograms/L during any of three stimulation tests was 61% at pubertal stage 1, 44% at pubertal stage 2, and 11% in pubertal stage 3.⁹⁶ We do not fully understand the reasons for such failure, but it is possible that varying levels of free fatty acids, even among normal weight children, may be contributing. Thus we feel it is scientifically and ethically justified to evaluate the response of normal weight children to growth hormone stimulation testing in the presence and absence of niacin. Given the lack of this type of testing in children, we do not know if children are susceptible to the effects of elevated free fatty acid concentrations, other factors, or both. Being able to compare the responses of obese children and normal weight children would provide better insight into this physiology.

Because there were no data demonstrating niacin's efficacy to suppress lipolysis, we first needed to conduct a small dose-establishing study. For dose-establishing study 1, only overweight subjects participated, so we could attempt to determine the dose of niacin needed to suppress FFA concentrations sufficiently in the sample of greatest interest. After an overnight fast, we administered three doses of niacin and measure FFA concentrations. We began with obese children studied using 250 mg niacin per dose; since failure to suppress FFA consistently below 200 uEq/L was seen, we studied 5 more children using 500 mg niacin per dose. We chose to start with 250 mg per dose based on a study by Stokes et al that administered adult subjects 1 g of niacin at the commencement of the study followed by 500 mg two and four hours later.⁷¹ These subjects had an average weight of 81 kg, suggesting they received 12.3 mg/kg as the first dose and a total of 24.7 mg/kg total. Our subjects will have a minimum weight of 30 kg; a dose of 250 mg would amount to a maximum of 8.3 mg/kg for a total maximum of 25 mg/kg after three doses. The 500 mg dose, would amount to a maximum of 16.6 mg/kg per dose or 50 mg/kg per day. Based on long-term data of children receiving niacin for treatment of hyperlipidemia, typical daily doses range from 550-2250 mg/day (7-98 mg/kg/day).⁹⁷ Thus, we believed our chosen doses were well within the safe administration range and might be effective in suppression of lipolysis.



Results from dose-establishing study 1 are shown in Figure 1. Seven children, mean age 10y, all with BMI over the 97th percentile, were studied. There was only temporary suppression of lipolysis with 250 mg niacin per dose; therefore as outlined in the experimental plan, only 2 subjects were studied with 250 mg niacin per dose. We observed that 500 mg niacin per dose led to greater suppression of lipolysis than 250 mg per dose (dose x time interaction $p=0.017$), but the 2 hour intervals between niacin doses were also accompanied by subsequent rises in FFA levels to near baseline concentrations. Small but non-significant increases in growth hormone concentrations were found with 500 mg niacin per dose (Figure 2). GHRH concentrations showed little change across time and no differences according to dose of Niacin (Figure 3). Somatostatin data were available only from patients given 500 mg Niacin. A nonsignificant decrease was observed during the first hour of Niacin treatment (Figure 4).



There were no serious adverse events, either unexpected or expected involving subjects participating in this protocol. As expected, flushing and nausea were observed frequently with the first dose of niacin, but, as also anticipated based on the extant literature, there was tachyphylaxis (sudden development of tolerance) such that side effects abated after the third dose of medication:

Table 1: Reported Adverse Events During Niacin Dose-Finding Study 1

Expected adverse reactions (%)	250 mg Niacin (n=2)							500 mg Niacin (n=5)						
	Time (hours)							Time (hours)						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Headache	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Flushing	0	100	100	100	50	50	0	0	80	40	80	40	20	0
Feeling warm	0	100	50	50	50	0	0	0	80	60	100	60	20	0
Nausea	0	0	0	0	0	0	0	0	0	20	0	0	0	0
Vomiting	0	0	0	0	0	0	0	0	0	20	0	0	0	0
Stomach Ache	0	0	0	0	0	0	0	0	20	20	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tingling	0	0	0	0	0	0	0	20	0	0	0	0	0	0
Light-headedness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Itchiness	0	100	100	0	0	0	0	0	60	20	60	40	0	0
Rashes	0	0	0	0	0	0	0	0	20	0	20	20	0	0

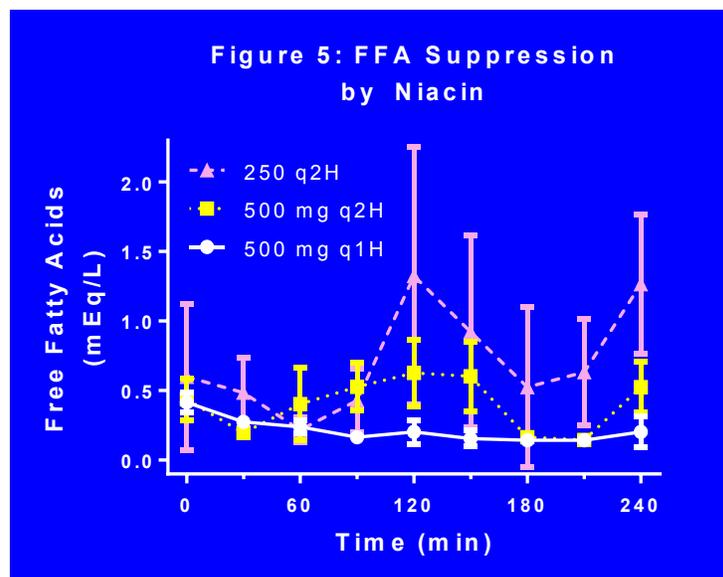
*Note: niacin doses administered at time 0, 2, and 4. Time 0 data reflect reactions before the first dose was administered.

Based on the preliminary results from Dose-Establishing study 1, we performed a second dose-establishing study examining 5 obese children, to determine whether niacin doses

administered more frequently will further suppress FFA. We administered niacin hourly, instead of every 2 hours, to increase FFA suppression throughout the entirety of the test. We tested only 500mg doses of niacin administered hourly for 4 hours (2,000 mg total), as we found clear evidence of insufficient suppression with 250mg niacin and greater suppression with 500mg niacin (Figure 1). To limit the niacin daily dose to 2,000 mg/day, a dose previously administered long-term to children for the treatment of hyperlipidemia⁹⁷, and accommodate nursing preference on the inpatient ward, we shortened the test to conduct a 4 hour study from 7:30am-11:30am.

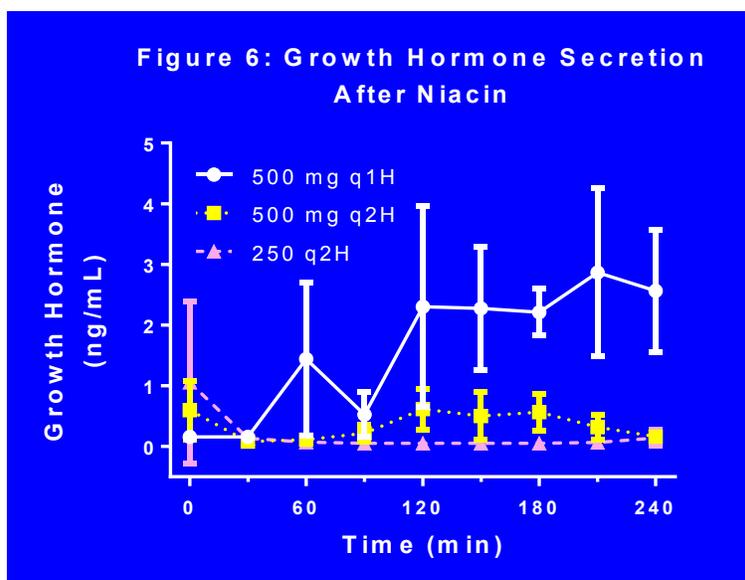
We specified that, after establishing the necessary dose of niacin, we would report back to the IRB before initiating the main study, which will be performed using both overweight and control subjects.

In the Dose-Establishing Study 2, a total of 5 obese children received 500 mg hourly for 4 hours. We found significant suppression of free fatty acids (Figure 5) to the pre-specified outcome (200 UEq/L = 0.2 mEq/L).



Dose p=0.048; Dose x time interaction p=0.018

We also observed stimulation of growth hormone concentrations (Figure 6).



Dose p=0.019; Dose x time interaction p=0.61

For the dose-finding studies, the goal is suppression of FFA concentrations. All 5 participants who received 500 mg qH demonstrated suppression of FFA below 200 μ Eq/L (0.2 mEq/L) during niacin administration. The suppression was much more consistent with the highest frequency of niacin administration:

Table 2: Suppression of FFA <2 mEq/L by Niacin

Group	Number of Subjects	Percentage of measurements post niacin with FFA <0.2 mEq/L
250 mg q 2H	2	11.5%
500 mg q 2H	5	43.1%
500 mg q 1H	5	67.5%

Adverse events during Dose Establishing Study 2 were largely as expected. All participants reported flushing or warmth, 60% tingling or rash, and 20-40% abdominal discomfort, nausea, or emesis.

Data Table 3: Reported Adverse Events During Niacin Dose-Finding Study 2

Expected adverse reactions (%)	500 mg Niacin every hour (n=5)				
	Time (hours)				
	0	1	2	3	4
Headache	0	20	0	0	0
Flushing	0	100	40	40	0
Feeling warm	40	100	40	20	20
Nausea	20	20	40	0	20
Vomiting	0	20	0	0	20
Stomach Ache	20	40	20	20	20
Diarrhea	0	0	0	0	0
Tingling	0	60	20	0	0
Light-headedness	0	0	0	0	0
Itchiness	0	60	40	20	0
Rashes	0	0	0	0	0

Note: niacin doses administered at time 0, 1, 2, and 3. Time 0 data reflect reactions before the first dose was administered. The symptoms at hour 4 were of short duration with only moderate severity. The symptoms reported at earlier hours were also of mild or moderate intensity and, with the exception of feeling warm or flushing, of short duration. All symptoms had resolved completely by the end of the 4th hour.

We therefore will initiate the main study protocol.

During the main study, two sets of GH stimulation tests will be performed on two consecutive days. On one day, the testing will be performed in conjunction with four hourly doses of niacin 500 mg, and on the other day with four doses of a placebo, with the order (niacin first day or second) randomly determined. Subjects will not receive sex hormone “priming” pretreatment (which is used by some clinicians to overcome the naturally lower occurring GH concentrations found in prepubertal children) due to the design of the study; no protocols have established methodology for priming when performing GH stimulation testing on sequential days. We were concerned that administration of sex-steroids could differentially affect the first stimulation test compared to the second. Additionally it might be difficult to assure compliance with the medication as it would be taken at home prior to the admission. Furthermore this protocol will be restricted to

prepubertal and early pubertal subjects to limit the degree of sex hormone influence. It should also be noted that the vast majority of pediatric growth hormone testing performed in the US is done in the absence of such sex steroid priming.

Stimulation testing will consist of arginine followed one hour later by L-dopa, both of which are standard medications used to assess GH secretion. Arginine is thought to inhibit somatostatin release, thereby allowing for GH secretion from the pituitary. Studies of the effect of arginine infusions on GHRH-stimulated GH release show enhanced GH response compared to GHRH alone.^{98,99} Furthermore, incubation of rat anterior pituitary cells with arginine shows no change in GH secretion,¹⁰⁰ suggesting arginine is not itself a direct GH secretagogue but likely works by decreasing somatostatin tone. Arginine has been reported to produce long term changes in free fatty acid concentrations¹⁰¹ but no short term changes were seen after a single infusion.¹⁰² In contrast, L-dopa (L-3,4-dihydroxyphenylalanine) is thought to increase GHRH secretion, thereby promoting pituitary GH secretion. Studies of humans given GHRH antagonists showed suppressed GH concentrations after L-dopa administration, suggesting L-dopa's actions were blocked.¹⁰³ In children with a robust GH response to L-dopa, GHRH plasma levels have also been found to rise in direct proportion to the GH response.¹⁰⁴ Furthermore, studies combining L-dopa infusions with GHRH administration do not show enhancement of GH secretion compared to GHRH alone.⁹⁸ Such results are not consistent with an effect of L-dopa on somatostatin release. We have chosen to use arginine and l-dopa in order to separately stimulate each hypothalamic hormone (GHRH and somatostatin) involved in growth hormone release and thus examine the physiologic effects of free fatty acids more carefully. We did not use GHRH as the stimulus because it would act directly on the pituitary and prevent us from examining the effects of niacin on the hypothalamus. We also chose not to use glucagon because it likely acts by two mechanisms, stimulating GHRH and inhibiting somatostatin via hypoglycemia.¹⁰⁵

We hypothesize that niacin will lead to a fall in free fatty acid concentrations in both groups and consequently a rise in stimulated GH secretion. We further hypothesize that the overweight subjects will demonstrate stimulated GH secretion profiles with niacin similar to those of control subjects who receive placebo.

In the first dose-establishing study, 7 children (two given 250 mg niacin x 3 doses every 2 hours, 5 given 500 mg niacin x 3 doses every 2 hours) were screened and enrolled. In the second dose-establishing study, 5 children to be given 500 mg niacin x 4 doses every hour will be screened and enrolled. In the main study, up to 66 children will be screened for study entry and enrolled until the goal of 40 completed subjects, 20 for each group, is met. Children will be recruited from two sites, the Hatfield Clinical Center at the NIH in Maryland and Children's National Medical Center in Washington, DC; however, testing will be performed exclusively at the NIH.

We have chosen to pilot a randomized, double blind, placebo controlled trial in order to minimize the opportunity for bias in our study and maximize the interpretation of our results. We used a cross-over design to help eliminate potential of temporal factors related to giving niacin or placebo first. We consider this study a pilot to examine new methodology for growth hormone testing in obese children, and thus we did not choose to start with an unblinded smaller study first. We also did not want to expose subjects to the risks, however minimal, of a medication not approved for children under age 16y that requires IND filing without powering the study for the possibility of finding significant results.

Objectives:

We propose to determine the effects of lipolytic inhibition on GH secretion in obese children as compared to the effects of such inhibition on GH secretion in short healthy children who are undergoing growth hormone stimulation testing. We plan to use our findings to better elucidate the

pathophysiology underlying diminished GH secretion in obesity as well as to pilot an improved testing of GH deficiency in overweight children.

Specific Hypotheses:

In the course of this study we will test the following hypotheses:

- Inhibition of lipolysis by niacin will lead to a decrease in plasma FFA in obese and control children without inducing significant adverse effects.
- In the control group of short children (that are not found to be growth hormone deficient) peak stimulated GH concentration will be significantly greater than that observed in obese children. GH secretion of non-obese short children will be significantly augmented by niacin treatment.
- In obese children, peak stimulated GH concentration will be blunted after placebo but with niacin these measurements will increase significantly to achieve values similar to those seen in the non-GH-deficient control subjects after placebo.

Study Design and Methods:

Inclusion Criteria:

Subjects will qualify for the overweight group (for the dose-establishing studies 1 and 2 and main study) if they meet the following criteria:

1. Good general health.
2. Age ≥ 7 and < 15 years.
3. Tanner stage I, II, or III for the breast among girls and testes < 10 mL for boys based upon an examination by a trained physician or nurse practitioner.
4. Weight > 30 kg.
5. Fasting plasma glucose < 100 mg/dL, 2 hour post-dextrose glucose < 140 mg/dL, and HgbA1C $\leq 6.4\%$.

6. Females who are age 10 or greater must have a negative pregnancy test.
7. Body mass index \geq 95th percentile determined by Centers for Disease Control age and sex specific data (given that most pathology of obesity does not usually emerge until children cross the 95th percentile).
8. No evidence of growth failure as defined as height $>$ 5th percentile.

Subjects will qualify for the non-overweight control group (for the main study only) if they meet the following criteria:

1. Recommended by a pediatric endocrinologist to undergo GH stimulation testing to establish the diagnosis of GH-deficiency.
2. Good general health.
3. Age \geq 7 and $<$ 15 years.
4. Tanner stage I, II, or III for the breast among girls and testes $<$ 10 mL for boys based upon an examination by a trained physician or nurse practitioner.
5. Weight $>$ 30 kg.
6. Fasting plasma glucose $<$ 100 mg/dL, 2 hour post-dextrose glucose $<$ 140 mg/dL, and HgbA1C \leq 6.4%.
7. Females who are age 10 or greater must have a negative pregnancy test.
8. Height $<$ 5th percentile.
9. BMI between the 5th and 85th percentiles determined by Centers for Disease Control age and sex specific data.
10. Birth weight and length not consistent with small for gestational age (SGA) criteria or a history of intrauterine growth restriction (IUGR) based on recall history.

Exclusion criteria (for the dose-establishing studies 1 and 2 and the main study):

Subjects will be excluded if they have any of the following:

1. Baseline creatinine \geq 1.0 mg/dl.

2. Significant cardiac or pulmonary disease likely to or resulting in hypoxia or decreased perfusion.
3. Hepatic disease with elevated liver function tests (ALT or AST) ≥ 1.5 the upper limits of normal.
4. Pregnancy.
5. Evidence for impaired glucose tolerance or Type 2 diabetes, including fasting plasma glucose ≥ 100 mg/dL, 2 hour post-dextrose glucose ≥ 140 mg/dL, or HgbA1C $> 6.4\%$.
6. Presence of other endocrinologic disorders leading to obesity (e.g. Cushing Syndrome).
7. Any disorder that is known to affect GH secretion (e.g. untreated hypothyroidism) or use of any medication known to affect GH levels (including glucocorticoids and GH itself).
8. Any other disorder that is known to affect stature including skeletal dysplasias.
9. Recent use (within two years) of anorexiants, stimulant medications, or other medications felt to impact growth.
10. Individuals who have, or whose parent or guardians have, current substance abuse or a psychiatric disorder or other condition that, in the opinion of the investigators, would impede competence or compliance or possibly hinder completion of the study.
11. Individuals receiving medical treatment other than diet for hypertension or dyslipidemia.
12. Individuals with evidence of precocious puberty as defined as palpable breast tissue noted in females before the age of 7, testicular size ≥ 4 cc in males before the age of 9, or bone age advancement more than 2 SD for chronologic age.
13. Individuals receiving androgen or estrogen hormone therapy.

Each subject will receive a written explanation of the purposes, procedures, and potential hazards of the study. Communication of this information and of the subject's assent as well as the consent of the parent or guardian will be documented in the medical record. All subjects will be

informed of their right to withdraw from the study. Prior to blood draws subjects will be offered application of ELA Max cream to the surface of the skin for a minimum of one-half hour, to anesthetize the skin surface.

Procedures of the study:

Initial Screening Evaluation for the dose-establishing studies 1 and 2 and the main study:

Performed in the outpatient clinic. Study subjects will be screened at the Clinical Center in Bethesda.

1. **Protocol review and signing of consent and assent forms.** All study procedures will be discussed and study risks and benefits explained.
2. **Complete medical history and physical exam** with Tanner pubertal staging, weight (in kg), and height (in cm) by stadiometer (3 repeated measures).
3. **Fasting Blood Work** including fasting insulin and glucose concentrations, and fasting lipid profile to include total cholesterol, LDL, HDL, and triglycerides.
4. **Additional blood tests** for complete blood count, HgbA1C, electrolytes, renal, mineral, and hepatic profiles, thyroid functions, gonadotropins, testosterone and SHBG, IGF-1, IGF binding protein 3 (IGFBP-3), sedimentation rate, total IgA, tissue transglutaminase (TTG) IgA, iron, vitamin D, urinalysis, and research samples to be saved (10 mL) for future genetic analyses of genes important for obesity and/or GH deficiency. Urine pregnancy test for all females age 10 and over.
5. **Glucose tolerance test:** Following the fasting blood work, an oral dextrose solution (75 g per 300 mL) will be administered in a dose of 1.75 g of dextrose per kg of body weight up to a maximum dose of 75 g. Two hours after this administration blood glucose and insulin levels will be drawn.
6. **Bone age X-ray** as part of the evaluation for pubertal development. The bone age is considered a more objective measure of pubertal staging, especially in obese children where the ability to

determine sexual maturation by physical exam can be compromised. The bone age is also useful for secondary analysis, including correlation of bone age, pubertal staging, growth hormone, and final height predictions. (This evaluation will not be performed in the dose-establishing study.)

7. **Hyperphagia questionnaire.** A hyperphagia questionnaire¹⁰⁶ that was originally designed for and validated in children and adults with PWS will be administered to the caregiver of all child subjects and any adult subjects who still reside with a caregiver to assess hyperphagic behavior, drive, and severity (see Appendix E).

Dose-Establishing Study 1

Only subjects meeting the inclusion and exclusion criteria during the screening visit will be evaluated. For dose-establishing study 1, we tested 2 subjects with the 250 mg niacin dose given every 2 hours. Since we did not see appropriate suppression of FFA concentrations (<200 uEq/L), we increased the dose to 500 mg every 2 hours and tested five more subjects. Since we did not see consistent suppression of FFA below 200 uEq/L with 500 mg every 2 hours, we will conduct another dose-establishing study lasting four hours, where niacin will be administered hourly (4 doses of 500 mg – see below). We will update the IRB on the results of dose-establishing study 2 before we initiate the main study.

The dose-establishing study visit 1 occurred within two months of screening visit to ensure consistency of physical exam and medical history throughout the study period. Patients were admitted the night before the first day of testing. Additionally, literature suggests that several vitamins influence growth hormone secretion;^{107,108 109,110,111,112} thus, if applicable, vitamin supplements were discontinued for three days prior to admission. All subjects were admitted to the NIH Clinical Center for an evaluation that included:

1. **Medical History and Physical Examination** with Tanner pubertal staging and anthropometric measurements including weight (in kg) and height (in cm) by stadiometer (3 repeated measures).
2. **Placement of an intravenous line** to be used for medication administration and blood sampling over a 24 hour time period.
3. **Repeat urine pregnancy test** in girls age 10 and older.
4. **Overnight Fast** beginning at 10 pm and ending after completion of testing the following day.
5. **Administration of niacin:** For dose-establishing study 1, niacin was given at 6am, 8am, and 10am. Each dose was either 250 mg or 500 mg as described above.
6. **Frequent blood sampling** from an indwelling intravenous line just prior to the first dose of niacin and then every 30 minutes for the next six hours. After completion of this sampling, the subjects were given an ad lib diet. Blood samples were analyzed for GHRH, somatostatin, GH, FFA, glucose (via bedside glucometer), and insulin concentrations. FFA assays were performed using the WAKO NEFA C diagnostic kit.
7. **Discharge:** Subjects were discharged following the completion of the frequent blood sampling.

Dose-Establishing study 2

The dose-establishing visit for dose-establishing study 2 will occur within two months of screening visit to ensure consistency of physical exam and medical history throughout the study period. Patients will be admitted the night before the first day of testing. Additionally, literature suggests that several vitamins influence growth hormone secretion;¹⁰⁷⁻¹¹² thus, if applicable, vitamin supplements will be discontinued for three days prior to admission. All subjects will be admitted to the NIH Clinical Center for an evaluation that will include:

8. **Medical History and Physical Examination** with Tanner pubertal staging and anthropometric measurements including weight (in kg) and height (in cm) by stadiometer (3 repeated measures).
9. **Repeat urine pregnancy test** in girls age 10 and older.
10. **Overnight Fast** beginning at 10 pm and ending after completion of testing the following day.
11. **Placement of an intravenous line** to be used for medication administration and blood sampling over a 5 hour time period.
12. **Administration of niacin** at 7:30am, 8:30am, 9:30am, and 10:30am. Each dose will be 500 mg.
13. **Frequent blood sampling** from an indwelling intravenous line just prior to the first dose of niacin and then every 30 minutes for the next four hours. After completion of this sampling, the subject will be given an ad lib diet. Blood samples will be analyzed for GHRH, Somatostatin, GH, FFA, glucose (via bedside glucometer), and insulin concentrations. GHRH and Somatostatin will be sampled once per hour. Based on our first dose-establishing study, we believe that hourly samples for these two hormones will be sufficient to measure the changes that these hormones undergo during testing.
14. **Discharge:** Subjects will be discharged following the completion of the frequent blood sampling.

Main Study: Randomization

Only subjects meeting the inclusion and exclusion criteria during the screening visit will be randomized. Subjects will be assigned to receive placebo x 4 doses on the first day of GH stimulation testing and niacin 500 mg x 4 doses on the second day or they will be assigned to receive niacin 500 mg x 4 doses on the first day and placebo x 4 doses on the second day. Subjects

will be stratified by group and sex in blocks of 4 such that roughly equal numbers of subjects in each stratum will be assigned to each placebo and active medication administration order.

Additionally randomization of obese subjects will be stratified by BMI (95-99th percentile or >99th percentile).

Main Study: Inpatient evaluation:

Only subjects meeting the inclusion and exclusion criteria during the screening visit will be evaluated as inpatients. A total of 40 subjects in the two main groups will complete inpatient evaluation, although up to 50 may initiate inpatient evaluation in order to achieve full enrollment. The inpatient visit will occur within two months of screening visit to ensure consistency of physical exam and medical history throughout the study period. Patients will be admitted the night before the first day of testing. Additionally, literature suggests that several vitamins influence growth hormone secretion;¹⁰⁷⁻¹¹² thus, if applicable, vitamin supplements will be discontinued for three days prior to admission. All subjects will be admitted to the NIH Clinical Center for an evaluation that will include:

1. **Medical History and Physical Examination** with Tanner pubertal staging and anthropometric measurements including weight (in kg) and height (in cm) by stadiometer (3 repeated measures).
2. **DXA scanning** for determination of lean body mass and body fat mass.
3. **Placement of an intravenous line** to be used for medication administration and blood sampling over a 48 hour time period.
4. **Repeat urine pregnancy test** in girls age 10 and older.
5. **Overnight Fast** beginning at 10 pm on both nights; ending after completion of testing each day.
6. **Growth Hormone Stimulation testing** consisting of arginine (500 mg/kg IV up to maximum of 30 g, infused over 30 min) at 8:30am, followed by L-dopa (500 mg by mouth)

at 9:30am. A dose of niacin (dose determine in the dose-establishing study) or placebo will be administered by mouth at 7:30am, 8:30am, 9:30am, and 10:30am . The study will be repeated the next day in the same fashion. The order of niacin 500 mg or placebo will be randomized. Blood will be sampled from an indwelling intravenous line at times -60, -30, 0, +30, +45, +60 minutes of arginine infusion for measurements of GH, free fatty acids, glucose (via bedside glucometer), and insulin concentrations. Blood will also be sampled at times -30, 0, +30, +45, +60, +75, +90, and +120 minutes of L-dopa administration for the same serum concentrations. GHRH and Somatostatin will be sampled hourly at 7:30am, 8:30am, 9:30am, 10:30am, and 11:30am, with two additional measurements at +30 minutes (9am) of arginine infusion and +30 minutes (10am) of L-dopa administration. After completion of this sampling, the subject will be given an ad lib diet.

7. **Discharge:** Subjects will be discharged following the growth hormone stimulation test on the second day.

Monitoring subjects and criteria for withdrawal of subjects from study:

The types, frequency, and duration of tests and visits are outlined on the flow diagrams at the end of this protocol document (Appendices B and C). Subjects will be examined at each of the two visits, which will be completed within a 2 month time period. If unanticipated toxicities are uncovered during the study, we will report these promptly to the IRB and FDA.

Subjects will be informed that they may withdraw from the study at any time.

Noncompliance with study procedures may lead to withdrawal of study subjects. Other events that will result in discontinuation of treatment include:

1. Inability to tolerate drug secondary to perceived or observed side effects.
2. Pregnancy.
3. Any verified abnormalities in laboratory values or physical status that cannot be explained by an intercurrent unrelated illness (or by obesity and insulin resistance itself)

that are considered WHO grade 2 or greater for gastrointestinal, hematologic, clinical hemorrhage, kidney/bladder, alopecia, pulmonary, cardiac, neurocerebellar, allergy, flu-like symptoms, metabolic, or eye toxicities; and grade 3 or greater for infection, anorexia, circulatory, neurologic, dermatologic, coagulation, endocrine, or performance status toxicities.¹¹³

Adverse event reporting:

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), 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. 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. Not 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.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

The PI will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs and report them to the Sponsor according to the timetable for reporting specified in the protocol (21 CFR 312.64(b)).

A post test questionnaire will be administered after the inpatient admission in the main study, and the questionnaire will also be administered every hour from 6am to Noon during the inpatient

admission in the dose-establishing study. A sample questionnaire is attached to the end of this document as Appendix D.

Waiver of Reporting to the IRB of anticipated minor protocol deviations, adverse events and deaths due to underlying disease or population under study unless determined to be an Unanticipated Problem

- For waiver of Reporting to the IRB of anticipated minor protocol deviations

The following anticipated minor deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than that which is anticipated to occur:

Problem	Expected Frequency
Rescheduled appointment for protocol visit	50%
Inability to obtain intravenous access after 2 attempts	25%
Venous sample at a time point not obtained	25%
Hemolysis or other issue preventing analysis of a blood or urine sample	25%
A research subject was enrolled but did not meet eligibility criteria	5%
A research subject met withdrawal criteria but was not withdrawn	5%
Subject had to make an additional visit for lab work (e.g. arrived too late for bloodwork, specimen result hemolyzed/equivocal, etc.)	30%
Inadvertent loss of samples or data	10%
Inadvertent collection of additional noninvasive data (e.g. extra survey)	5%
Appointment conducted >2 weeks beyond anticipated date	10%

Participant did not take all medication doses as prescribed	10%
Lost medication	5%

If the rate of these events exceeds the rate specified by the protocol, the events will be classified and reported as though they are Unanticipated Problems.

- **For waiver of Reporting to the IRB of anticipated adverse events:**

The following anticipated non-UP adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in this population:

Problem	Expected Frequency
Pain during intravenous catheter insertion	60%
Bruising, fainting or lightheadedness after a blood draw	20%
Red or white skin reaction to ELA Max numbing cream	100%
Phlebitis after intravenous catheter	20% (1 subject)
Occurrence of adverse response to niacin administration	See Table 2 for time point based frequencies

- ***For waiver of Reporting to the IRB of anticipated adverse events on an FDA-regulated trial:***

The following anticipated adverse events will not be reported to the IRB unless they occur at a rate or severity greater than that known to be associated with Niacin. Examples of expected adverse events include but are not limited to those events detailed in the FDA-approved package insert for niacin and in the protocol’s risk section. The waivers that apply here are:

Table 2: Time Point Based Expected Adverse Reactions During Niacin Administration

	Time (hours after Niacin administration)
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Expected adverse reactions (%)	1	2	3	4	5	6
Headache	20	0	0	0	0	0
Flushing	100	100	100	50	50	0
Feeling warm	100	100	100	60	20	0
Nausea	20	20	0	0	0	0
Vomiting	20	20	0	0	0	0
Stomach Ache	20	20	0	0	0	0
Diarrhea	20	0	0	0	0	0
Tingling	20	20	0	0	0	0
Light-headedness	20	0	0	0	0	0
Itchiness	100	100	60	40	0	0
Rashes	20	20	20	20	0	0

If the rate or severity of these events exceeds the rate or severity anticipated in the protocol or FDA-approved package insert, the events will be classified and reported as though they are Unanticipated Problems.

Because we anticipate that other mild symptoms may occur in the course of participation in the protocol that are unrelated to study drug or participation, we will not submit Unanticipated Problems for mild signs/symptoms (CTCAE Grade ≤ 2), such as rhinorrhea, nasal congestion, watery eyes, cough, lightheadedness, fever blister, etc., that occur infrequently ($\leq 10\%$). Such events will be reported at the time of the IRB Annual Review.

Data safety monitoring board (DSMB): A DSMB will be assembled for oversight of this protocol by the Clinical Director, NICHD, with membership and scope determined by applicable institutional guidelines. The DSMB will meet to evaluate all serious adverse events and will at a minimum convene yearly. The DSMB will examine whether there is evidence of safety for niacin administration. The DSMB will review adverse event questionnaire data, and reports of side effects from all visits. These reports will also be supplemented by a manual review by the investigators, who will add any other events not captured by these systems. Results will be presented as an A vs. B analysis prepared by the data manager. The DSMB will be asked to prepare a detailed report to

supply to the IRB that describes any adverse events discussed, and that explains the DSMB's vote to allow the study to continue or recommendation to end the study.

Data Analysis

Primary research outcome will be niacin-induced changes in arginine and L-dopa stimulated GH concentrations, measured as both area under the curve and peak values, in the overweight group compared with the control group. Secondary research outcomes will include changes in free fatty acid, glucose, and insulin concentrations during the GH stimulation tests. Since non-completion of the study is expected to be due to technical failures (e.g., nonfunctioning IVs) rather than subject factors, all analyses will be limited to subjects who complete both placebo and niacin studies. We do expect that some members of the control group will not reach a peak GH concentration of 7 ng/mL during the stimulation testing. We will report all control subjects in the final analyses. Analyses will be performed with age, Tanner stage, and initial BMI-SDS as covariates. Furthermore, tertiary analyses will also be performed to compare subjects in the Tanner I, Tanner II, and Tanner III groups to determine if any differences exist among these pubertal development stages. This analysis will be for exploratory purposes only; the study is not powered for this subgroup analysis. Additionally if any members of the control group have abnormally elevated lipid levels, we will specifically examine these subjects and their growth hormone concentrations. The primary outcome analysis will therefore be a repeated measures ANCOVA with subject group (obese and control) as the between factor, treatment (niacin or placebo) as the repeated measure, GH concentration (peak and AUC) as the outcome variables, and age, sex, and Tanner pubertal stage as covariates. We will also model the subject group by treatment interaction but no other interaction terms will be entered into the primary analysis.

Determination of Sample Size

Using data from the available studies that used acipimox, we performed power calculations based on the difference in effect on peak GH secretion found with and without use of acipimox in

obese adults. Following acipimox, this cohort showed an increase of 10.3 micrograms per liter in peak GH concentrations with a standard deviation of 6.1. Thus, at a power of .80 and an alpha of .05, the sample size necessary to detect a 10 microgram per liter difference in GH secretion is 14 in each group (e.g. obese versus control), and at a power of .95 and an alpha of .05, the sample size necessary to detect a 10 microgram per liter difference in GH secretion is 22 in each group. Given these calculations, we will aim for 20 subjects per group who complete all aspects of the study.

Allowing for 20% of subjects to fail to complete the main study for various reasons, the total number of subjects needed to be studied is 50 (25 in each group). We expect that we will need to screen no more than 66 children to find the 50 who meet the inclusion and exclusion criteria. Subjects will be randomized with the assistance of the NIH Investigational Drug Management and Research Section (IDMS); we anticipate sex and race/ethnicity to be similar in each study arm. For the pilot dose-finding studies, we will study no more than 5 per group (15 total) and screen at most an additional 10 subjects who do not qualify for these studies.

Pharmaceutical information

Packaging and Labeling:

Niacin will be purchased from Upsher-Smith Laboratories, Inc, as 500 mg tablets, USP, that have been manufactured according to GMP. Each table contains 500 mg of nicotinic acid. In addition, each tablet contains the following inactive ingredients: croscarmellose sodium, hydrogenated vegetable oil, magnesium stearate and microcrystalline cellulose. From 2011-2016, niacin, and the niacin placebo were packaged, supplied, and distributed to the inpatient staff by the NIH pharmaceutical development section. The study was placed on hold when use of all PDS-prepared compounds was quarantined in December 2016. Afterwards, starting in mid- 2017, all study compounds will be prepared following the same procedures by Pine Pharmaceuticals. The niacin study medication will be packaged as 250 mg capsules using a Swedish orange/caramel

opaque #1 capsule. The same capsule components used for the niacin will be used for the placebo.

From 2011-2016 PDS, and subsequently, Pine Pharmaceuticals, supplies inert placebo capsules that match the weight of the unlabeled niacin capsules. Pine Pharmaceuticals will perform bioburden testing per the United States Pharmacopeia (USP) <61> and <62> standards. Purity and stability testing will be performed by the NIH Investigational Drug Management and Research Section. Labels for medication will be computer generated by the NIH CRIS system.

Randomization

For randomizing order of administration of niacin and placebo, investigators will assign consecutive code numbers to participants from prespecified lists stratified by group, sex, and BMI (95-99th percentile or >99th percentile). The NIH Clinical Center Investigational Drug Management and Research Section will use permuted blocks with stratification to generate the allocations that translate code numbers into study group assignments by using a pseudo-random number program. Pharmacy personnel not otherwise involved with the conduct of the study will dispense study capsules in containers that appear identical and differ only by participant code number. No participant, investigator, or other medical or nursing staff interacting with participants will be aware of study group assignments during the trial.

Arginine hydrochloride 10% solution is approved by the FDA for growth hormone testing. This agent is available through the NIH pharmacy and is on the formulary. It will be used at a dose of 500 mg/kg IV up to maximum of 30g infused over 30 min on two consecutive days. This dosing corresponds to 5 mL/kg, with a maximum dose of 300 mL on two consecutive days.

L-dopa is not approved by the FDA for growth hormone testing, but is approved for other uses. L-dopa has been used clinically for many years for the purpose of growth hormone testing. L-dopa powder will be obtained from Spectrum Chemicals, which has supplied us with a Certificate of GMP. From 2011-2016, L-dopa was packaged, supplied, and distributed to the inpatient staff by the NIH pharmaceutical development section as 250 mg capsules. The analytical group of

Pharmaceutical Development Service conducted testing according to USP standards for medications used in this protocol prior to release for human consumption. Starting in mid-2017, Pine Pharmaceuticals will compound the 250 mg capsules and perform bioburden testing per the United States Pharmacopeia (USP) <61> and <62> standards. Purity and stability testing will be performed by the NIH Investigational Drug Management and Research Section, which will track and dispense all medications to the inpatient staff.

Unblinding Procedure

In this trial, all participants, Study Site staff, and pathology and laboratory personnel are blinded to the individual assignment of the order in which niacin and placebo are administered. Participants will learn their individual assignments (i.e., be unblinded) when the trial is complete. In rare instances, it may be necessary to unblind a participant's order of assignment before completion of the trial, to a physician, to the Study Site, or to the participant.

In general, however, participants should not be unblinded. Anytime a staff member learns the treatment assignment of a participant, the staff member is at risk of being influenced by that information. For example, if the unblinded participant was known to have taken the active agent and reported a toxicity, the staff member might make inferences about the effects of niacin on that participant, and possibly on other participants as well. The management of future participants may be influenced by this information. Maintaining the double-blind design offers the best protection against this potential bias.

In the unlikely event that a Serious and Unexpected Suspected Adverse Reaction (SUSAR) occurs and a physician must be unblinded to the treatment group to which an affected trial subject belongs, the Investigational Drug Management and Research Section (IDMS) will be contacted by a study investigator (usually by the Principal Investigator). The study investigator will request that IDMS release the needed information and specify the individual to receive the information. IDMS will normally supply unblinding information only to a physician who is not a co-investigator on the

study and make clear that group assignment must not be given to study investigators. This procedure is intended to ensure that the identity of the investigational medicinal product is revealed only insofar as is necessary. As much as possible, the blinding should be maintained for the investigators and for anyone responsible for data analysis or interpretation. A subject's treatment group should not be revealed to a study investigator except in a medical emergency, i.e. when this appears necessary to ensure the subject's safety and would be instrumental in further treatment decisions. If a subject's treatment is unblinded, this must be documented by IDMS in their written records. In all cases, the reasons and rationale for unblinding will be also documented in writing and maintained in the study file.

Unblinded data are also available to the Data Safety Monitoring Committee (DSMC). In the case of a SUSAR, the DSMC is informed and will thus have the opportunity to review subject-level data and make any changes to the experimental plan that are required. In the case of a SUSAR, the DSMC may elect to reveal the treatment group to which a subject belongs, request additional safeguards, or stop the study. The DSMC will assist investigators in deciding if a SUSAR requires any of the study investigators to become unblinded so that the FDA can be informed of subject assignment because of a SUSAR.

Study Administration and Data Collection, Management, and Storage

The study will be conducted by the research team as listed above. Human biospecimens will be collected using procedures appropriate for the type of biospecimen being collected and will be handled in accordance with the U.S. Occupational Safety and Health Administration's Bloodborne Pathogens Standard for Samples. The principal investigator and the co-investigators will have access to identifiable participant records. Data will be coded by subject number, but will be readily associated with identifiable participant records. Records containing personal identifiers will be maintained consistent with the security measures required by the NIH. Data will either be transferred directly from the electronic medical record into the NICHD Clinical Trials Data Base (CTDB), or for data where direct transfer is unavailable, will be double-entered by hand into

CTDB. All data will be kept on secure computers in the SGO office, whose doors will be locked when staff is not present. All paper data will also be kept in locked areas in the SGO office. Serum and Plasma samples not analyzed immediately (as described above) will be stored in locked freezers by the NICHD sample management team supervised by the NICHD Clinical Director and tracked/managed through CTDB. Human biospecimens in storage will have a unique identifier, will be labeled with a printed label and will contain a barcode. Samples will be tracked using a computer-based inventory system that records the location and detailed information of every specimen in the repository.

Sample freezers will be operated using facility environments that include ambient temperature controls, good air circulation, lighting, and security. Systems are in place to allow for local and remote temperature monitoring of freezers. The NIH has an emergency preparedness plan that covers equipment failures and power interruption, including back-up storage capacity and back-up power generators.

Parents of participants give explicit permission regarding sample use in the consent form of the study, indicating if samples may be used for future experimental testing related to growth hormone response, body weight and metabolism without additional consent.

At the conclusion of the study, samples will be retained for future experimental testing related to growth hormone response, body weight and metabolism.

The PI will inform the IRB if samples are lost or destroyed before they are assayed for free fatty acids and growth hormone. Loss or destruction of samples saved only for future unspecified research will not be routinely reported, unless such loss also entails a breach of the Privacy Act.

Protection of Participants' Privacy and Confidentiality

We will follow all relevant NIH policies and HRPP SOPs. Samples and data will be coded. Only individuals involved in consenting and patient care will have access to identifiers. Records

containing personal identifiers will be maintained consistent with the security measures required by the NIH. Access to the code will be limited to the study investigators. Electronic data will be password protected and accessible only by authorized study personnel. Electronic data will be backed up daily and stored at a secure server. All hardcopy data will be locked in Dr. Yanovski's storage cabinets in research offices at the NIH Clinical Research Center and will be accessible only by authorized study personnel. All data will be collected specifically for the purpose of the current proposed research project. Any email communications that involve PII will be sent using encryption or the secure email system of the NIH. Since even when precautions are taken to ensure participant confidentiality, data collection in any study is accompanied by threat to privacy and confidentiality, in the unlikely event of a potential breach of confidentiality, the PI will immediately inform the IRB.

Limitations to the Study Design

We have identified several possible limitations to our study, including:

- 1) Given that the GH stimulation tests are being performed on consecutive days, it is possible that effects of the first day's testing could carry-over to the second day and affect the results. However, many studies in the adult literature have used similar study designs. Furthermore, Cavallo et al¹¹⁴ showed no difference in response to GH stimulation testing on two consecutive days. Additionally, by randomizing the order of placebo or niacin given during the stimulation tests, we believe any effects of order will be detected.
- 2) Niacin is known to cause flushing shortly after administration. We anticipate that this side effect may conceivably lead the subjects and investigators to become aware of which day the niacin and placebo capsules were administered. We do not believe the blinding is critical to the success of the study given that we are only measuring objective hormonal data.

Human Subject Protections:

a) Rationale for research subject selection:

This is a study to examine the effect of niacin on GH secretion in pediatric obesity and in short stature. School-age children are chosen as the study group because of the importance of GH on normal growth and development of childhood. Children of various racial/ethnic groups will be recruited to ensure that its possible beneficial effect is applicable across racial and ethnic subgroups. The mechanism by which obesity changes GH secretion is important as a guide to appropriately diagnosing and treating GH deficiency in children. The impact of niacin on GH secretion in both groups will aid in development of improved GH provocative tests among children.

b) Strategies/procedures for recruitment:

Participants will be recruited by sending letters to appropriate-aged children in the Montgomery County, Fairfax County, and Washington, DC school systems, by advertisements in local papers, and by referral from physicians who see obese children and short children.

c) Justifications for exclusions:

All exclusions are either based on identification of problems in the subject's physical or mental health, or based on the ability of the subject to complete protocol studies or are required for the goals of the study. We have chosen to study only prepubertal and early pubertal children for several reasons. First, we are aware of only one study of prepubertal children, which did not include obese children, and did not show any increased response to l-dopa stimulation in the presence of acipimox.⁸⁶ Thus, we feel that the prepubertal population has not been sufficiently studied. Secondly, most growth evaluation is performed when children are prepubertal or in early puberty, creating a need to understand this physiology carefully. We aim to pilot niacin as a supplemental part of growth hormone testing in obese children in order to improve the sensitivity of the growth hormone stimulation test in this population. Developing a test for the prepubertal or early pubertal population is particularly useful. We included early pubertal subjects (Tanner stage II and Tanner

stage III) because the growth hormone concentrations in response to secretagogues do not vary significantly from prepubertal levels⁹⁶ and are not different between males and females in prepuberty or early puberty.¹¹⁵

d) Risks/Benefits Analysis

i. Risks and Discomforts:

1. **Physical Examination** by a health care provider will be performed with the child unclothed. Although appropriate measures will be taken to protect privacy, some children may find this embarrassing.
2. **Total blood withdrawal** will remain within the NIH guideline of 5mL/kg/day and up to 9.5mL/kg every 6 weeks. Blood collection by venipuncture is associated with mild discomfort, which will be attenuated by offering administration of ELA Max cream before venipunctures, and the possibility of local bruising and extravasation. The risk of infection or fainting is extremely small.
3. **Dextrose solution** administration is without known risks but may cause mild feelings of nausea.
4. **Bone age X-ray** is without known risks. The total radiation exposure to the left hand and wrist for a volunteer undergoing bone age X-ray is 20 millirem. **Maximum total radiation exposure** is estimated to be 0.007 millirem per year, which is well below the recommended radiation exposure for child volunteers (no more than 500 millirem in one year). This protocol has been approved by the NIH Radiation Safety Committee.
5. **DXA scanning** involves passing a collimated X-ray beam of 45 and 105 keV through the subject and collecting the X-rays that pass through the patient with a standard X-ray detector. Because the delivery and attenuation of the X-rays are measured separately for the two principal energies, the procedure allows the differentiation of substances in their path if the attenuation coefficients of these substances are known. The procedure requires that the

child lie still for 15-20 minutes on a padded table that is part of the instrument. According to the manufacturer's specifications, DXA scanning for body composition delivers no more than 1 millirem total body radiation. Volunteers undergo DXA scanning in the Nuclear Medicine Department using the Hologic QDR4500A machine. To perform the DXA scan, the subject will be placed between the collimated X-ray source and the detector. This is achieved by having the subject lie down on a cushioned table. The X-ray source and detector are contained in a C-arm construction that is moved to cover the patient. At all times the child can see, and be seen by, a person standing nearby. A parent may safely be in the room and be in visual and auditory contact with the child. The subject is also in constant voice contact with the operating personnel. Fat, bone, and muscle in many body regions will be determined during a total body scan. **Maximum total radiation exposure** is estimated to be 0.02 millirem per year, which is well below the recommended radiation exposure for child volunteers (no more than 500 millirem in one year). This protocol has been approved by the NIH Radiation Safety Committee.

6. **Niacin:** We successfully applied for FDA approval for the use of niacin in children under an investigational new drug application (IND) with Jack Yanovski, MD, PhD as sponsor. Niacin is currently FDA-approved in the United States, but only for adults. Niacin has been used off-label to treat hyperlipidemia in children.⁹⁷ A retrospective chart review of 21 children, age 4 to 14, found one subject who developed a febrile illness with elevation in serum aminotransferase who was discontinued off niacin after two months. Reversible adverse effects of niacin included flushing (71%), itching (19%), abdominal pain (14%), nausea (14%), vomiting (14%), headache (14%), and constipation (5%). 29% also demonstrated reversible dose-related elevations in AST concentrations. Niacin is known to cause transient skin flushing likely due to prostacyclin formation leading to cutaneous blood flow.¹¹⁶ In some trials testing GH secretion in adults, flushing has been observed after niacin

with rapid resolution.^{66,67,73,117,118} In the event of intolerable flushing as reported subjectively by the patient, we will administer a single dose of 10 mg/kg of ibuprofen to help alleviate the reaction. Nausea, vomiting, and temporary tightness of the chest have also been noted in some subjects.^{66,67,117} No other side effects have been noted in these studies; however, in uses of niacin to treat hyperlipidemia, the following other side effects have infrequently been reported: atrial arrhythmias,¹¹⁹ dental pain,¹²⁰ liver injury,^{94,121-123} myalgia,¹²⁴ myopathy,^{94,95} skin rashes,⁹⁵ peptic ulcers,⁹⁵ gastrointestinal bleeding as well as bleeding from other sites,⁹⁵ diarrhea,⁹⁵ onset of diabetes and worsening of diabetic control,^{94,95,125-127} infections,^{93,95} blurred vision and macular degeneration.¹²⁸ Additionally the effects of niacin in pregnancy are unknown.

7. **L-dopa:** L-dopa is a precursor to dopamine that is normally synthesized in vivo from tyrosine in neurons. It has been used for GH stimulation testing for over thirty years, although its current use is primarily outside the United States at this time. L-dopa has been well-tolerated with side effects reported only as transient nausea, emesis, and headaches.¹²⁹⁻¹³¹ L-dopa is also used as chronic treatment for Parkinson disease, and more severe side effects are seen with this treatment regimen.
8. **Arginine** is a naturally-occurring amino acid used frequently in clinical settings for testing of GH secretion. Although generally well tolerated, there have been reports of thrombophlebitis,¹³² flushing and histamine release,¹³³ necrosis of the skin in one patient likely due to overly concentrated formulation,¹³⁴ release of GH, insulin, glucagon, and prolactin, nausea and vomiting, thrombocytopenia in one patient with unclear causal effect,¹³⁵ anaphylaxis in one patient,¹³⁶ numbness and headache,¹³⁵ and elevations in blood urea nitrogen and creatinine concentrations.¹³⁵ In patients with cirrhosis elevation in body temperature occurred.¹³⁷ Hyperkalemia has been observed in patients with diabetes, liver disease, and renal insufficiency,^{138,139} and hypophosphatemia has been seen in diabetic

patients.¹⁴⁰ Arginine is a category B in the FDA's pregnancy rating, meaning that no controlled pregnancy studies have been performed in humans.

ii. Benefits

There may be some direct benefit for the subjects involved from the medical evaluations each subject will undergo. Abnormal results will be communicated to the participants and their parents or guardians and will be made available to the primary care physician. Providing GH stimulation testing for children with short stature who require evaluation for GH deficiency may be a benefit for some participants, but since most will not have GH deficiency, most will not experience a direct benefit from this testing. Treatment will not routinely be provided for any condition noted during evaluation. We will refer participants to appropriate treatment programs at the conclusion of the study. Even if no individual benefit exists, the knowledge obtained will be important for understanding childhood growth and development.

iii. Assessment of Risk/Benefit Ratio

Overall, there are a number of potential benefits, not just to study participants but also to the larger population of children who are at risk for metabolic dysregulation due to obesity and short stature. Study participation will enable the participant to be screened for clinically-significant pathology, and in cases where identified, may facilitate treatment. The procedures for participants are described above. Taken together, we believe these represent a minor increment above minimal risk (detailed below in **Approval under 45-CFR-46, Subpart D 46.406**). The investigators have further taken many steps to minimize potential discomfort, for instance by administration of ELA max cream before blood is drawn. Children also routinely undergo venipuncture, which in children not infrequently requires multiple sticks to obtain samples. Furthermore, children are routinely required to complete questionnaires during school. We believe the studies outlined in this protocol are reasonably commensurate with those children may be exposed to in their actual or expected

medical, dental, psychological, social, or educational experiences. We therefore believe that the benefits to individual participants and to our understanding of short stature and obesity outweigh the minor increment above minimal risk.

iv. Alternatives to Participation

The current proposal is a physiological study that does not treat any known condition; as a result, there are no alternatives other than non-participation. Participants and their families will be informed that they will not receive treatment as a result of study participation. For participants interested in weight management, treatment referrals will be provided.

e) Compensation

Compensation will be offered to study participants in the amounts of \$60 for completing the screening visit, \$100 for completing the dose-establishing study's inpatient visit, and \$200 for completing the main study's inpatient visit. NIH guidelines indicate that compensation for all visits is appropriate and potentially unethical to not provide, and our compensation rates are consistent with these guidelines.

f) Participation of Children and Other Vulnerable Populations:

i. Approval under 45-CFR-46, Subpart D 46.406

We propose that this research project be approved under the provisions of 45-CFR-46, Subpart D 46.406. Research can be carried out under the provisions of 45-CFR-46, Subpart D, 46.406 when an IRB finds that more than minimal risk to children is presented by an intervention or procedure that does not hold out the prospect of direct benefit for the individual subject, or by a monitoring procedure which is not likely to contribute to the well-being of the subject only if the IRB finds that four conditions are met: (a) The risk represents a minor increase over minimal risk; (b) the intervention or procedure presents experiences to subjects that are reasonably commensurate

with those inherent in their actual or expected medical, dental, psychological, social, or educational experiences; (c) the intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition; and (d) adequate provisions are made for soliciting assent of the children and permission of their parents or guardians, as set forth in 46.408.

We believe this protocol clearly meets the criteria of 45-CFR-46, Subpart D46.406:

(a) The risk represents a minor increase over minimal risk

The protocol's procedures presently include the following: outpatient and inpatient visits, history and physical examination, anthropometric measurements, placement of intravenous lines, blood draws, administration of oral niacin and L-dopa and intravenous arginine, urine collections, bone age x-ray, and DXA scan. Each of these procedures, including intravenous infusions of arginine and oral administration of L-dopa, has been performed safely and without significant sequelae in pediatric studies. In the medical literature, there are reports of children, many of whom do not have other medical conditions, who have undergone each of these tests in IRB-approved studies.

Individually, many of the procedures proposed as part of this protocol may possibly be considered to represent minimal risk. For example, a panel convened in 1993 by the Secretary of Health found that placement of two intravenous catheters, administration of insulin and dextrose for the purpose of inducing mild hypoglycemia, and monitoring of blood glucose measurements every 5 minutes for many hours were of "minimal risk." The investigators have further taken many steps to minimize potential discomfort, for instance by administration of ELA max cream before blood is drawn. Given the large pediatric experience with each of the proposed procedures, we believe there can be little question, that the proposed procedures in the aggregate represent, at most, a minor increment over minimal risk.

(b) The intervention or procedure presents experiences to subjects that are reasonably commensurate with those inherent in their actual or expected medical, dental, psychological, social, or educational experiences

Among the procedures that are part of this protocol are: venipuncture, administration of ELA Max cream, oral and intravenous medication, removal of samples at frequent intervals from an intravenous line, temporary movement restriction for periods up to 20 minutes, and x-rays. Children are routinely exposed to vaccination injections that today often require multiple injections on a single day. Children also routinely undergo venipunctures, which in children not infrequently require multiple sticks to obtain samples. Dental procedures may entail: 1) administration of topical and injected medications (some of which can cause pain), 2) restriction of movement for significant periods of time, 3) discomfort from the manipulations, and 4) exposure to x-rays. Many children have orthodontic braces placed on their teeth, including night braces, which are worn for many hours and can be uncomfortable during the night. Given these facts, we believe the studies outlined in this protocol are reasonably commensurate with those children may be exposed to in their actual or expected medical, dental, psychological, social, or educational experiences.

(c) The intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition

There are two groups of children studied in this protocol: children who are obese and children with short stature who are being evaluated for GH deficiency. We believe both groups meet the definition of having a condition, about which the study will yield generalizable knowledge.

Childhood and adolescent obesity is an enormously important problem for the US. Such children are at immediate risk for a number of comorbid conditions associated with obesity.¹⁴¹ The immediate consequences include orthopedic (slipped capital femoral

epiphysis and Blount's disease), neurologic (pseudotumor cerebri), pulmonary (asthma and sleep disorders including sleep apnea), gastroenterologic (gallstones and steatohepatitis), endocrinologic (insulin resistance, type 2 diabetes, hyperandrogenemia, early puberty, abnormalities in GH secretion, and menstrual abnormalities), metabolic (dyslipidemia), and cardiovascular (elevated blood pressure) disorders. Rates of childhood hypertension, type 2 diabetes, hyperlipidemia, asthma, sleep apnea, and orthopedic disorders such as slipped capital femoral epiphysis are all climbing, in direct proportion with the increasing prevalence of obesity. Obese children are 9 times more likely to develop hypertension in adulthood, and 8 times more likely to have dyslipidemias that predispose them to heart disease. Children with excessive body weight are also at increased risk for cardiovascular and all-cause mortality in adulthood. There are also significant psychosocial and economic difficulties associated with pediatric obesity.¹⁴²⁻¹⁴⁵ Thus, there can be no question that obesity during childhood is a disorder.

Evaluation of height is an important aspect of the pediatric examination. Height, and more importantly growth, are general indicators of overall well-being and health in children. Chronic disease often manifests with poor growth velocity and short stature. Compromised growth may be the only clinical sign of an underlying disorder, including Turner syndrome, hypothyroidism, renal tubular acidosis, and celiac disease. Additionally, isolated partial GH deficiency may be found during a short stature evaluation. Thus, short stature is an indication for further testing, which often includes GH axis evaluation.¹ Short stature may also have behavioral effects. Short adults may have difficulty performing basic activities of daily living such as reaching the stove top or driving, and children show delay in visual-motor skills.¹⁴⁶ Psychological effects of short stature are not well established, but are often present in the cohort of children who are referred to pediatric endocrinology.¹⁴⁷ Given that short stature may represent an underlying medical problem, may lead to physical

impairment, and should prompt testing and possible endocrine referral, short stature is a pediatric disorder.

It is also clear that this protocol will yield generalizable data, since subject selection is not limited by race, ethnicity, or sex.

Because administration of arginine, l-dopa, and niacin are likely to be considered greater than minimal risk but without the prospect of direct benefit to individual subjects, a normal weight, normal height control group (i.e., a group with no medical condition) could be studied only under section 45-CFR-46 subpart D 46.407 (Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children). This regulation requires approval of the Secretary of DHHS following a determination that the research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of children.

(d) Adequate provisions are made for soliciting assent of the children and permission of their parents or guardians, as set forth in 46.408

Provision has been made for soliciting the assent of children and the permission of parents. The consent and assent forms for this study have been carefully reviewed. We believe they accurately portray the procedures being performed, and meet the requirements of 45-CFR-46. Consent is obtained by the principal investigator or associate investigators.

Because this study is proposed under Section 46.406 of 45CFR 46, Subpart D, namely greater than minimal risk with no prospect of direct benefit, but likely to yield generalizable knowledge about the subject's disorder or condition, both parents are required to provide permission for the minor's participation in the study unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child. When the second parent is not present to sign a

witnessed consent form, one parent will sign the consent form and explicit written permission will be obtained (whenever possible) from the other parent of the child before any study procedures are carried out. When only one parent is available to give permission for participation, this fact will be documented.

iii Consent/Assent Process:

Each participant will receive a written explanation of the purposes, procedures, and potential hazards of the study. Communication of this information and of the participant's assent as well as the consent of the parents or guardians will be documented in the medical record. Written, active informed consent will be obtained in-person during the Outpatient Screening Visit from parents/guardians, and assent will be obtained from youth by the lead associate investigator or designated associate investigators/key personnel. All participants will be informed of their right to withdraw from the study. The purpose of the project, all testing procedures, and study components, and possible risks and inconveniences will be described in detail, in language understandable to the parent(s)/guardian(s). The investigator will also explain the study to the minor who is of a younger age and level of understanding. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. When the NIH informed consent and assent forms are signed, a witness will sign the consent document to attest to the validity of the signature of the parent(s)/guardian(s). Parents or guardians will be given a signed copy of the consent and assent form for their records. In certain instances, the PI or designee will obtain informed consent by a witnessed telephone conversation with the parents/guardians of protocol-eligible subjects who cannot travel to the NIH Clinical Center for a considerable period of time. Consent may be obtained

via telephone and/or another electronic process, rather than in person. In such cases, the written signed consent will be faxed and/or mailed and made part of the patients' NIH records.

iv. Non-English–Speaking Participants

If a non-English speaking family is unexpectedly eligible for enrollment, the parent will be provided with the CC Short Written Consent Form for Non-English Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12 and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreter will interpret all oral communications (English to target language and conversely) between Investigator and Participant, facilitate discussions, and ensure understanding.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home. The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter.

We request prospective IRB approval of the use of the short form for up to a maximum of 5 separate encounters in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we may have that consent document translated into the given inherent language.

g) Conflicts of Interest

NIH guidelines on conflict of interest have been distributed to all investigators. There are no conflicts-of-interest to report for NIH or non-NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions.

h) Monitoring Plan

1. Purpose

The purpose of this Monitoring Plan is to describe the rationale and process for the collection, recording, and verification of data for NICHD Protocol #11-CH-0004

2. Objectives

- a. To establish a monitoring plan to ensure the protocol data are in compliance with Good Clinical Practice (GCP), NICHD Institutional Review Board (IRB) and Data Safety Monitoring Committee policies, and Federal regulations.
- b. To ensure the validity, accuracy and integrity of the data

3. Study Staff Responsibilities

Jack A. Yanovski, MD, PhD (the principal investigator) is responsible for all aspects of the study.

Delegation of responsibility will be documented on a study staff ***Signature and Delegation of Responsibility Log.***

STUDY STAFF NAME	TITLE	DELEGATED RESPONSIBILITIES
Jack Yanovski, MD, PhD	Co-investigator	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
*Delegated Study Tasks: 1. Obtain Informed Consent 2. Obtain Medical History 3. Perform Physical Exam 4. Assess Eligibility Criteria	5. Prescribe Study Drug/device 6. CRF Completion 7. CRF Queries 8. Query completion	9. Maintain Regulatory Docs 10. Maintain IRB documents 11. Data Monitoring 12. Safety Monitoring

Hope Decederfelt, of the NIH Investigational Drug Management and Research Section (IDMS), will maintain records of randomized study medication dispensed, maintain records of subject identification codes assigned, and have responsibility for releasing randomized

codes to investigators at the conclusion of the randomized medication phase. IDMS will also have responsibility for the management of niacin and placebo capsules manufactured for the study by IDMS, including the performance of stability testing of drug.

4. Source Documentation and Case Report Forms

Jack A. Yanovski, MD, PhD, is responsible for coordinating data collection and will review the data for accuracy and completeness within seven days of each subject visit.

The Sponsor (*Jack A. Yanovski, MD, PhD*) will conduct initial monitoring. Patient consent documents, primary outcome and safety laboratory results and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. As case report forms are entered electronically into the NICHD Clinical Trials Database, the computer system contains logs indicating changes made and the circumstances leading to these changes.

The NICHD Data Safety Monitoring Committee will also review data from this trial. An outside monitor (Amarex) will also review study data periodically as directed by the NICHD Clinical Director.

FDA regulatory requirements (annual reports, adverse events reporting, etc) related to IND # 111512 (Niacin + L-dopa + L-arginine) will also be monitored. The medical records of active subjects (defined as subjects receiving study medication) will be monitored quarterly or more frequently as required. The FDA issues will be monitored at least annually. Any major findings will be summarized in writing and reported to the NICHD Institutional Review Board, if indicated. Investigator credentials, training records, and the delegation of responsibility log will also be reviewed on an annual basis.

5. IRB and DSMC Documentation

All IRB documentation can be found in PTMS. The Principal Investigator, Jack A. Yanovski, MD, PhD, is responsible for maintaining IRB and DSMC correspondence related to this protocol, including records of all reviews of the study and submissions to the IRB and DSMC.

6. FDA Documentation

Jack A. Yanovski, MD, PhD is responsible for maintaining FDA correspondence, including forms 1571 and 1572 and other correspondence (e.g., annual reports, amendments, safety reports) in the electronic binder for IND #111512 (Niacin + L-dopa + L-arginine).

Copies of all such correspondence are also maintained in PTMS as part of the NICHD protocol record.

7. Adverse Event Procedures and Documentation

Serious adverse events are defined by the FDA as any untoward medical occurrences that: (1) result in death, (2) are life threatening, (3) require (or prolong) hospitalization, (4) cause persistent or significant disability/incapacity, (5) result in congenital anomalies or birth defects, or (6) are other conditions which in the judgment of the investigators represent significant hazards. All serious adverse events will be reported by the PI verbally and in writing as soon as possible to the NICHD Clinical Director using a form provided by the Clinical Center. All serious adverse events will also be reported to the Data Safety and Monitoring Committee (DSMC), the IRB, and the FDA within 7 calendar days for death or life-threatening adverse events, and within 15 days for all other serious adverse events. Adverse events are defined by the FDA as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen]. All adverse events (serious and non-serious, expected and unexpected) will be reported annually to the IRB and DSMC for review. Electronic data sheets or summary language will be supplied for IRB review of adverse event reports at continuing reviews.

The PI will report adverse events in PTMS, to the IRB and to the DSMC. Jack A. Yanovski, MD, PhD will inform the FDA in writing.

8. Study Completion

Upon completion of the study *Jack A. Yanovski, MD, PhD* will retain possession of the electronic IND binder and electronic Protocol Binder in a secure location. FDA requires records be retained for at least 2 years after study completion. HHS regulations require that subjects' records be maintained for at least three years after completion of a study. Since subjects of this investigation are children age 6-14 years, even longer record retention is required. All records will be retained until all participants have reached age 23 years.

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Appendix A

Medications Used in Growth Hormone Stimulation Testing^{131,148}						
Test	Dose	Timing of peak GH	Mechanism	Side effects	Cutoff GH value for $\geq 90\%$ sensitivity	Cutoff GH value for $\geq 90\%$ specificity
Arginine	0.5 g/kg IV to max of 40 g, infuse over 30 min	30-60 min	Inhibits somatostatin	Late hypoglycemia		
L-Dopa	125 mg for < 13.5 kg 250 mg for 13.5-31.5 kg 500 mg for > 31.5 kg	20-120 min	α -adrenergic stimulation of GHRH	Nausea, emesis, headache	26.6	11.1
GHRH	1 or 2 mg/kg, infuse over 1 min	15-30 min	Stimulates pituitary somatotrophs	Flushing		
Glucagon	0.03 mg/kg to max of 1 mg, give IM or SC	2-3 h	Increases insulin	Late hypoglycemia	29.4	7.5
Insulin	0.05-0.1 units/kg, give as bolus	30-60 min	Stress response to hypoglycemia presumed to decrease somatostatin	Severe hypoglycemia	23.4	2.2
Clonidine	5 mcg/kg to max of 250 mcg	60 min	α -2 agonist stimulates GHRH and inhibits somatostatin	Drowsiness, hypotension	30.5	8.2

Appendix B

Safety Monitoring for Niacin Growth Hormone Study			
Study Months	Baseline Evaluation	Dose-Establishing Studies Inpatient Admission	Main Study Inpatient Admission
Screening Visit: baseline blood work; Sign consent	Yes		
Fasting baseline blood work	Yes		
Urine pregnancy test	Yes	Yes	Yes
Medical history	Yes	Yes	Yes
Physical Exam	Yes	Yes	Yes
Bone age X-ray	Yes (only for subjects in the main study)		
DXA scan			Yes
Adverse Drug Event Assessment	Yes	Yes	Yes
Review of systems	Yes	Yes	Yes

Appendix C

Flow Diagram for Niacin Growth Hormone Study

DOSE-ESTABLISHING STUDY 1

Recruitment Clinic Visit

(performed at NIH Clinical Center):

Medical history and physical examination.
Fasting blood work
Oral glucose tolerance test
Bone age x-ray
Study entry only if subject meets inclusion criteria, and subject has & HGBA1C of $\leq 6.4\%$, fasting blood glucose of <100 , and 2 hour post-dextrose glucose of <140
Sign consent and assent forms

Within 2 months

Inpatient Stay:

Admission Day:

Admission to Clinical Center by 4:00 PM
Review medical history
Repeat physical exam
Placement of intravenous line
Begin fast at 10 pm

Study Day:

6am, 8am, and 10am: niacin (250 or 500 mg per dose)
6am, 6:30am, 7am, 7:30am, 8am, 8:30am, 9am, 9:30am, 10am, 10:30am, 11am, 11:30am, Noon: Blood draw
Noon: completion of test, resume ad lib diet then discharge

DOSE-ESTABLISHING STUDY 2

Recruitment Clinic Visit

(performed at NIH Clinical Center):

Medical history and physical examination.
Fasting blood work
Oral glucose tolerance test
Bone age x-ray
Study entry only if subject meets inclusion criteria, and subject has & HGBA1C of $\leq 6.4\%$, fasting blood glucose of <100 , and 2 hour post-dextrose glucose of <140
Sign consent and assent forms

Within 2 months

Inpatient Stay:

Admission Day:

Admission to Clinical Center by 4:00 PM

Review medical history

Repeat physical exam

Placement of intravenous line

Begin fast at 10 pm

Study Day:

7:30am, 8:30am, 9:30am, 10:30am niacin (500 mg per dose)

7:30am, 8am, 8:30am, 9am, 9:30am, 10am, 10:30am, 11am,

11:30am: Blood draw (measuring FFA, insulin, GH, and glucose)

7:30am, 8:30am, 9:30am, 10:30am, 11:30am: Blood draw (measuring Somatostatin and GHRH)

Noon: completion of test, resume ad lib diet then discharge

MAIN STUDY

Recruitment Clinic Visit

(performed at NIH Clinical Center):

Medical history and physical examination.
Fasting blood work
Oral glucose tolerance test
Bone age x-ray
Study entry only if subject meets inclusion criteria, and subject has & HGBA1C of $\leq 6.4\%$, fasting blood glucose of <100 , and 2 hour post-dextrose glucose of <140
Sign consent and assent forms

Within 2 months

Inpatient Stay:

Admission Day:

Admission to Clinical Center by 4:00 PM
Review medical history
Repeat physical exam
Placement of intravenous line
Begin fast at 10 pm

Day 1:

7:30am and 8:30am: Placebo or niacin 500 mg
7:30am, 8am, and 8:30am: Blood draw (Note GHRH and Somatostatin measured 7:30am and 8:30am)
8:30am: Arginine stimulation test per routine protocol - 500mg/kg up to 30g
8:30am, 9am, 9:15am, 9:30am: Blood draw (Note: GHRH and Somatostatin measured 9am)
9:30am: Placebo or niacin 500 mg and start L-dopa stimulation test – 500 mg
9:30am, 10am, 10:15am, 10:30am, 10:45am, 11am, 11:30am: Blood draw (Note: GHRH and Somatostatin measured 9:30am, 10am, 10:30am, and 11:30am)
10:30am: Placebo or niacin 500 mg
11:30am: completion of test, resume ad lib diet
Afternoon: DXA scan in nuclear medicine
10pm: begin fast

Day 2:

7:30am and 8:30am: placebo or niacin 500 mg
7:30am, 8am, and 8:30am: Blood draw (Note GHRH and Somatostatin measured 7:30am and 8:30am)
8:30am: Arginine stimulation test per routine protocol - 500mg/kg up to 30g
8:30am, 9am, 9:15am, 9:30am , : Blood draw (Note: GHRH and Somatosatin measured 9am)

9:30am: Placebo or niacin 500 mg and start L-dopa stimulation test – 500 mg
 9:30am, 10am, 10:15am, 10:30am, 10:45am, 11am, 11:30am: Blood draw (Note: GHRH and Somatostatin measured 9:30am 10am 10:30am, and 11:30am)
 10:30am: Placebo or niacin 500 mg
 11:30am: completion of test, resume ad lib diet then discharge

Outline of Main Study Blood Draws

Time	Study Hour	Medication	Time point Arginine	Time point L-Dopa	Blood Drawn
7:30am	0	Niacin	-60		GHRH, Somatostatin, Insulin, GH, Glucose, FFA
8:00am	30		-30		Insulin, GH, Glucose, FFA
8:30am	60	Niacin, Arginine	0		GHRH, Somatostatin, Insulin, GH, Glucose, FFA
9:00am	90		+30	-30	GHRH, Somatostatin, Insulin, GH, Glucose, FFA
<i>9:15am</i>			+45		Insulin, GH, Glucose, FFA
9:30am	120	Niacin, L-dopa	+60	0	GHRH, Somatostatin, Insulin, GH, Glucose, FFA
10:00am	150			+30	GHRH, Somatostatin, Insulin, GH, Glucose, FFA
<i>10:15am</i>				+45	Insulin, GH, Glucose, FFA
10:30am	180	Niacin		+60	GHRH, Somatostatin, Insulin, GH, Glucose, FFA
<i>10:45am</i>				+75	Insulin, GH, Glucose, FFA
11:00am	210			+90	Insulin, GH, Glucose, FFA
11:30am	240			+120	GHRH, Somatostatin, Insulin, GH, Glucose, FFA

Appendix D

Post-Test Questionnaire

To be administered hourly from 6am-Noon during the inpatient stay of the dose-establishing study and at the end of the inpatient stay of the main study

Please check off the symptoms that the patient experienced during the time the patient as staying at the NIH. Then indicate how long it lasted and how severe it was.

Symptom	Present?	Duration	Severity (please choose one)		
			1 (mild)	2 (moderate)	3 (severe)
Headache	<input type="checkbox"/>				
Flushing of face	<input type="checkbox"/>				
Feeling warm	<input type="checkbox"/>				
Nausea (feeling like vomiting)	<input type="checkbox"/>				
Vomiting	<input type="checkbox"/>				
Stomach ache	<input type="checkbox"/>				
Diarrhea or loose stools	<input type="checkbox"/>				
Tingling in arms or legs	<input type="checkbox"/>				
Light-headed or faint	<input type="checkbox"/>				
Itchiness	<input type="checkbox"/>				
Rash or skin problems	<input type="checkbox"/>				
Other (please list):					

Appendix E: (completed once at baseline)

Eating Behavior Questionnaire

Participant's Name:

Date:

Relationship of person completing questionnaire to subject (circle one):

Mother Father Other _____

(1) How upset does your child generally become when denied a desired food?

- Not particularly upset at all
- A little upset
- Somewhat upset
- Very upset
- Extremely upset

(2) How often does your child try to bargain or manipulate to get more food at meals?

- A few times a year
- A few times a month
- A few times a week
- Several times a week
- Several times a day

(3) Once your child has food on their mind, how easy is it for you or others to re-direct your child away from food to other things?

- Extremely easy, takes minimal effort to do so
- Very easy, takes just a little effort to do so
- Somewhat hard, takes some effort to do so
- Very hard, takes a lot of work to do so
- Extremely hard, takes sustained and hard work to do so

(4) How often does your child forage through the trash for food?

- Never
- A few times a year
- 1-2 times a month
- 1-3 times a week
- 4 to 7 times a week

(5) How often does your child get up at night to seek food?

- Never
- A few nights a year
- 1-2 nights a month
- 1-3 nights a week
- 4 to 7 nights a week

(6) How persistent is your child in asking or looking for food after being told "no" or "no more"?

- Lets go of food ideas quickly and easily
- Lets go of food ideas pretty quickly and easily

- Somewhat persistent with food ideas
- Very persistent with food ideas
- Extremely persistent with food ideas

(7) Outside of normal meal times, how much time does your child spend talking about food or engaged in food-related behaviors?

- Less than 15 minutes a day
- 15 to 30 minutes a day
- 30 minutes to an hour
- 1 to 3 hours a day
- more than 3 hours a day

(8) How often does your child try to steal food (that you are aware of?)

- A few times a year
- A few times a month
- A few times a week
- Several times a week
- Several times a day

(9) When others try to stop your child from talking about food or engaging in food-related behaviors, it generally leads to:

- No distress or upset
- Mild distress or upset
- Moderate distress or upset
- Severe distress or upset
- Extreme distress, behaviors can't usually be stopped

(10) How clever or fast is your child in obtaining food?

- Not particularly clever or fast
- A little clever or fast
- Somewhat clever or fast
- Very clever or fast
- Extremely clever or fast

(11) To what extent do food-related thoughts, talk, or behavior interfere with your child's normal daily routines, self-care, school, or work?

- No interference
- Mild interference; occasional food-related interference in completing school, work, or hygiene tasks
- Moderate interference; frequent food-related interference in completing school, work, or hygiene tasks
- Severe interference; almost daily food-related interference in completing school, work, or hygiene tasks
- Extreme interference, often unable to participate in hygiene tasks or to get to school or work due to food-related difficulties

(12) How old was your child when they first showed an increased interest in food? _____ (write N/A if interest in food has never been a problem)

(13) How variable is your child's preoccupation or interest in food?

- Hardly ever varies
- Usually stays about the same
- Goes up and down occasionally
- Goes up and down quite a lot
- Goes up and down all the time

Adapted from Dykens EM, et al. *Obesity*. 15(7):1816-1826 (2007). Used with permission