

# Short-term Effects of Leptin Withdrawal or Initiation in Lipodystrophy Independent of Energy Intake

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## Protocol Overview

|                                                                             |                                                                                                     |
|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Protocol Title:                                                             | Short-term Effects of Leptin Withdrawal or Initiation in Lipodystrophy Independent of Energy Intake |
| Abbreviated Title:                                                          | Leptin Independent of Energy Intake                                                                 |
| IRB:                                                                        | NIDDK/NIAMS                                                                                         |
| Research Type:                                                              | Phase II Clinical Trial                                                                             |
| Multi-site Collaboration:                                                   | No                                                                                                  |
| Intramural Collaboration:                                                   | No                                                                                                  |
| Ionizing Radiation Use:<br>(X-rays, e.g., CT; radioisotope, e.g. PET; etc.) | Research Indicated                                                                                  |
| Investigational New Drug/Device:                                            | Yes                                                                                                 |
| Patient Self-Referral Allowed:                                              | Yes                                                                                                 |
| List Protocol On Web:                                                       | Yes                                                                                                 |
| Is tissue being collected for research purposes:                            | Yes                                                                                                 |

**Conflict of Interest**

The protocol involves no drugs/devices/products that may lead to payments and/or royalties to be paid to the investigators or the NIH.

The investigators have no equity, consultative, or other financial relationship with a non-NIH source related to this protocol which might be considered a conflict of interest.

**Time Frame**

Start Date: 11/1/2012

End Date: 12/31/2019

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## Precis – Abstract

### *Background*

Leptin is an adipocyte-derived hormone that can be thought of as a signal from adipose tissue to the rest of the body conveying information about long-term nutritional status. Patients with lipodystrophy have leptin deficiency secondary to lack of adipose tissue, and thus represent a natural model for studying the effects of leptin deficiency and replacement in humans. Leptin replacement in lipodystrophy ameliorates metabolic and endocrine abnormalities, including reducing food intake, improving insulin resistance and diabetes, reducing ectopic lipid, and normalizing reproduction. The reduction in energy intake induced by leptin replacement is likely responsible for part of the improvements observed in glucose and lipid metabolism. The clinical effects of leptin that are independent of changes in energy intake, and the mechanisms underlying these effects, have been poorly explored in humans.

### *Aim*

The primary aim of this study is to determine the energy intake-independent effects of leptin on energy metabolism in lipodystrophic subjects. The major aspects of energy metabolism to be studied are:

1. Lipid metabolism, including fasting lipids, lipolysis and fatty acid turnover, and ectopic lipid storage.
2. Glucose metabolism, including fasting glucose, endogenous glucose production, and insulin sensitivity
3. Energy expenditure, including total and resting energy expenditure, skeletal muscle work efficiency, and spontaneous physical activity

In addition, the effects of leptin on endocrine and autonomic function will be examined, including effects on the thyroid, gonadal, and adrenal axes, as well as blood pressure, body temperature, and heart rate variability.

### *Methods*

This is a non-randomized, parallel group study. Two groups of patients aged 14 to 70 years with lipodystrophy will be studied: leptin naïve and leptin treated. Minors will only be included in the leptin naïve arm. All subjects will be stabilized on a weight maintenance diet for 5 days (Period 1). After this, leptin will be withdrawn from leptin treated subjects, and leptin will be initiated in leptin naïve subjects for a period of 14 days (Period 2). The same isocaloric diet will be continued throughout both Periods, permitting study of leptin's effects independent of energy intake.

All subjects will undergo metabolic testing on admission, at the end of Period 1, and throughout Period 2, to generate a detailed short-term time course of the effects of leptin initiation or withdrawal. At the end of Period 2, leptin will be continued in the leptin naïve subjects, and restarted in the leptin treated subjects. Repeat metabolic testing will be performed 6 months (ideally 5-9 months but may be extended to 12 months in foreign subjects) after leptin initiation in the leptin-naïve cohort to generate information on leptin's long-term effects.

## Study Objectives

The primary aim of this study is to determine the energy intake-independent effects of leptin on energy metabolism in lipodystrophic subjects.

The null hypothesis is that, when caloric intake is fixed, leptin has no effects on energy metabolism.

The alternative hypothesis is that, when caloric intake is fixed, leptin does have measureable effects on energy metabolism.

The major aspects of energy metabolism to be studied are:

1. Lipid metabolism, including fasting lipids, lipolysis and fatty acid turnover, and ectopic lipid storage.
2. Glucose metabolism, including fasting glucose, endogenous glucose production, and insulin sensitivity
3. Energy expenditure, including total and resting energy expenditure, skeletal muscle work efficiency, and spontaneous physical activity

In addition, the effects of leptin on endocrine and autonomic function will be examined, including effects on the thyroid, gonadal, and adrenal axes, as well as blood pressure, body temperature, and heart rate.

## Introduction

### Leptin

Leptin is an adipocyte-derived hormone that can be thought of as a signal from adipose tissue to the rest of the body conveying information about long-term nutritional status. In particular, low leptin levels serve as a signal of inadequate nutritional stores. Rodents and humans with leptin deficiency due to mutations of the leptin gene or its receptor exhibit many of the same physiological changes observed in starvation, including hyperphagia, infertility, decreased immune function, and, in rodents, decreased energy expenditure; these abnormalities can be reversed with leptin replacement<sup>1-10</sup>. Due to the constant “starvation signal” from leptin deficiency, both rodents and humans with congenital leptin deficiency exhibit extreme obesity, which can be greatly ameliorated with exogenous administration of leptin<sup>4-7,9</sup>. One mechanism by which body weight is reduced after leptin replacement is via appetite reduction, with spontaneous reductions in food intake of ~50% in both rodents<sup>6,9</sup> and humans<sup>5</sup>. In rodents, there is also evidence that leptin replacement decreases body weight by increasing energy expenditure. Although leptin-induced alterations in energy expenditure are likely to be much more important in rodents than in humans due to differences in small versus large animal biology, there is limited evidence of an effect in humans. In weight-reduced healthy humans, leptin replacement to pre-weight-loss levels appears to increase energy expenditure<sup>11,12</sup>. Similarly, in patients with congenital leptin deficiency, leptin treatment appears to prevent the typical weight-loss induced fall in energy expenditure<sup>5,13</sup>.

In addition to its roles in regulating body weight, immune function, and reproduction, leptin is important for normal glucose and lipid metabolism<sup>5,7,9,14,15</sup>. Leptin replacement in deficiency states improves glucose and lipid metabolism, including reversal of insulin resistance and diabetes<sup>7,9,14,16,17</sup>, and reduction in hepatic and circulating triglycerides<sup>7,14,15</sup>. The physiologic and molecular mechanisms by which leptin alters insulin and lipid metabolism have been explored in rodent models, and to a much lesser extent in humans. One major effect of leptin is reduction of hepatic gluconeogenesis, which appears to be mediated via the central nervous system through effects on the vagus nerve<sup>18</sup>. Leptin-induced decreases in lipogenic enzymes, including stearoyl-coenzyme A desaturase 1, fatty acid synthase, and HMG CoA reductase have been found in liver and adipose tissue in rodents<sup>15,19</sup>. Recently, insulin-like growth factor binding protein 2 (IGFBP2) has been proposed as a major mediator of leptin’s antidiabetic effects, with IGFBP2 overexpression reversing diabetes in leptin-deficient animals. In addition, leptin may have effects on glycemia independent of improvements in insulin sensitivity,

via overlapping post-receptor signaling pathways between insulin and leptin, including phosphatidylinositol 3-kinase<sup>20</sup>.

Leptin also has effects on other organ systems, including bone. In rodent models, leptin deficiency is associated with decreased cortical bone in the vertebrae and limbs, and increased trabecular bone in the vertebrae. These effects are reversed with leptin administration; hence, leptin replacement results in overall increased bone mineral density<sup>21</sup>. This effect has not been well-studied in humans.

#### Leptin in lipodystrophy

Lipodystrophies, in which there is partial or complete absence of adipose tissue (and hence, adipokines such as leptin), represent another model for understanding the actions of leptin in a state of deficiency and replacement. Unlike patients with congenital leptin deficiency, patients with lipodystrophy are non-obese, but have more extreme manifestations of the metabolic syndrome due to factors such as ectopic lipid storage<sup>22,23</sup>. Leptin replacement in lipodystrophy reduces food intake<sup>24,25</sup> and ameliorates most metabolic abnormalities, including insulin resistance<sup>25-27</sup> and diabetes<sup>26,28,29</sup>, hypertriglyceridemia<sup>27-29</sup>, and hepatic steatosis<sup>25,30</sup>. Based on data derived largely from studies conducted in the intramural research program of the NIDDK, the FDA approved metreleptin (recombinant methionyl leptin) for the treatment of generalized lipodystrophy in February, 2014; however, leptin remains an experimental therapy in other forms of lipodystrophy, including partial lipodystrophy.

Limited information is available about the time course of leptin's effects in lipodystrophy. In seven patients with generalized lipodystrophy, declines in glucose and triglyceride levels reached statistical significance by 7 days after leptin initiation<sup>31</sup>. In these subjects, non-significant increases in insulin sensitivity (measured using the hyperinsulinemic, euglycemic clamp) were seen one month after starting leptin, and significant increases after 2 months.

More detailed mechanistic studies of leptin's effects on glucose and lipid metabolism after 3-8 months have been performed on three patients with lipodystrophy (one acquired generalized and two congenital generalized)<sup>25</sup>. Using stable isotope infusions in combination with the hyperinsulinemic, euglycemic clamp, these subjects in the leptin deficient state were shown to have elevated hepatic glucose production and severe hepatic insulin resistance compared to healthy controls. After leptin replacement for 3-8 months, hepatic insulin resistance decreased to nearly that of controls, and insulin-stimulated glucose disposal doubled. These changes were associated with an 86% reduction in hepatic triglyceride content and a 33% reduction in muscle triglyceride, suggesting that reduction in ectopic lipid may have contributed to the improvements in insulin sensitivity. Non-significant decreases in glycerol turnover were also observed following leptin treatment, suggesting that leptin increased lipolysis in any existing adipose tissue or within ectopic lipid stores. In a single subject, a 30% reduction in muscle fatty acyl CoA levels was observed after leptin replacement, offering support for the hypothesis that these compounds contribute to insulin resistance by interfering with insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation, a key step in the insulin signaling cascade. In summary, leptin appears to improve plasma glucose and triglycerides within 1 week, while improvements in insulin sensitivity may be detectable by one month, with continued improvement thereafter, and changes in ectopic lipid storage may not be detectable for several months after leptin initiation.

The effect of leptin on lipid metabolism has also been examined in a randomized, controlled trial of leptin for 4 months in 17 men with HIV associated lipodystrophy<sup>32</sup>. In this trial, the authors did not find any effects of leptin treatment on lipid metabolism, including fasting triglycerides, rates of lipolysis, and fatty acid oxidation. This suggests that there may be differences in the effect of leptin on lipid metabolism in HIV patients versus the non-HIV infected population studied at NIH. These differences may be related to the severity of the phenotype (typically milder in HIV) or to the doses of leptin used in the HIV study (0.02-0.04 mg/kg/day, which is 25-50% of the lowest doses used in NIH studies).

### Energy intake independent effects of leptin

In lipodystrophy, as in other models of leptin deficiency, leptin replacement causes a reduction in spontaneous food intake by approximately 50%<sup>24,25</sup>. It is likely that reduced energy intake is at least partially responsible for observed changes in glucose and lipid metabolism in lipodystrophic patients, as caloric restriction or fasting has long been known to improve glycemia control in patients with diabetes. Prior to the development of insulin, starvation followed by severe carbohydrate restriction was state of the art treatment for diabetes<sup>33</sup>. Marked improvement in blood glucose with less severe caloric restriction (330 – 1800 kcal per day for 3-21 days) has been shown by multiple researchers since that time<sup>34-41</sup>. Two studies in this group<sup>36,41</sup> demonstrated an approximately 50% rise in insulin sensitivity, suggesting that improvements in insulin sensitivity may be a major mechanism underlying lower blood glucose levels following caloric restriction.

In rodent models of leptin deficiency, pair-feeding studies have been performed to assess the energy intake-independent effects of leptin replacement. Leptin effects in rodents that have been shown to be at least partly independent of energy intake include increased energy expenditure<sup>15</sup>, increased body temperature<sup>9</sup>, decreased insulin resistance<sup>9,14,16-18</sup>, and decreased blood glucose<sup>9,14,16,17</sup>. In contrast, leptin's effects on triglycerides and free fatty acids in rodents were not independent of energy intake<sup>15</sup>.

In humans, the energy intake-independent effects of leptin have been assessed in only two studies. A single patient with acquired, generalized lipodystrophy was studied while taking leptin, and during leptin withdrawal, while maintaining constant energy intake<sup>29</sup>. In this patient, blood glucose did not change during leptin withdrawal, a rise in serum insulin (indicative of worsening insulin resistance) was seen after four days, and a rise in triglycerides after seven days. Although based on a single subject, these data suggest that leptin's effects on both insulin resistance and lipids may be partly independent of energy intake in humans. Liebel's group studied the energy intake-independent effects of leptin in ten healthy subjects following 10% weight loss, a model of relative leptin deficiency<sup>11</sup>. Subjects in the weight-reduced state were studied before and after five weeks of leptin replacement to pre-weight-loss levels, while maintaining constant energy intake. Leptin replacement resulted in an increase in energy expenditure to pre-weight-loss levels that appeared to be due to changes in skeletal muscle work efficiency, thyroid hormones, and catecholamines. Because these subjects did not have abnormalities of glucose or lipid metabolism, the energy intake-independent effects of leptin on these parameters were not examined.

## **Subject Eligibility Assessment and Enrollment**

### *Inclusion Criteria*

1. Age 14-70 years (children under age 18 will only be enrolled in the leptin-naïve arm of the study [Inclusion criterion 3b, below])
2. Clinically-significant lipodystrophy as summarized below:
  - a. Lipodystrophy identified by the study physician during physical examination as an absence of fat outside the range of normal variation.
  - b. Circulating leptin levels < 12.0 ng/mL in females and < 8.0 ng/mL in males
  - c. Presence of at least one of the following metabolic abnormalities:
    - i. Diabetes as defined by the 2007 American Diabetes Association criteria:
      1. Fasting plasma glucose  $\geq$  126 mg/dL, or
      2. 2-hour plasma glucose  $\geq$  200 mg/dL following a 75 gram 91.75 gm/kg) oral glucose load, or
      3. Diabetic symptoms with a random plasma glucose  $\geq$  200 mg/dL.
    - ii. Fasting insulin >30  $\mu$ U/mL
    - iii. Fasting hypertriglyceridemia >200 mg/dL
3. Either:



- a. Leptin naïve, with plans to initiate leptin treatment during the current study. For the purpose of this study, “leptin naïve” will be defined as having received no exogenous leptin in the 4 months prior to study participation. Thus, subjects who previously received leptin therapy, discontinued, and wish to restart are eligible.

Or

- b. Leptin treated, meaning the subject has taken a stable dose of exogenous leptin for a minimum of 4 months prior to study entry (adults over age 18, only)

### *Exclusion Criteria*

In leptin treated subjects only, the following exclusion criteria apply:

1. Poorly controlled diabetes at study entry (hemoglobin A1c  $\geq 9\%$ ).
2. Poorly controlled hypertriglyceridemia at study entry (serum triglycerides  $> 800$  mg/dL).
3. Extreme hypertriglyceridemia prior to leptin (preleptin triglycerides greater than 2000 mg/dL).
4. History of chronic or recurrent acute pancreatitis ( $>1$  episode), or a single episode of pancreatitis while receiving leptin treatment.
5. Lipase greater than the upper limit of normal (491 units/L) at study entry.
6. Known presence of neutralizing antibodies to leptin.

In all subjects (leptin treated and leptin naïve), the following exclusion criteria apply:

7. Known HIV infection or HIV-associated lipodystrophy.
8. History of diabetic ketoacidosis.
9. Active inflammatory disease (e.g. dermatomyositis).
10. Change in diabetes or lipid-lowering medications within the past 6 weeks.
11. Estimated glomerular filtration rate  $< 30$  mL/minute.
12. Current or recent (past 2 weeks) use of systemic glucocorticoids.
13. Inadequately controlled hypothyroidism (TSH  $< 0.4$  or  $> 4$  mIU/L) or change in thyroid medication in the past 8 weeks.
14. Pregnancy or breast-feeding.
15. Psychiatric disorder impeding competence or compliance.
16. Any medical condition or medication that will increase risk to the subject (e.g. ischemic heart disease, decompensated liver disease) or that will interfere with interpretation of study data (e.g. Cushing's syndrome).
17. Current alcohol or substance abuse.
18. Subjects who have a known hypersensitivity to E. coli derived proteins.
19. Subjects with acquired lipodystrophy and a hematologic abnormality such as neutropenia and/or lymphadenopathy.

## **Study Design and Statistical Analysis**

### **Design**

This is a prospective, non-randomized, study. Two groups of subjects will be studied. In the leptin naïve group, subjects with lipodystrophy who have not received leptin therapy in the past 4 months will be studied first in the leptin deficient state (Period 1), and second during the first 14 days of leptin initiation (Period 2). In the leptin treated group, subjects with lipodystrophy who have been receiving stable doses of leptin for a minimum of 4 months will be studied first in the leptin replete state (Period 1), and then during a 14 day period of leptin withdrawal (Period 2). Throughout both periods, subjects will consume an isocaloric diet (described in detail under Study Implementation, below), thus controlling for any effects of leptin on energy intake. Measurement of metabolic and endocrine parameters will be performed on admission, at the end of Periods 1 and 2, and at



intermediate time points during Period 2. Subjects in the leptin naïve group will be restudied 6 months (range, 5-9 months) after starting leptin. At that time, options for continued treatment with leptin will be discussed and/or facilitated (see Long-term follow up, below). Given budgetary constraints, foreign subjects will be given the option to return in 12 months and will be asked to have lab work completed locally 3-6 months after starting leptin.

## **Randomization**

Although the optimal method to study the time course of leptin's effects would be a randomized, double-blinded, placebo controlled, cross-over study, such a study design is not feasible in this population. Lipodystrophy is an extremely rare disease, reported in only about 1000 patients worldwide<sup>42</sup>. The NIH study of leptin treatment in lipodystrophy currently follows approximately 65 active patients. A randomized, cross-over design would require a subset of these patients to undergo multiple visits to NIH of approximately 16 days, separated by wash-out periods of approximately 4 months. Subjects newly starting on leptin could not be enrolled, as leptin could not be ethically withheld during the washout period. The likelihood of enrolling a sufficient number of subjects to complete such a study is very low. Therefore, we have chosen a more feasible study design in which the effects of short-term leptin initiation and withdrawal can be examined in a non-randomized fashion.

## **Outcome Measures**

The primary aim of this study is to determine the energy intake-independent effects of leptin on energy metabolism in lipodystrophic subjects. Important aspects of energy metabolism to be studied include glucose metabolism, lipid metabolism, and energy expenditure. For each of these three aspects of energy metabolism, we will measure a primary, secondary, and one or more exploratory outcomes, as follows:

### Glucose Metabolism

- **Primary outcome:** Total body insulin sensitivity (measured as glucose disposal rate during a hyperinsulinemic, euglycemic clamp)
- **Secondary outcomes:**
  - Hepatic insulin sensitivity (measured as suppression of endogenous glucose production during a hyperinsulinemic, euglycemic clamp)
  - Fasting plasma glucose
  - Fasting C-peptide
- **Exploratory outcomes:**
  - Insulin-like growth factor binding protein 2 (IGFBP2) and other novel mediators of leptin action on glycemia

### Lipid Metabolism

- **Primary outcome:** Rate of lipolysis (measured using stable isotope tracers)
- **Secondary outcomes:**
  - Fasting plasma triglycerides
- **Exploratory outcomes:**
  - Rate of fatty acid turnover (measured using stable isotope tracers)
  - Liver triglyceride (measured using MRI and MRS)
  - Muscle triglyceride (measured using MRS)

### Energy Expenditure

- **Primary outcome:** Total 24 hour energy expenditure (measured using indirect calorimetry in the metabolic chamber)
- **Secondary outcomes:**
  - Resting energy expenditure (measured using indirect calorimetry with a hood)

- Skeletal muscle work efficiency (measured as the exercise-induced incremental change in energy expenditure above resting energy expenditure)
- **Exploratory variables:**
  - Spontaneous physical activity (measure using portable accelerometers)
  - Thyroid function
  - Urine and plasma catecholamines
  - Cardiac autonomic nervous system tone (measured using heart rate variability analysis)

Additional outcomes include the following:

Thermal regulation

- Core versus peripheral body temperature
- Thermal comfort (measured using visual analog scales)

Endocrine

- LH pulsatility analysis

Other

- Blood pressure (measured using 24 hour ambulatory monitoring)
- Hunger and satiety (measured using visual analog scales)
- Markers of bone turnover ( $\beta$ -CTX and P1NP) will be measured to look for potential effects of leptin on bone formation and resorption.

## Statistical Considerations

### Sample Size Justification

This is an exploratory study of the energy-intake independent effects of leptin initiation and withdrawal in lipodystrophy. Preliminary data are not available to generate estimates of power for each outcome to be tested. The primary comparisons to be tested for each outcome of interest are results at the end of Period 1 versus the end of Period 2, analyzed separately for each group of subjects (leptin-naïve versus leptin treated). Thus, sample size calculations are based on paired, before and after, comparisons within each group of subjects. The data and references used for power calculations are shown in the table, below. Data in the table using the 10% weight loss model are given as percent change from pre-weight loss values, while data from lipodystrophy patients are given as absolute differences between leptin treated and untreated states. All calculations assumed a sample size of 10 and an alpha of 0.05, without correction for multiple comparisons. Because the standard deviations (SDs) of differences between on and off leptin conditions were not reported in publications, the SDs of the difference (used to calculate power for paired comparisons) was estimated in two ways: First, the SDs of differences are calculated by assuming that the SDs of on and off leptin reported in the literature are independent (Power 1 in the table). Second, a “worst-case scenario” was assumed that the SDs on and off leptin in the literature are perfectly positively correlated (Power 2 in the table). The first scenario is the commonly used method, while the second case is the most conservative calculation.

| Outcome                                                                                                     | Model         | Duration of leptin | Off-leptin mean (SD) | On-leptin mean (SD) | Power 1 | Power 2 |
|-------------------------------------------------------------------------------------------------------------|---------------|--------------------|----------------------|---------------------|---------|---------|
| Total energy expenditure <sup>11</sup> (% $\Delta$ in kcal/day)                                             | 10% wt loss   | 5 wk               | -22% (15.8)          | -6% (4.7)           | 0.92    | 0.79    |
| Skeletal muscle work efficiency <sup>11</sup> (% $\Delta$ in kcal/min expended during exercise vs. at rest) | 10% wt loss   | 5 wk               | 20% (12.6)           | 3% (2.5)            | 0.99    | 0.97    |
| Resting energy expenditure <sup>24</sup>                                                                    | lipodystrophy | 4 mo               | 35.5 (8.2)           | 33.6 (6.7)          | 0.14    | 0.11    |
| T3 <sup>11</sup> (% $\Delta$ )                                                                              | 10% wt loss   | 5 wk               | -9% (6.3)            | 3% (3.2)            | 0.99    | 0.99    |

|                                                                                                                                  |               |      |             |             |      |      |
|----------------------------------------------------------------------------------------------------------------------------------|---------------|------|-------------|-------------|------|------|
| T3 <sup>43</sup> (nmol/L)                                                                                                        | lipodystrophy | 4 mo | 124 (37)    | 114 (26)    | 0.18 | 0.13 |
| T4 <sup>11</sup> (% Δ)                                                                                                           | 10% wt loss   | 5 wk | -7% (7.9)   | -1% (1.3)   | 0.77 | 0.66 |
| fT4 <sup>43</sup> (pmol/L)                                                                                                       | lipodystrophy | 4 mo | 1.05 (0.15) | 1.03 (0.15) | 0.09 | 0.08 |
| TSH <sup>43</sup> (μU/mL)                                                                                                        | lipodystrophy | 4 mo | 1.37 (0.37) | 1.24 (1.1)  | 0.10 | 0.09 |
| Norepinephrine <sup>11</sup> (% Δ)                                                                                               | 10% wt loss   | 5 wk | -12% (15.8) | -7% (12.6)  | 0.19 | 0.14 |
| Epinephrine <sup>11</sup> (% Δ)                                                                                                  | 10% wt loss   | 5 wk | -40% (35)   | 28% (47)    | 0.98 | 0.83 |
| Sympathetic nervous system tone <sup>11</sup><br>(% Δ in mean ECG R-R interval after<br>atropine + esmolol vs. atropine alone)   | 10% wt loss   | 5 wk | -25% (22.1) | 1% (1.6)    | 0.98 | 0.97 |
| Liver volume <sup>24</sup> (cm <sup>3</sup> )                                                                                    | lipodystrophy | 4 mo | 3055 (1051) | 2433 (763)  | 0.45 | 0.29 |
| Total body insulin sensitivity <sup>31</sup><br>(glucose infusion rate during clamp,<br>mg*kg <sup>-1</sup> *min <sup>-1</sup> ) | lipodystrophy | 1 mo | 2.5 (0.79)  | 3.7 (1.1)   | 0.87 | 0.63 |
| Total body insulin sensitivity <sup>25</sup><br>(glucose infusion rate during clamp,<br>mg*kg <sup>-1</sup> *min <sup>-1</sup> ) | lipodystrophy | 4 mo | 1.2 (0.35)  | 5.4 (1.6)   | 1    | 1    |
| Hepatic insulin sensitivity <sup>25</sup> (%<br>suppression of endogenous glucose<br>production during clamp)                    | lipodystrophy | 4 mo | 40 (10.4)   | 82 (8.7)    | 1    | 1    |
| Fasting glucose <sup>31</sup> (mg/dL)                                                                                            | lipodystrophy | 7 d  | 172 (20)    | 120 (12)    | 1    | 0.99 |
| Fasting triglycerides <sup>31</sup> (mg/dL)                                                                                      | lipodystrophy | 7 d  | 700 (272)   | 260 (98)    | 0.99 | 0.98 |
| Fasting triglycerides <sup>31</sup> (mg/dL)                                                                                      | lipodystrophy | 14 d | 700 (272)   | 110 (38)    | 1    | 0.99 |
| Rate of lipolysis <sup>25</sup> (glycerol turnover,<br>μmol*kg fat mass <sup>-1</sup> *min <sup>-1</sup> )                       | lipodystrophy | 4 mo | 78 (36)     | 25 (7)      | 0.99 | 0.99 |

Based on these calculations, a sample size of 10 subjects in each group (leptin-naïve and leptin-treated) will provide greater than 80% power (in most cases greater than 95%) for the following primary and secondary outcomes:

#### Glucose Metabolism

1. Total body insulin sensitivity
2. Hepatic insulin sensitivity
3. Fasting plasma glucose

#### Lipid Metabolism

1. Rate of lipolysis
2. Fasting triglycerides

#### Energy Expenditure

1. Total energy expenditure
2. Skeletal muscle work efficiency

Exploratory outcomes for which we anticipate greater than 80% power include epinephrine and sympathetic nervous system tone. Discrepant data exist for the effects of leptin on thyroid function (specifically, T3) based on the model used (lipodystrophy versus 10% weight loss), and this study should help to clarify whether this effect does or does not exist in lipodystrophy. We do not anticipate observing significant changes in resting energy expenditure; however, because this procedure is minimal risk, we feel that it will be important to document negative results for this outcome. Certain outcome measures, such as liver triglyceride (for which liver size was a proxy in the table, above), and insulin sensitivity, appear to progressively change over time with leptin replacement. For these outcomes, a 2 week intervention may be insufficient to observe statistically significant results. For other outcome measures, such as intramyocellular triglyceride, preliminary data on the short-term effects of leptin are not available, but effects, if present, are unlikely to be detectable by two weeks.

Therefore, in addition to obtaining data after 2 weeks of leptin withdrawal or initiation, we will restudy subjects in the leptin-naïve group 6 months (range is 5-9 months) after leptin initiation to look for changes that may not be detectable by 2 weeks.

Because some subjects may not complete the study, or may not have interpretable data for each outcome, we plan to enroll up to 20 subjects in each group in order to have 10 completers per group.

### **Statistical Methods for Data Analysis**

Data will be analyzed separately for the leptin naïve and leptin treated subjects. This is done because the effects of leptin withdrawal after long-term treatment are likely to be different from the effects of leptin initiation after long-term deficiency.

#### *Analysis of Primary Outcomes*

Measurements in each of the primary outcomes will be analyzed using the paired t-test (for normally distributed variables) or Wilcoxon paired test (for skewed variables) to test if there is any significant difference between the two study periods (on and off leptin) for each group (leptin naïve and leptin treated), respectively. In addition, mixed models incorporating baseline (pre-diet) parameters as covariates will be used to adjust for any effects of the inpatient, metabolic diet, independent of leptin's effects. For variables with more than 2 measurements during Period 2, repeated measure linear models (Mixed model or GLM) will be used to study the time effect, using pooled data from the 2 periods. Potential covariates to be explored in the model include: Age, sex, race, percent body fat, lean mass, body weight, type of lipodystrophy, and endogenous leptin level. To account for any deviation from the isocaloric protocol diet, actual caloric intake will be included as a covariate in mixed model analyses.

Additional correlations between variables within and between each of the major outcome categories (glucose metabolism, lipid metabolism, and energy expenditure) will be analyzed using either Pearson's correlation or simple linear regressions. These include the following:

1. Association between change in total body insulin sensitivity and changes in hepatic insulin sensitivity, fasting plasma glucose, and C-peptide
2. Association between change in rate of lipolysis, and changes in fasting triglycerides and fatty acid turnover
3. Association between insulin sensitivity and ectopic lipid stores, and rates of lipolysis and fatty acid turnover
4. Association between change in total energy expenditure and changes in resting energy expenditure, skeletal muscle work efficiency, spontaneous physical activity, thyroid function, catecholamines, and cardiac autonomic nervous system tone.

#### *Analysis of Secondary and Exploratory Outcomes*

Methods for analysis of secondary outcomes will be similar to those for primary outcomes. In addition, we will perform analysis to study the following associations between categories of outcome measures:

1. Association between the Ectopic Lipid measurements with Glucose and Lipid measurements
2. Association between the Endocrine measurements with Energy Expenditure measurements

#### *Analysis of long-term data*

Additional follow up data will be obtained and analyzed 6 to 12 months after leptin initiation for the leptin naïve patients. Long-term follow-up measurements for this group will be compared with the baseline using paired comparisons. Time-dependent effects will also be investigated using repeated measures analysis. The analysis methods to be used are similar to those used in the primary outcome analyses.

#### *By Group Comparisons*

Although most of the analyses will be performed separately for leptin naïve vs. leptin treated patients due to the consideration stated previously, exploratory analyses will be carried out to compare the two study groups. These analyses include:

1. Comparison of outcomes and baseline characteristics by group will be performed using t-test or rank test, depending on the distribution of measurement compared
2. Model analysis using general linear models will be performed by including group as a factor

## **Study Implementation**

Subjects will be admitted to the Metabolic Research Unit in the NIH Clinical Center. They will remain as inpatients throughout the 19 day duration of the short-term portion of the study. After admission, they will undergo a history and physical examination, and baseline labs will be obtained. Baseline phenotyping will include measurement of resting energy expenditure, hemoglobin A1c, and fasting lipid panel, glucose, insulin, and C-peptide. Fasting triglycerides and hemoglobin A1c will be used to assess subject eligibility in the leptin treated group. Additional labs used to assess subject eligibility in all subjects include electrolytes, BUN, creatinine, CBC, and a pregnancy test for female patients of reproductive potential. Height, weight, blood pressure, resting pulse, temperature, and anthropometric data (e.g. skin fold measurements, waist circumference, etc.) will be obtained. Additionally, the clinical testing that was formerly included in 02-DK-0022 will be done: ECHO, OGTT, liver/abdominal and pelvic ultrasounds.

## **Diet**

After baseline phenotyping, subjects will be placed on a low fat (20±5% protein, 25% fat, 55±5% carbohydrate), weight maintenance diet from the metabolic research kitchen. Total caloric requirements will be estimated by the research dietitian. Due to the low proportion of body fat in people with lipodystrophy, all male and female subjects' energy needs will be estimated using the Mifflin St. Joer equation for males with an activity factor of 1.3. Upon admission, resting energy expenditure will be measured and dietary energy will be adjusted as needed. The diet may be adjusted during the first few days, but will be continued for the remainder of the study without change.

In the leptin naïve group, appetite is expected to decrease following leptin initiation during Period 2, although the early time course of this effect is not known. Subjects will be encouraged to eat all food presented to them by the metabolic kitchen in order to maintain the isocaloric nature of the diet throughout both periods. However, if they are unable to consume all food, the uneaten portion will be returned to the metabolic kitchen and weighed to document the extent of deviation from the isocaloric diet. Analogously, in the leptin-treated group, subjects may feel hungry after leptin withdrawal, but will only be permitted to eat the food provided by the metabolic kitchen.

In prior studies of leptin replacement in lipodystrophy, subjects had a mean weight loss of only 2.8 kg (4.6% of initial body weight) after 4 months of leptin replacement. Hence, changes in body weight during the course of the 3 week inpatient portion of this study are not expected to be clinically significant. Because each subject will be compared with him/herself in the leptin deficient versus leptin replaced state, maintaining constant (isocaloric) food intake during both conditions will allow assessment of the energy-intake independent effects of leptin.

## **Medications**

### ***Leptin Treatment***

Any subject starting leptin on this study will get it through NIH under IND, regardless of whether or not they fit in an FDA approved category. After the follow-up visits, subjects who are eligible will be transitioned to commercial drug supply through their physician. Subjects undergoing leptin withdrawal may receive leptin under IND or FDA approved commercial drug, depending on the timing of their study participation.

Details of leptin administration and dosing for the subjects who are leptin treated are as follows:

Formulation, storage conditions/stability, and drug reconstitution are detailed in the package insert (Appendix C). For patients receiving metreleptin through the NIH Pharmacy, the following additional conditions will apply:

*Storage Conditions and Stability:*

The study drug must be stored in a secure location under controlled conditions at the study site or pharmacy before dispensing to subjects. Metreleptin must be stored at 2-8<sup>0</sup> C prior to reconstitution. It is recommended that the refrigerator be connected to a back-up power source, and a temperature alert system. Aegerion Pharmaceuticals must be notified if any test material is exposed to excessive or uncontrolled temperatures; possible replacement of the affected material will be considered. Study staff must be instructed in proper storage of the study drug.

*Storage Temperature Monitoring:*

Records of the storage conditions should be maintained throughout the study by either a continuous temperature recording system, a regularly maintained temperature alarm system or by regular visual inspection of a calibrated thermometer placed inside the refrigeration unit.

*Expiration Dating:*

As per standard practice for experimental biologic pharmaceuticals, Aegerion Pharmaceuticals will conduct periodic stability assays to monitor product stability and determine appropriate expiration dating of the study drug. The appropriate Aegerion Pharmaceuticals representative shall communicate this information to the investigator.

*Availability*

Metreleptin is being supplied by Aegerion Pharmaceuticals.

*Initial Drug Shipment and Re-Supply*

A signed and completed Drug Request Form must be emailed, at least 5 days prior to the expected delivery date, to Aegerion Supply Chain for approval and processing at [Orders.GAP@aegerion.com](mailto:Orders.GAP@aegerion.com). At the end of the study, and after full accountability is performed on study drug or site closure, study drug destruction will occur at NIH.

*Supply of Drug*

The study test drug will be shipped to the investigator's institution and should be checked for amount and condition of the drug received. This data will be entered into the proof of receipt letter. The proof of receipt letter should be faxed to the appropriate Aegerion Pharmaceuticals representative at the number indicated on the letter and the original retained in the pharmacy files.

*Side effects:*

Side effects of metreleptin observed in all clinical studies, including in patients with generalized or partial lipodystrophy, are detailed in the package insert (Appendix C)

*Dosing of metreleptin:*

*Leptin treated subjects:* Leptin treated subjects take leptin as once or twice daily subcutaneous injections, at doses between 0.06 and 0.24 mg per kg per day. The total daily dose and dosing interval is determined by both clinical response and the total volume of medication to be delivered. During Period 1, the leptin treated group will continue their previous dose of leptin ( $\leq 0.24$  mg/kg/day), and injections will be given at approximately 8 am, and 8 pm (for patients on twice daily dosing).



*Leptin naïve subjects:* For leptin naïve patients, leptin will be initiated at a dose of 5 mg twice daily in subjects weighing at least 50 kg. This dose is equivalent to 0.14 mg/kg/day for a 70 kg individual. For subjects weighing less than 50 kg, leptin will be initiated at a dose of 0.15 mg/kg/day, divided twice daily. For comparison purposes, a typical starting dose of leptin given to lipodystrophy patients who were previously enrolled in protocol 02-DK-0022 is 0.08-0.1 mg/kg/day, and the range of doses used in these patients is between 0.06 and 0.24 mg/kg/day. Thus, the starting dose of leptin used in this study is slightly higher than that which was typically used in protocol 02-DK-0022, but well within the typical dose range. This higher starting dose was chosen to maximize the probability of observing effects from leptin within the short, 2 week duration of this study. We do not anticipate a significantly increased risk of adverse events related to this higher starting dose (see Risks). The first dose of leptin will be given at approximately 8 am on the first day of Period 2 (Day 0). Dosing will continue unchanged throughout Period 2. At discharge, leptin doses may be adjusted based on individual patient responses, and future dose adjustments will be conducted as outlined below.

#### ***Dose escalation:***

The dose of metreleptin will be increased beyond 5 mg twice daily (or 0.15 mg/kg/day in patient under 50 kg) only if there is a clear decline in metabolic status without alternative explanations for the metabolic change (such as an infection, noncompliance or dietary indiscretion). It is important to note this because of the wide range of variation in the clinical presentation of these patients, it is impossible to define pre-determined thresholds of metabolic parameters that would appropriately guide dose modifications. Instead, the PI will use best clinical judgment to make dose modifications based on the constellation of metabolic and clinical data available for each patient. Dose escalations will be capped such that the total dose administered will not exceed 0.24 mg/kg/day for any patient without seeking prior permission from the FDA, the NIDDK/ NIAMS IRB and Aegerion Pharmaceuticals, the manufacturer of Metreleptin. If subjects do not tolerate a higher dose level, they can continue the study at the next lowest tolerated dose. Based on previous clinical experience with Metreleptin, tolerance to Metreleptin at doses between 0.12-0.24 mg/kg/day is not expected to be an issue.

#### ***Dose reduction:***

The dose of metreleptin will be decreased if a subject experiences excessive weight loss (BMI below the normal range for age and sex, or a lesser degree of weight loss that is distressing to the patient). Doses will also be decreased if a subject experiences injection site reactions that cannot be controlled with antihistamines and/or analgesics.

#### ***Leptin Withdrawal***

For leptin treated subjects, the last dose of leptin will be given on the final day of Period 1 (Day -1) at either 8 am or 8 pm, depending on whether the subject receives once or twice daily dosing. No leptin will be given during Period 2 (Days 0-13). Leptin will be resumed on the morning of Day 14 at the previous dose. In patients who were treated with leptin under protocol 02-DK-0022, we typically recommend tapering of leptin over a 1 week period in patients who stop leptin treatment in an outpatient setting. In contrast, in this protocol, leptin will be stopped without a taper under inpatient observation. The risks of stopping leptin are discussed under Risks.

#### ***Pre-study Medications***

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

#### ***Glucose Management***

*Leptin naïve subjects:* Leptin naïve subjects who take insulin or insulin secretagogues may be at risk of hypoglycemia after leptin initiation due to improvements in insulin sensitivity. Bedside blood glucose monitoring will be performed prior to meals and at bedtime (or more frequently if clinically indicated) in diabetic



subjects. Adjustments in insulin doses will be made on an as-needed basis to maintain blood glucose within a target range of 100-180 mg/dL.

*Leptin treated subjects:* Bedside blood glucose monitoring will be performed prior to meals and at bedtime (or more frequently if clinically indicated) in all subjects in this group. In leptin-treated subjects who take insulin, home insulin doses will be continued throughout the study. We anticipate that subjects in the leptin treated group (regardless of whether they require insulin at home) may have worsened insulin sensitivity during leptin withdrawal, and hence, may experience hyperglycemia. As changes in blood glucose are a major outcome of this study, insulin will not be started, nor will home insulin doses be increased, unless a subject has fasting blood glucose greater than 300 mg/dL, non-fasting glucose > 500, or a 200 mg/dL increase from baseline in either fasting or non-fasting glucose. Unlike patients with type 1 diabetes, patients with lipodystrophy are at extremely low risk of developing diabetic ketoacidosis; hence, the risks associated with short-term hyperglycemia are minimal (see Risks).

### **Standardized Exercise**

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

### **Study Procedures**

Subjects will undergo metabolic and endocrine testing at the end of Period 1 and at the end of Period 2. Certain tests will also be performed at earlier time points during Period 2, in order to clarify the time course of leptin initiation and withdrawal on these outcomes. Explanations for the timing of these tests are provided in the detailed test descriptions. A summary of the test schedule is in the table, below. Tests associated with Period 1 and the follow-up are highlighted in gray. The exact timing of each test may vary slightly due to practical scheduling issues. A sample patient calendar is given in Appendix A.

|                              | Period 1 |    |    |    |    | Period 2 |   |   |   |   |   |   |   |   |   |    |    |    |    |    |
|------------------------------|----------|----|----|----|----|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Study Day                    | -5       | -4 | -3 | -2 | -1 | 0        | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Metabolic Chamber            |          |    |    |    | x  |          |   |   |   |   |   |   | x |   |   |    |    |    | x  |    |
| 24 hr urine                  |          |    |    |    | x  |          |   |   |   |   |   |   | x |   |   |    |    |    | x  |    |
| DEXA                         |          |    |    |    |    | x        |   |   |   |   |   |   |   |   |   |    |    |    |    | x  |
| Skin temperature             |          |    | x  |    | x  |          |   |   |   |   | x |   |   |   |   |    | x  |    | x  |    |
| Exercise test                |          |    | x  |    |    |          |   |   |   |   | x |   |   |   |   |    | x  |    |    |    |
| Resting Energy Expenditure   | x        |    |    | x  |    |          | x |   |   |   | x |   |   |   | x |    |    | x  |    |    |
| Blood pressure monitoring    |          |    |    | x  | x  | x        | x |   |   |   |   |   |   |   |   |    |    | x  | x  |    |
| Heart rate monitoring        |          |    |    | x  | x  | x        | x |   |   |   |   |   |   |   |   |    |    | x  | x  |    |
| Euglycemic Clamp             |          |    |    | x  |    |          |   |   |   |   |   |   |   |   |   |    |    | x  |    |    |
| Glucose & lipid turnover     |          |    |    | x  |    |          |   |   |   |   |   | x |   |   |   |    |    | x  |    |    |
| LH pulsatility               |          |    | x  |    |    |          |   |   |   |   |   |   |   |   |   |    |    | x  |    |    |
| MRI/MRS                      |          |    |    | x  |    |          |   |   |   |   |   |   |   |   |   |    | x  |    |    |    |
| Fasting research blood tests | x        |    |    |    | x  | x        |   | x |   | x |   | x |   | x |   | x  |    | x  | x  |    |
| Safety labs                  | x        |    |    |    | x  | x        |   | x |   | x |   | x |   | x |   | x  |    | x  | x  |    |
| Actigraphy                   |          |    | x  | x  | x  | x        | x | x | x | x | x | x | x | x | x | x  | x  | x  | x  |    |
| Vital signs                  | x        | x  | x  | x  | x  | x        | x | x | x | x | x | x | x | x | x | x  | x  | x  | x  | x  |
| Anthropometrics              | x        |    |    |    |    | x        |   |   |   |   |   |   |   |   |   |    |    |    |    | x  |
| Bedside glucose monitoring   | x        | x  | x  | x  | x  | x        | x | x | x | x | x | x | x | x | x | x  | x  | x  | x  | x  |
| Pregnancy test               | x        |    |    |    |    |          |   |   |   |   |   |   |   |   |   |    | x  |    |    |    |
| Echocardiogram               |          | x  |    |    |    |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |
| Ultrasounds                  | x        |    |    | x  |    |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |
| OGTT                         | x        |    |    |    |    |          |   |   |   |   |   |   |   |   |   |    |    |    |    |    |

### Long-term follow-up

Certain effects of leptin may not be detectable, or may be submaximal, within the 2-week intervention period in this study. These include changes in liver steatosis and insulin sensitivity. For this reason, we wish to study the longer-term (6 months) effects of leptin in subjects participating in this study. Therefore, we plan to restudy subjects in the leptin naïve group and bring them in for follow-up 6 months (range, 5-9 months) after starting leptin. Given budgetary and logistical issues, foreign patients may be seen for follow-up 12 months after initiating leptin therapy. We will ask them to obtain lab work locally and send us the results. It will not be possible to maintain an isocaloric diet for 6 months after leptin initiation in the free-living environment.

Therefore, long-term follow-up data on leptin's effects will not help to answer questions about the energy intake independent effects of leptin. Nonetheless, these data will provide valuable additional information about the time course of leptin's actions in lipodystrophy. Testing during the follow-up visits will mimic that performed during Period 1.

### **Metabolic Testing**

1. Exercise testing: Testing will be performed at the end of Period 1, in the middle and end of Period 2, and during the long-term follow-up visit for subjects in the leptin-naïve group. Skeletal muscle work efficiency will be measured as the increment in energy expenditure above resting energy expenditure during exercise, with lower incremental increase in energy expenditure corresponding to greater muscle work efficiency. This test will be conducted on a cycle ergometer. Following a 10 minute warm-up period, subjects will pedal at 60 rpm with the resistance adjusted to generate 10W of power for 4 minutes. The resistance will then be increased such that the power generated is 25W for 4 minutes, then 50W for 4 minutes. Breath-by-breath oxygen consumption and carbon dioxide production will be collected and averaged every 30 seconds using a metabolic cart (ParvoMedics TrueOne2400, Sandy UT). Heart rate will be monitored by electrocardiograph.
2. Free-living physical activity (actigraphy) will be quantified minute-by-minute using small, portable pager-type and watch accelerometers at the subjects' hip and wrist. Overall physical activity levels, daily changes, amount of time spent in sedentary, moderate, vigorous intensity categories and activity-associated energy expenditures will be extracted [35]. Actigraphy will be conducted continuously throughout the study, beginning on Day -5, and ending on Day 14, and during the long-term follow-up visit for subjects in the leptin-naïve group
3. Resting Metabolic Rate (RMR): The RMR will be measured upon awakening before breakfast after a minimum 8 hour fast, in a resting supine position using the standard technique of the Clinical Center. This is done using a microprocessor-controlled indirect calorimetry device (ParvoMedics TrueOne2400, Sandy UT) that measures oxygen consumption and carbon dioxide production. The procedure involves positioning a canopy over the patient's head that allows the patient to exchange gas with an open-circuit design. The recording should be for at least 20 minutes after achieving a steady curve. This measurement is done under conditions of minimal physical activity. The amount of carbon dioxide generated and oxygen consumed by the patient is determined by calorimeter. RMR measurements will be obtained after admission to assist with estimation of caloric requirements, once at the end of Period 1, and approximately every 4 days during Period 2 in order to look for any time-course effects of leptin initiation of withdrawal. RMR will also be measured during the long-term follow-up visit for subjects in the leptin-naïve group
4. DEXA scan for total body composition: Measurements of fat-free mass will be obtained and used to normalize energy expenditure measurements. Measurements will be obtained at the end of Period 1 and the end of Period 2, and during the long-term follow-up visit for subjects in the leptin-naïve group, immediately after leaving the metabolic chamber. A DEXA scan (iDXA, GE Healthcare, Madison WI) will be performed to determine total and regional body fat and lean soft tissue masses, bone mineral content and density. DEXA produces photons at two different energy levels, 40 and 70 KeV. The photons pass through tissues and attenuate at rates related to elemental composition. Bone mineral, with highly attenuating calcium and phosphorous, is readily distinguished from soft tissues. The different elemental profiles of fat and bone-mineral free lean components allows for the analysis of soft tissue fat content, so that bone mineral, fat, and bone mineral fat-free lean components may be resolved.
5. Blood pressure monitoring: Blood pressure will be monitored every 30 minutes using an automated ambulatory blood pressure monitor. Because leptin's effects on blood pressure have been reported to be rapid and transient following leptin initiation (Sadaf Farooqi, unpublished observations), testing will be

focused on the 48 hours immediately before and after leptin initiation and withdrawal. An additional 48 hours of monitoring will be performed at the end of Period 2, and during the long-term follow-up visit for subjects in the leptin-naïve group, to look for longer term effects.

6. Heart rate variability: Heart rate will be measured and recorded using a portable ECG Holter monitor, which will be downloaded and analyzed for heart-rate variability using power spectrum analyses. Testing will be performed in conjunction with blood pressure monitoring.
7. Visual Analog Scales (hunger, thermal regulation): Patients will be given a questionnaire to determine their level of thermal comfort and hunger using visual analog scales (VAS), as shown in Appendix III. The hunger VAS question has been characterized previously, and is digitized exactly onto an iPad app<sup>44</sup>. Testing does not require supervision, and will be performed on a daily basis after lunch.
8. Skin temperature: Wireless probes (iButtons, Maxim Inc., Sunnyvale, CA) will record skin temperature with data reported each minute. The positions of the skin sensors at five sites (deltoid, hand, pectoralis major, anterior thigh, and shin) will be according to the ISO standards<sup>45</sup>. Skin temperature monitoring will be performed prior to, during, and for one hour following each exercise test, as well as throughout each stay in the metabolic chamber.
9. Metabolic chamber: The room calorimeter is a specially constructed room designed to assess the metabolism of human subjects. Designed as a walk-in “pull” calorimeter, it is an open circuit unit that draws conditioned room air into the room calorimeter at the same flow rate as it is extracted into the gas analysis system<sup>42</sup>. Each of the three rooms is equipped with toilet and sink with privacy screen, treadmill, bed, desk, and computer with access to television and other forms of entertainment. In addition to the detailed measurements using portable activity monitors, a general physical activity level is also measured continuously through a wall mounted monitoring device (microwave sensor). Food and fresh water is passed through an air-lock drawer system. In this rapid-response room calorimeter, the sleeping energy expenditure, diet induced thermogenesis<sup>43</sup> and the specific energy costs of prescribed non-intense physical activity types<sup>44</sup> will be assessed. A recent study of our first room calorimeter showed better than  $\pm 85$  kcal/day (95% Confidence Interval) reproducibility in total energy expenditure in 10 healthy normal subjects (total energy expenditure ranged from 1551 to 3559 kcal/day), and  $\pm 0.023$  in average and sleeping RQ. Compared to the literature, this is the most precise system to date. Continuous nursing supervision with telemetry cardiac monitoring is a standard procedure to enhance patient safety<sup>46-48</sup>. Subjects will wear standardized clothing while in the metabolic chamber in order to increase uniformity in measurements of skin temperature and thermal comfort. Subjects will have a 30 minute treadmill walking period in the chamber in order to bring out any effects of altered skeletal muscle work efficiency on total energy expenditure. Subjects will stay in the metabolic chamber at the end of Period 1, in the middle and end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group.
10. Glucose and Lipid Turnover: Stable isotope tracers will be used to measure glucose and lipid turnover. Endogenous glucose production will be measured using steady-state infusion of [6,6-<sup>2</sup>H<sub>2</sub>] glucose tracer. The fractional rate of gluconeogenesis will be measured using steady-state oral dosing of deuterated water (<sup>2</sup>H<sub>2</sub>O)<sup>49</sup>. The rate of lipolysis will be measured using steady-state infusion of deuterium-labeled <sup>2</sup>H<sub>5</sub>-glycerol, and the fatty acid turnover rate will be measured using steady-state infusion of [U-<sup>13</sup>C<sub>16</sub>] palmitate. Subjects will fast after 8 pm. A total of 3 grams per kg lean body mass of <sup>2</sup>H<sub>2</sub>O will be given in four divided doses every two hours at 9 pm, 11 pm, 1 am, and 3 am in order to enrich the subject's body water pool to approximately 0.5% <sup>2</sup>H<sub>2</sub>O. Beginning at ~5 am, a primed [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion will be given for 3 hours, after which blood samples to measure isotope enrichment will be measured over a period of 30 minutes at steady state. Two hours after the [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion begins, a primed <sup>2</sup>H<sub>5</sub>-glycerol infusion, and unprimed [U-<sup>13</sup>C<sub>16</sub>] palmitate infusion will be given for one hour, and

isotope enrichment will be measured over a period of 30 minutes at steady state. Tracer studies will be performed at the end of Period 1, in the middle and end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group. To assess the effects of hyperinsulinemia on glucose turnover, the glucose tracer infusion will be continued during the euglycemic, hyperinsulinemic clamp study, below, with blood samples for tracer enrichment obtained during the steady-state period of the clamp.

11. Euglycemic Hyperinsulinemic Clamp: This is the gold-standard test to measure insulin sensitivity. For insulin treated subjects, an overnight insulin drip may be given beginning at 6 pm the night prior to the clamp to maintain euglycemia. After an 8-hour fast, insulin will be infused at a dose of 120 mcU/m<sup>2</sup>\*minute. This dose is designed to suppress endogenous glucose production and near-maximally stimulate glucose uptake in this group of very insulin resistant subjects. For a small number of subjects with extreme insulin resistance (requiring more than 200 units of insulin per day), higher doses of insulin may be used during the clamp study to create a state of hyperinsulinemia above the subject's baseline state. 20% dextrose enriched with 2.5% [6,6-<sup>2</sup>H<sub>2</sub>] glucose tracer will be infused at a variable rate to maintain blood glucose at approximately 90 mg/dL. If subjects remain hyperglycemic without dextrose infusion after 1 hour of insulin infusion (by which time maximum insulin effect is expected), the clamp will be considered "failed" and will be prematurely terminated. During the insulin infusion, blood samples (0.5 mL) will be obtained every 5 minutes to measure blood glucose at the bedside. At steady state, the rate of dextrose infusion provides an estimate of insulin-stimulated glucose disposal. Additional samples will be drawn to measure the glucose and lipid tracers (described above), insulin, and other hormone levels. Due to the blood volumes and inconvenience of this test to participants, it will be performed once at the end of Period 1, once at the end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group. If technical problems occur with a clamp study (e.g. IV failure or failure to attain steady-state glucose and insulin levels), the clamp may be repeated if blood volume limits permit.
12. Fasting research blood tests: Fasting glucose, insulin, C-peptide, markers of insulin action (e.g. IGFBP2), lipid panel, free fatty acids, and high-sensitivity C-reactive protein will be measured every other day. Markers of bone turnover (□-CTX and P1NP) will be obtained twice at the end of Period 1, twice at the end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group. Additional blood samples will be stored for future analysis of leptin and inflammatory markers—as well as used to measure circulating RNA and DNA during periods in which patients do or do not receive leptin, to determine the effects of leptin on circulating nucleic acids and pharmacopigenomics of leptin therapy.
13. Biochemical evaluation: A baseline biochemical evaluation, which includes: electrolytes, BUN, creatinine, LDH, CPK, SGOT, SGPT, bilirubin, alkaline phosphatase, calcium, magnesium, albumin, protein, iron, and transferrin, will be conducted during Period 1 and at follow-up.
14. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS): Hepatic, muscle and central body fat will be measured by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). MR studies will be performed at the end of Period 1, at the end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group. Studies are performed on a 3T MRI scanner using phased-array coils. B0 shim parameters are optimized with a breath hold B0 mapping method (1). Navigator gated T2 weighted (T2w) and breath-hold T1 weighted (T1w) images in coronal and axial orientations are used to prescribe a 20x20x20 mm<sup>3</sup> spectroscopy volume in an area with high signal to noise ratio, free of obvious fatty structures or blood vessels. After additional manual shimming, and optimization of transmit power and WS RF level 32 signals are averaged with a minimum repetition time (TR) of 3s and 35 ms echo time (TE) with WET water suppression (WS) followed by 4 signals averaged with WS RF power set to zero. Spectral data are fitted with AMARES (2) using a model of four resonances for the lipid signals on the WS spectra. Additionally, the apparent T2's of the summed

lipid CH2 and CH3 resonances lipid and the T2 of water are measured with five quick expiration breath hold non-WS spectra averaging two signals each at TR 3s and TE 24, 36, 48, 96 and 144 ms. These T2 values are used to correct the water and total lipid signals for signal decay in the 35 ms echo delay. Fat fraction is then calculated as from the T2 corrected signals Swater and Slipid as: fat fraction = Slipid/(Swater+Slipid). Visceral and subcutaneous fat measurements will be obtained using standard T1 Spin Echo technique which presents fat as a bright signal that can be segmented for area and volume measurements.

15. 24 hour urine studies: Urine will be collected during each stay in the metabolic chamber (at the end of Period 1, in the middle and end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group). Samples will be analyzed for nitrogen excretion to assess macronutrient oxidation, as well as excretion of glucose, protein and minerals.
16. Oral glucose tolerance test: After a 12-hour fast preceding the test, subjects will be given 75 gram (1.75gm/kg in pediatric patients less than 40kg) oral glucose solution. Venous glucose, plasma insulin and free fatty acid levels (3 cc blood to be drawn at each draw) will be obtained from blood samples drawn at -10, 0, 30, 60, 90, 120 and 180 minutes during the oral glucose tolerance test.

### **Endocrine Evaluation**

1. LH pulsatility will be assessed using overnight sampling. Blood will be sampled from an indwelling IV catheter every 10 minutes between 11 pm and 7 am. Arithmetic mean levels for LH will be calculated. Analysis of pulsatile secretion of LH will be determined using a modification of the Santin and Bardin Method<sup>50</sup>, which requires a 20% increase from nadir to peak LH concentrations for pulse detection. LH pulse frequency will be determined by calculating the number of LH pulses during the sampling interval. LH pulse amplitude will be calculated from the difference between the nadir and peak hormone levels. Due to the blood volume requirements of this test, it will be performed once at the end of Period 1, once at the end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group.
2. Thyroid function will be assessed as serum levels of total and free thyroxine, triiodothyronine (T3), and thyroid stimulating hormone (TSH). Thyroid function tests will be performed at the end of Period 1, in the middle and end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group.
3. Catecholamines: Plasma and 24 hour urine catecholamines will be measured to assess effects of leptin on adrenal medullary function. Testing will be performed at the end of Period 1, in the middle and end of Period 2, and during the follow-up visit for subjects in the leptin-naïve group.

### **Safety Labs**

1. Amylase and lipase will be drawn approximately every other day in the leptin withdrawal group at the time of the Fasting Research Blood draws.
2. Pancreatic elastase will be measured in stool samples approximately every other day (depending on when subjects are able to provide stool samples).
3. Urine or serum pregnancy testing will be obtained on female subjects with reproductive potential after admission, and will be repeated prior to radiation exposure (for DEXA scans) as needed. If the study participant is a minor, and she is found to have a positive pregnancy test, we will inform her. If the patient grants us permission to do so, we will discuss this with her parent(s) so that they can help their daughter make good medical decisions.
4. Immunogenicity testing: As a post marketing requirement, blood will be drawn at baseline and at the 6 month follow-up visit for measurement of neutralizing antibodies to leptin.



## **Imaging Studies**

1. Ultrasound of pelvis: This will be performed on female patients to evaluate the effects of hyperinsulinism and hyperandrogenism on the ovaries. The ovarian volumes, number of cysts and cyst-volumes will be recorded.
2. Echocardiogram: This will be performed to evaluate the presence of cardiomyopathy or heart failure before starting leptin. In the NIDDK long term leptin study those with generalized lipodystrophy frequently had signs of hypertrophic cardiomyopathy.
3. Ultrasound of abdomen or liver: This will be performed to evaluate size and echogenicity of the liver and other abdominal organs before starting leptin and at follow-up.
4. Ultrasound of thyroid: This will be performed at Baseline on all enrolled patients with insulin resistance to screen for possible papillary thyroid carcinoma (PTC) since this patient population may be at a higher risk of developing PTC. The testing may also uncover information for future analysis.
5. Other imaging studies as clinically indicated:
  - a. Long bone X-rays to evaluate osseous lesions and to provide further definition as to the natural history of such lesions.

## **Follow-up**

### Post-Study Treatment

Subjects newly starting leptin who decide not to complete the entire 19 days of the study will be given the option to continue leptin and to come back for follow-up and evaluation in six months.

Patients who have generalized lipodystrophy and who reside within the US may continue leptin treatment as an FDA-approved drug through their home endocrinologist. These patients may also continue to receive care at NIH via protocol 76-DK-0006 (Studies of Molecular Genetics of Insulin Secretion, Insulin Action, and Diabetes Mellitus). However, leptin drug supply will not be offered through this study.

Patients who have partial lipodystrophy are not eligible to receive leptin treatment as an FDA-approved drug. In addition, patients with generalized lipodystrophy who do not live in the US cannot obtain FDA-approved drug despite being on-label indication. After completing 6 months of leptin treatment in this study, these patients will have the following options:

1. Taper leptin over 1 week and discontinue the drug
2. Receive leptin through other clinical trials (at NIH or elsewhere) if such trials are available
3. Receive leptin through a compassionate use IND

These choices will be discussed with patients and study staff will assist with decision-making, based on drug availability and the patient's clinical improvement (or lack thereof) with leptin therapy. Regardless of the option chosen, partial lipodystrophy patients and non-US generalized lipodystrophy patients may also continue to receive care at NIH via protocol 76-DK-0006.

### Post-Study Obligations

There are no anticipated post-study obligations.

## **Human Subject Protection**

### **Data and Safety Monitoring Plan**

The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team. Serious adverse events will be reported to the NIDDK/NIAMS IRB per NIH guidelines (below). Overall accrual and adverse event information will be reported to the NIDDK/NIAMS IRB annually.



As required by FDA 21 CFR 312.50 (and NIH OHSRP's SOP 23), trial procedures will be subject to review and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements with the NIDDK quality assurance program plan. Audit and/or monitoring visits results will be reported to the Principal Investigator/Sponsor for further reporting to the FDA consistent with applicable regulations. The specific monitoring plan will be developed with the Principal Investigator and frequency of monitoring visits determined by such factors as study enrollment, data collection status and regulatory obligations. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

### **Adverse Events, Protocol Deviations, and Unanticipated Problems**

Adverse events, protocol deviations, unanticipated problems (UP), serious adverse events (SAEs), sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded and summarized at the time of annual review. Serious unanticipated problems and serious protocol deviations, will be reported to the NIDDK/ NIAMS 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 NIDDK/ NIAMS IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Non-serious protocol deviations will only be reported to the NIDDK/ NIAMS IRB (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

### **Adverse Events**

An **Adverse Event (AE)** is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The causal relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

### ***Non-Serious Adverse Event Collection and Reporting***

The collection of non-serious AE information should begin once a subject's written consent to participate in the study has been obtained. Any significant worsening noted during interim or final physical examinations,

electrocardiogram, x-ray filming, any other potential safety assessment required or not required by protocol should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious (see Section “Serious Adverse Events Reporting & Collection”). Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

#### *Laboratory Test Result Abnormalities*

The following laboratory test result abnormalities should be captured on the non-serious AE CRF page or SAE Report Form as appropriate:

1. Any laboratory test result that is clinically significant or meets the definition of an SAE
2. Any laboratory test result abnormality that required the subject to have study drug discontinued or interrupted
3. Any laboratory test result abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

All identified non-serious AEs must be recorded and described on the non-serious AE page of the case report forms (CRF). Completion of supplemental CRFs may be requested for AEs and/or laboratory abnormalities that are reported/identified during the course of the study.

#### **Serious Adverse events**

A Serious adverse event is defined as one of the following (*in accordance with FDA 21 CFR312.32 and the NIH intramural guidance for principal investigators on reporting adverse events*)

- death from any cause,
  - life threatening event, i.e., an event that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred.
  - any event that requires or prolongs in-patient hospitalization
  - any event that results in persistent or significant disability/incapacity
  - any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study.
  - other medically important events that in the opinion of the investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.
- Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE. Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events will be handled as SAEs (per Aegerion Pharmaceuticals request via United Biosource Corporation, the CRO contracted by Aegerion to perform Pharmacovigilance activities on their behalf).

#### **NOTE:**

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure

- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

### *Pregnancy*

All pre-menopausal women included in the study will be asked to use an effective method of contraception. If necessary, a gynecology consultation will be obtained to assist the patients to choose an appropriate method of contraception.

Should a patient become pregnant while taking metreleptin, metreleptin may be continued during pregnancy under the following circumstances:

1. The investigators feel that metreleptin withdrawal would constitute a harm to mother and fetus.
2. The patient understands that the effects of metreleptin in pregnancy are unknown, and wishes to continue.
3. The patient receives prenatal care from a high-risk obstetrician experienced in managing diabetes during pregnancy. Management of diabetes must be the primary responsibility of the patient's local health care providers, although the NIH study team will provide advice as appropriate regarding metreleptin use and use of concentrated insulin (U-500) if necessary.
4. The patient and her local health care team agree to remain in close contact with the NIH study team and respond to requests for clinical information needed to document the course of pregnancy and its outcome.

Should a patient become pregnant while on study, per 45 CFR 46.204, (1) no inducements, monetary or otherwise, will be offered to terminate a pregnancy, (2) individuals engaged in research will not be involved in any decisions as to timing, method, or procedures used to terminate a pregnancy, and (3) individuals engaged in the research will have no part in determining viability of a neonate.

The investigator must immediately notify the Aegerion Pharmaceuticals (or designee) Medical Monitor of this event and complete and forward a Pregnancy Surveillance Form to United Biosource Corporation (UBC) (or designee) within 7 business days via email to [AegerionPV@UBC.com](mailto:AegerionPV@UBC.com) and Francine.Galibert@UBC.com.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form. Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

### *Overdose*

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

### *Serious Adverse Event Collection and Reporting*

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 30 days of discontinuation of dosing. The investigator should report

any SAE that occurs after these time periods and that is believed to be related to study drug or protocol-specified procedure.

An SAE report should be completed for any event where doubt exists regarding its seriousness. If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

SAEs, whether related or not related to study drug, (including pregnancies) must be reported to Aegerion Pharmaceuticals on an SAE Report Form or similar form (e.g. CIOMS, MedWatch) and submitted as soon as possible but not more than 7 calendar days in the event of a death or a life-threatening event or in 15 calendar days for all other SAEs. SAE reports within 7 calendar days are to be transmitted via email:

**SAE Email Address:** [AegerionPV@UBC.com](mailto:AegerionPV@UBC.com) and [Francine.Galibert@ubc.com](mailto:Francine.Galibert@ubc.com)

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported). If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent to the Aegerion Pharmaceuticals (or designee) using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

Per NIH HRPP SOP 16, all serious adverse events will be reported to the NIDDK/NIAMS IRB at the time of annual review. The PI will report all deaths (that are not Unanticipated Problems) to the NIDDK CD and NIDDK/NIAMS IRB as soon as possible, but not more than 7 days after the PI first learns of the event, unless otherwise specified by the NIDDK CD and documented in the protocol.

#### *Deaths*

Per NIH HRPP SOP 16, the PI will report all deaths (that are not Unanticipated Problems) to the NIDDK Clinical Director and the NIDDK/NIAMS IRB as soon as possible, but not more than 7 days after the PI first learns of the event, unless otherwise specified by the NIDDK CD and documented in the protocol.

#### **Rationale for Subject Selection**

Subjects eligible for this study include mature adolescents and non-elderly adults with lipodystrophy. All subtypes of lipodystrophy (i.e. generalized, partial, acquired, and congenital) will be eligible. Although restricting the study to one or two lipodystrophy subtypes (such as the most severe forms: acquired and congenital generalized) would likely give greater homogeneity in the patient population, such restrictions would dramatically decrease feasibility in this extremely rare condition. Because each subject will be compared to him/herself in this within-subjects design, the effects of inter-subject heterogeneity will be less important.

For leptin-treated patients who are withdrawing from leptin, this study is of greater than minimal risk, and without prospect of direct benefit to subjects, but likely to yield generalizable knowledge about the subjects' condition, e.g. lipodystrophy. For those in the leptin naïve arm of the study, there is a prospect of direct benefit. Based on these criteria, mature adolescents ages 14-17 years will be permitted to participate in the leptin-naïve arm of the study. Even though there is the potential for clinical benefit, younger children and adults who are unable to give informed consent are unlikely to possess the emotional maturity to participate in such a complex and time-consuming study. Because the risk of pancreatitis with leptin withdrawal represents greater than a minor increment over minimal risk, children will not be enrolled in the leptin withdrawal arm of the study. As we gain more experience in risks of leptin withdrawal in adults, we may request NIDDK/NIAMS IRB permission to include children in this arm in the future. Adults over age 70 years are excluded in order to decrease inhomogeneity related to an aging population. Because there is no prospect for direct benefit, adults who are unable to give informed consent will not be eligible.

### Withdrawal Criteria

1. **Withdrawal of consent.** A subject wishes to withdraw from the study as stated in the informed consent (all subjects reserve the right to withdraw from the study without prejudice).
2. **Adverse event.** A subject experiences an adverse event that in the investigator's opinion necessitates withdrawal from the study. Specific examples of adverse events that would result in withdrawal are:
  - a) Pancreatitis during the leptin withdrawal period in the leptin treated group. Pancreatitis is a clinical diagnosis, and will be suspected in the presence of moderate to severe abdominal pain accompanied by nausea and/or vomiting. If pancreatitis is suspected, amylase and lipase levels will be drawn, and GI consultation obtained. However, even if amylase and lipase levels are normal, in the presence of typical signs and symptoms of pancreatitis, leptin will be restarted.
  - b) Diabetic ketoacidosis during the leptin withdrawal period in the leptin treated group
3. **Investigator decision.** An investigator feels it is in the subject's best interest to terminate participation. The detailed reasoning behind this decision will be documented.
4. **Protocol violation.** Includes subject noncompliance, pregnancy, study entry criterion violation, or start of an unacceptable concomitant medication.

Subjects in the leptin treated group, who undergo leptin withdrawal during this study, will be immediately restarted on leptin if they withdraw from the study for one of the above reasons.

### Risks/Benefits Analysis including Considerations of Alternatives to Participation

#### Benefits

1. Leptin Initiation: Leptin naïve subjects enrolled in this study will initiate leptin treatment under this treatment protocol. Since the patients included in this protocol have metabolic abnormalities, they have some likelihood of considerable clinical benefit from this trial. The potential benefits of leptin in lipodystrophy are discussed in detail in protocol 02-DK-0022. In brief, leptin has been shown to cause short-term improvements (within 14 days, the duration of the current protocol) in blood glucose and triglyceride levels<sup>31</sup>. Long-term benefits (over 3 years) of leptin in lipodystrophy have been demonstrated under protocol 02-DK-0022, and include decreased hemoglobin A1c, triglycerides, ALT and AST<sup>51</sup>. Given the clear benefits, in February 2014 the FDA approved leptin (Myalept) for treatment of individuals with generalized lipodystrophy. A review of the data obtained in 02-DK-0022 showed that leptin also leads to clinically important improvements in patients who have partial lipodystrophy with significant metabolic disease.
2. Leptin Withdrawal: Subjects undergoing leptin withdrawal as part of this study will not have any real or potential personal medical benefit accruing from study participation. However, they will contribute to important research that has the potential to improve medical treatment of lipodystrophy patients in the future.

#### Risks/Discomforts

1. General: Some patients may find the time needed to complete the research studies an inconvenience in their routine lives.
2. Leptin Initiation: Leptin naïve subjects enrolled in this study will initiate leptin treatment under this treatment protocol. The risks of leptin are discussed in detail in the drug package insert (Appendix C). In brief, adverse effects of leptin include mild to moderate injection site reactions, headache, temporary flu-like symptoms, and the development of non-neutralizing antibodies that do not interfere with clinical efficacy. Three subjects who were enrolled in 02-DK-0022 developed antibodies that may block the effects of leptin. All three subjects had congenital generalized lipodystrophy. Two of these subjects had episodes of sepsis after the development of neutralizing antibodies (multiple episodes in one patient and a single, unverified episode in the other patient). Both patients had risk factors for sepsis including



cirrhosis with portal hypertension, and severe periodontal disease. It is unknown if these sepsis episodes were related to the presence of neutralizing antibodies. The subject with multiple sepsis episodes was withdrawn from leptin in order to improve quality of life, as her life expectancy is limited. She did not experience worsening of metabolic status after leptin withdrawal, suggesting a possible loss of efficacy of leptin replacement in conjunction with her neutralizing antibodies. The second patient has been lost to follow-up and no further clinical information is available. The third patient has not had sepsis. This patient requires the maximum dose of leptin (0.24 mg/kg/day) to maintain good metabolic control, suggesting a possible partial loss of efficacy associated with neutralizing antibodies. At this point the clinical effects of this type of blocking antibody are not established. In patients who make very little leptin, such as those with generalized lipodystrophy, a blocking antibody might make the injected leptin medication ineffective, and health status would return to where it was before starting leptin treatment. As immunodeficiency has been reported in patients who make no leptin (congenital leptin deficiency), sepsis might be related to neutralizing antibodies. In patients who make more leptin, such as those with partial lipodystrophy, a blocking antibody might not only make the injected leptin medication ineffective, it might also block the leptin the body makes, resulting in worse health status than before starting leptin treatment. In this study, leptin will be initiated at a slightly higher dose (0.14-0.15 mg/kg/day) than the typical starting dose of leptin given in protocol 02-DK-0022 (0.08-0.1 mg/kg/day). This higher dose will be used to more rapidly observe leptin's biological effects within the short, 2 week duration of this study. The dose of 0.14-0.15 mg/kg/day is well within the dose ranges that were used to treat lipodystrophy patients enrolled in protocol 02-DK-0022 (up to 0.24 mg/kg/day) and, based on our experience, is not likely to increase the risk of leptin related adverse events.

3. Leptin Withdrawal: In the current protocol, we anticipate worsening of metabolic parameters, particularly blood glucose and triglycerides, during leptin withdrawal. Risks associated with short-term worsening of blood glucose and triglycerides are discussed below.
  - a. Hyperglycemia: Leptin withdrawal for 2 weeks may increase blood glucose in many patients. To reduce this risk, patients will be continued on their home antihyperglycemic regimens, including oral medications and/or insulin. In addition, only patients with good to fair diabetes control (hemoglobin A1c < 9%) will be enrolled. Unlike patients with type 1 diabetes, patients with lipodystrophy are at extremely low risk for ketoacidosis in the context of hyperglycemia, as diabetes in lipodystrophy is typically due to insulin resistance rather than insulin deficiency. Subjects with a history of diabetic ketoacidosis will be excluded. Because blood glucose is an important endpoint, we will not initiate insulin, or increase insulin doses, in the case of mild hyperglycemia. However, for safety reasons, we will initiate or increase insulin if a subject has fasting blood glucoses greater than 300 mg/dL, non-fasting glucose >500 mg/dL, or a 200 mg/dL increase from baseline in either fasting or non-fasting glucose. A two-week period of hyperglycemia will not significantly impact the risk of long-term diabetes-related complications. Acute hyperglycemia may be associated with mild patient discomfort due to polydipsia, polyuria, blurred vision, or non-specific malaise associated with sensing a high blood sugar.
  - a. Hypertriglyceridemia: Leptin withdrawal for 2 weeks may increase blood triglycerides in many patients. To reduce this risk, patients will be continued on their home lipid-lowering regimens (e.g. statins or fibrates). In addition, only patients with fair triglyceride control (serum triglycerides < 800 mg/dL) will be enrolled. The main risk of short-term hypertriglyceridemia is acute pancreatitis. Pancreatitis is discussed in more detail, below.
  - b. Pancreatitis: There have been 3 serious adverse events associated with short-term leptin withdrawal in the course of the 12 years (approximately 500 patient-years) that leptin treatment has been given to lipodystrophy patients at NIH. Each of these SAEs is described below:
    - i. NIH-1: This is the first lipodystrophy patient treated with leptin, and with the most severe lipid abnormality, possibly due to the copresence of another lipid disorder coupled with lipodystrophy. This subject had triglycerides greater than 30,000 mg/dL leading to recurrent pancreatitis that was treated with three-times weekly

plasmapheresis. In this subject, inpatient leptin withdrawal for 10 days during a 1900 kcal/day diet led to a rise in triglycerides from 400 to 800 mg/dL. Leptin was restarted after 10 days due to the development of nausea, vomiting, and abdominal pain consistent with acute pancreatitis (but without elevated amylase or lipase). Leptin was then restarted, and the patient recovered uneventfully. Of note, ~10 years after this SAE, the patient was diagnosed with superior mesenteric artery syndrome, and her symptoms of episodic abdominal pain, nausea, and vomiting have substantially improved since this was surgically treated. Thus, this patient's symptoms during leptin withdrawal on a controlled diet may not have represented true pancreatitis.

- ii. NIH-25: This was a 15 year old patient with congenital, generalized lipodystrophy with no known history of pancreatitis. The patient was admitted to an outside hospital with acute pancreatitis following a period of poor compliance with leptin therapy (taking 4-5 of 14 prescribed doses per week). Leptin was not given in the hospital. On hospital day 3, the patient suffered a cardiac arrest and died. The cause of death was listed as shock from pancreatitis.
- iii. NIH-38: This 36 year-old patient with partial lipodystrophy and a history of recurrent pancreatitis was admitted to an outside hospital for dehydration related to colitis. Amylase and lipase were normal on admission. The outside hospital did not administer leptin, and after 2-3 days of leptin withdrawal, the patient developed elevated amylase and lipase consistent with acute pancreatitis. Leptin was restarted, and the patient recovered without incident.

Because of the risk of pancreatitis with leptin withdrawal, our group has recommended that patients discontinuing leptin in the outpatient setting (in the absence of physician supervision) gradually taper the dose over one week. In this study, in order to more rapidly test the effects of leptin withdrawal, we plan to discontinue leptin without tapering. Leptin has been discontinued in at least 25 other lipodystrophy patients, either based on patient decision (e.g. non-compliance or personal choice) or joint patient-investigator decision. In half of these patients, leptin was tapered over 1 week, and in the other half it was stopped without a taper. None of these patients had any serious adverse event resulting from acute leptin withdrawal, although long-term (months to years) worsening of metabolic parameters (A1c, triglycerides, ALT/AST) and other adverse events related to the natural history of lipodystrophy were observed. Based on these observations, we estimate that the risk of pancreatitis during short-term leptin withdrawal is low, but non-zero. The following measures are being taken to minimize the risk of pancreatitis to patients undergoing leptin withdrawal in this study:

- i. Subjects at increased risk of pancreatitis are excluded, as follows:
  - a. **Triglycerides over 2000 mg/dL at initiation of leptin treatment.** This will exclude patients with extreme hypertriglyceridemia at baseline, who would be more likely to rebound to high levels with leptin withdrawal, regardless of their current triglyceride control or history of pancreatitis.
  - b. **Prior history of more than one episode of pancreatitis, or 1 or more episodes of pancreatitis after starting leptin.** Even in the presence of a known risk factor such as hypertriglyceridemia, having recurrent pancreatitis (more than one episode) suggests that a patient may be at intrinsically greater risk of pancreatitis. We have therefore chosen a very conservative cutoff of more than one prior episode. This will permit enrollment of subjects who have had a single, isolated episode of pancreatitis prior to starting leptin. Subjects who have had pancreatitis while receiving leptin are presumed to be at greater risk with leptin withdrawal, and will not be enrolled. Unfortunately, a careful literature review reveals no data to more precisely quantify the risk of future pancreatitis based on past pancreatitis. The spectrum between acute, recurrent acute, and chronic pancreatitis is poorly understood, but even in patients with a risk factor such as hypertriglyceridemia,



additional “hits” (such as genetic modifiers) may be necessary to trigger recurrent or chronic pancreatitis<sup>52</sup>.

- c. **Current triglycerides over 800 mg/dL.** Our gastroenterology consultants felt that a patient’s risk of pancreatitis is more likely to be related to their pre-leptin triglycerides and their past history of pancreatitis, rather than their current triglyceride level. The level of hypertriglyceridemia that is likely to occur after leptin withdrawal in a patient with triglycerides of 800 at the onset of the study is not known. In a single patient, the effect of 10 days of leptin withdrawal on triglycerides during a controlled diet was measured. In this patient (NIH-1), triglycerides rose from 400 to 800 mg/dL. If this response is typical, we would anticipate that, in a patient enrolled with triglycerides of 800 mg/dL, the triglyceride level might increase to 1200 mg/dL after leptin withdrawal, which is just above the threshold of risk for pancreatitis. However, it is important to note that pancreatitis is a rare complication of hypertriglyceridemia, even when serum triglycerides are elevated above 1000 mg/dL. The risk of subjects reaching this degree of triglyceride elevation will be reduced by only enrolling those with triglycerides <800 mg/dL, as well as keeping subjects on a fat and carbohydrate controlled diet during leptin withdrawal.
- d. **Current lipase > 491 units/L (the upper limit of normal in the NIH assay).** While both amylase and lipase are often used clinically in the assessment of pancreatitis, amylase is a non-specific test that may be elevated for other reasons. Subjects with elevated lipase at study entry will be considered to have laboratory findings consistent with pancreatitis, and will be excluded.
- ii. The key element that will minimize risk to subjects is that leptin withdrawal will be performed under close inpatient observation with a controlled diet that should help maintain lower blood glucose and triglycerides. Clinical and laboratory monitoring of subjects for pancreatitis will be performed throughout the period of leptin withdrawal, as follows
  - a. Safety labs will be monitored approximately every other day (when research labs are obtained). These will include amylase, lipase, and stool pancreatic elastase. Elevated lipase (greater than the upper limit of normal, 491 units/L) will be a withdrawal criterion. Elevated amylase will be used only as a supportive criterion. Stool pancreatic elastase will be measured primarily for research purposes, as a marker of pancreatic exocrine function. Although triglycerides will be measured along with these labs, isolated elevation of triglycerides, without signs or symptoms of pancreatitis or elevated lipase, will not be a withdrawal criterion.
  - b. If pancreatitis is clinically suspected (either based on signs/symptoms consisting of moderate to severe abdominal pain with nausea or vomiting, or based on lipase), the following will take place:
    - i. Amylase and lipase levels will be drawn
    - ii. GI consultation will be obtained
  - c. If a diagnosis of pancreatitis is likely or confirmed based on labs and/or GI consultation, the following additional actions will be taken
    - i. Appropriate treatment will be initiated based on GI recommendations (usually, NPO and pain medications)
    - ii. Leptin will be restarted immediately
    - iii. The patient will be withdrawn from the study
    - iv. The IRB will be notified per guidelines for SAEs

- 4. **Blood Sampling:** Peripheral blood draws (venipuncture) performed during this study for research will not exceed 10.5 mL/kg, or 550 mL (whichever is smaller) per 8-week period for adults. For pediatric

patients, blood draws will not exceed 5 mL/kg in a single day, or 9.5 mL/kg or 550 mL (whichever is smaller) per 8 week period. Patients may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

5. Oral Glucose Tolerance Test: Patients will be required to drink a very sweet glucose containing beverage. This may be associated with mild nausea or distaste. Due to serial blood draws, an intravenous line will be placed, with attendant risks of discomfort and possible skin infection.
6. Euglycemic Hyperinsulinemic Clamp Study: In order to assess insulin sensitivity, patients will receive an intravenous infusion of insulin and – via a second intravenous access – increasing amounts of glucose to maintain euglycemia. Insertion of the necessary intravenous lines may cause the above-mentioned discomfort and a possible skin infection. Insulin infusion can induce hypoglycemia and hypokalemia. Either event is very unlikely, since patients undergo frequent blood glucose measurements and are constantly observed for the presence of hypoglycemic symptoms by an experienced nurse and/or physician. However, in order to prevent complications, two patent, well-functioning intravenous lines are required during this procedure.
7. Glucose and Lipid Turnover: The stable isotopes C is not associated with any toxicity at the doses used in these studies <sup>53</sup>. Deuterium at the doses given may cause temporary dizziness. This risk will be minimized by dividing the <sup>2</sup>H<sub>2</sub>O dose into four aliquots, and by giving the doses at night while subjects lie in bed. If subjects need to get out of bed (e.g. to use the bathroom) the patient will first sit upright in bed for a few minutes prior to standing, and a nurse will be available to supervise and assist. Palmitate must be complexed to albumin prior to delivery, and thus carries the risks associated with use of human blood products. To minimize exposure to human albumin, uniformly labeled <sup>13</sup>C<sub>16</sub> palmitate will be used, permitting use of the minimum possible dose of this tracer.
8. Resting Energy Expenditure: There are no significant risks associated with measurement of resting energy expenditure. Some patients may feel uncomfortable from lying still with a plastic hood over the head for approximately 30 minutes.
9. Actigraphy, heart rate variability, blood pressure monitoring, and skin temperature: There are no significant risks associated with actigraphy, or monitoring of heart rate, blood pressure, or skin temperature. Some patients may find it an inconvenience to wear the devices.
10. DEXA Scan: The effective radiation dose with total body DEXA scanning is 0.00001 rem. This is equivalent to average daily background radiation from natural sources, and is not thought to increase lifetime cancer risk. Subjects in this study will have up to three DEXA scans (total radiation dose 0.00003 rem) per year.
11. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS): While MR scanning is thought to be safe, the procedure may cause anxiety in some patients since current equipment used at the Clinical Center uses a closed tube. Adult patients will be offered sedatives such as Valium if they express worry about being in a closed space. Conscious sedation will not be employed on adolescents to perform any of these tests. Parental support will be sought if adolescents find it hard to stay immobile for the duration of the studies. No intravenous contrast will be used in these studies.
12. 24 hour urine collection: Urine collection may be slightly inconvenient for subjects; however, there is no health risk.

13. Metabolic Chamber: Some inconvenience can reasonably be expected as a result of spending an extensive time (24 hours) in the live-in room calorimeter. However, the risk to subjects' health is minimal.
14. Ultrasounds: The pelvic ultrasound may be uncomfortable due to pressure on a full bladder but there are no risks.
15. Echocardiogram: The echocardiogram may be inconvenient but there are no risks to the subject's health.

### Alternatives to participation

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

### Financial Compensation

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

| Procedure          | Inconvenience Units | Compensation | Short-term (3 week) study |               | Long-term follow-up visit |              |
|--------------------|---------------------|--------------|---------------------------|---------------|---------------------------|--------------|
|                    |                     |              | Occurrence                | Total         | Occurrence                | Total        |
| Inpatient per diem | n/a                 | \$40         | 20                        | \$800         | 4                         | \$160        |
| Metabolic Chamber  | 5                   | \$50         | 3                         | \$150         | 1                         | \$50         |
| DEXA               | 1                   | \$10         | 2                         | \$20          | 1                         | \$10         |
| Exercise test      | 1                   | \$10         | 3                         | \$30          | 1                         | \$10         |
| Euglycemic Clamp   | 5                   | \$50         | 2                         | \$100         | 1                         | \$50         |
| Tracer studies     | 2                   | \$20         | 3                         | \$60          | 1                         | \$20         |
| LH pulsatility     | 3                   | \$30         | 2                         | \$60          | 1                         | \$30         |
| MRI/MRS            | 3                   | \$30         | 2                         | \$60          | 1                         | \$30         |
| <b>Total</b>       |                     |              |                           | <b>\$1280</b> |                           | <b>\$410</b> |

### Consent/Assent Procedures

Written consent/assent will be obtained from each subject after detailed explanations of the planned procedures by the principal or an associated investigator in language understandable to the subject. The consent process will take place prior to any study procedures. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions the subject may have, taking care to minimize or eliminate the perception of coercion or undue influence. The participant and the investigator will sign the current IRB-approved informed consent document. A copy of the consent will be given to the subject for future reference and the consenting process will be documented in the electronic medical record (CRIS).

Given the prospect of direct benefit for minor subject participants, only one parent/guardian or LAR will need to give written informed consent prior to any screening visits, study procedures or treatments. The Principal Investigator or other designated qualified protocol investigators will explain the study in

language understandable to the parent/guardian or LAR. If appropriate, 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. A witness will also sign the consent document to attest only to the validity of the signature of the parent/guardian or LAR, not the validity or quality of the consent. If appropriate, the investigator will have the minor will provide assent, and if possible, will sign the current IRB approved assent document. The investigator will sign the assent as well. A copy of the consent and assent will be given to the minor and his/her parent/guardian or LAR for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS)

For patients currently enrolled on the protocol, that are turning of legal age (i.e. 18 years of age) and who are able to provide consent, we plan to follow the procedures outlined in NIH SOP 14D and the same procedures described above in order for the patient to continue receiving study drug without interruption.

We do not plan or anticipate the enrollment of non-English speaking subjects. However, they are not excluded from participation either. If there is unexpected enrollment of a research participant for which there is no translated extant IRB-approved consent document, the Principal Investigator and/or those authorized to obtain informed consent will use the short form consent process as described in MAS Policy M77-2, NIH SOP 12, 45 CFR 46.117 (b), and 21 CFR 50.27 (b). The summary that will be used is the English version of the extant IRB-approved consent document. We request prospective IRB approval of the use of the short form consent process for up to a maximum of 5 requests (either for individual participants or families of participants) 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 subjects and/or families speaking a single language, we will request an additional use of the short form from the IRB and will notify the Board that we plan to have any consent documents frequently used with that population translated into the language(s) they speak.

Subjects have the right to withdraw participation from this protocol at any time.

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

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

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

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

**Material Transfer Agreement/Collaboration(s):**

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

Collaboration has been established with Dr. Ravinder J. Singh at the Mayo Clinic in Rochester, Minnesota. The purpose of the collaboration is to assess whether steroid synthesis and metabolism is altered in patients with lipodystrophy and effected by metreleptin treatment. This collaboration will involve transfer of coded human plasma and urine samples (not data), review of the coded data derived, and interpretation of the results.

Collaboration has been established with Dr. Elizabeth Parks at the University of Missouri School of Medicine. The purpose of the collaboration is to measure rates of de novo lipogenesis during periods in which patients do or do not receive leptin, using stable isotope tracer techniques. This will involve transfer of coded human frozen plasma, and/or isolated triglyceride-rich particles derived from frozen plasma, review of the coded data derived and interpretation of the results.

Collaboration has been established with Professor Stephen O’Rahilly in Cambridge, England. Our direct physician contacts are Dr. Robert Semple and Dr. David Savage. The purpose of this collaboration is to share coded de-identified clinical data collected from subjects enrolled under this protocol at NIH. The Laboratory at Cambridge University will use the coded data, including laboratory, anthropometric, imaging and demographic data, to characterize the phenotype of patients affected by suspected mutations of the insulin receptor or lipodystrophy and to study the association between phenotypes and causative genetic mutations, in order to better understand the pathogenesis and natural history of these diseases, improve the diagnosis, and predict and assess response to treatment.

Collaboration has been established with Elif Oral, MD at the University of Michigan in Ann Arbor. Dr. Elif Oral, is a former NIDDK investigator, and familiar with our patient population. The purpose of this collaboration is to improve the diagnosis of patients with lipodystrophy by characterizing their phenotype compared to control subjects, and to determine biomarkers which may predict response to treatment. To reach these goals, Dr. Oral will be provided coded de-identified blood samples (serum, plasma) and coded de-identified clinical data to analyze body composition and additional clinical data (including laboratory, anthropometric, imaging and demographic data) from study participants enrolled at NIH under this protocol.

Collaboration has been established with Abhimanyu Garg, MD at the University of Texas Southwestern. This collaborator is a former associate investigator of our original leptin protocol, and is a world’s expert on syndromes of lipodystrophy. The purpose of this agreement is to transfer coded de-identified metabolic and clinical data, including laboratory, anthropometric, imaging and demographic data, to characterize the phenotype of patients affected by lipodystrophy and to study the association between phenotypes and causative genetic mutations, in order to better understand the pathogenesis and natural history of this disease, improve the diagnosis, and predict and assess response to treatment.

# Appendices

## Appendix A: Sample Patient Calendars

### Short-term portion of study (leptin naïve and leptin treated subjects)

|           | Period 1                    |                 |                      |                   |                  |                   | Period 2         |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
|-----------|-----------------------------|-----------------|----------------------|-------------------|------------------|-------------------|------------------|-----------------|---------------|---------------|---------------|-------------------|---------------|-------------------|------------------|---------------|---------------|-------------------|------------------|---------------------------------|------------------|--|
| Study Day | -6                          | -5              | -4                   | -3                | -2               | -1                | 0                | 1               | 2             | 3             | 4             | 5                 | 6             | 7                 | 8                | 9             | 10            | 11                | 12               | 13                              | 14               |  |
| Time      |                             |                 |                      |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 6:00      |                             | Fasting labs    |                      |                   | LH sampling      | Fasting labs      | Chamber          |                 | Fasting labs  |               | Fasting labs  |                   | Fasting labs  |                   | Chamber          |               | Fasting labs  |                   | Fasting labs     | LH sampling                     | Chamber          |  |
| 6:30      |                             |                 |                      |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 7:00      |                             | REE             |                      |                   |                  | Start 24 hr urine | Stop 24 hr urine | REE             |               |               |               | REE               |               | Start 24 hr urine | Stop 24 hr urine | REE           |               |                   | Stop 24 hr urine | Fasting labs/ Start 24 hr urine | Stop 24 hr urine |  |
| 7:30      |                             |                 |                      |                   | Start tracers    | Start skin temp   | DEXA             |                 |               |               |               |                   | Start tracers |                   |                  |               |               |                   | Start tracers    | Start skin temp                 | DEXA             |  |
| 8:00      |                             | Start diet      |                      |                   | REE              | Chamber           | Fasting labs     |                 |               |               |               |                   |               | Chamber           | Fasting labs     |               |               |                   | REE              | Chamber                         | Stop Actigraph   |  |
| 8:30      |                             |                 |                      |                   |                  | VAS (fasting)     | Stop skin temp   |                 |               |               |               |                   |               | VAS (fasting)     |                  |               |               |                   | VAS (fasting)    |                                 | Stop skin temp   |  |
| 9:00      | Oral Glucose tolerance Test | Start Actigraph | Pelvic Ultrasound    |                   |                  |                   | Start BP monitor |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 | Dis-charge       |  |
| 9:30      |                             |                 |                      |                   |                  |                   |                  | Stop BP monitor |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 10:00     |                             |                 | Abdominal ultrasound | Exercise test     |                  |                   |                  |                 |               |               |               | Exercise test     |               |                   |                  |               |               | Exercise test     |                  |                                 |                  |  |
| 10:30     |                             |                 |                      |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 11:00     |                             |                 |                      | Skin temp monitor |                  |                   |                  |                 |               |               |               | Skin temp monitor |               |                   |                  |               |               | Skin temp monitor |                  |                                 |                  |  |
| 12:00     |                             |                 |                      |                   | Clamp            |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   | Clamp            |                                 |                  |  |
| 13:00     | Admit                       | VAS (p-lunch)   | VAS (p-lunch)        | VAS (p-lunch)     |                  | VAS (p-lunch)     | VAS (p-lunch)    | VAS (p-lunch)   | VAS (p-lunch) | VAS (p-lunch) | VAS (p-lunch) | VAS (p-lunch)     | VAS (p-lunch) | VAS (p-lunch)     | VAS (p-lunch)    | VAS (p-lunch) | VAS (p-lunch) | VAS (p-lunch)     | VAS (p-lunch)    | VAS (p-lunch)                   |                  |  |
| 14:00     |                             |                 |                      | MRI               |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               | MRI               |                  |                                 |                  |  |
| 15:00     |                             |                 | Thyroid ultrasound   |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 16:00     |                             |                 |                      |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 17:00     |                             |                 |                      |                   | Start BP monitor |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   | Start BP monitor |                                 |                  |  |
| 18:00     |                             |                 |                      |                   |                  | Stop BP monitor   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  | Stop BP monitor                 |                  |  |
| 19:00     |                             |                 |                      |                   |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   |                  |                                 |                  |  |
| 20:00     | Exercise                    | Exercise        | Exercise             | Exercise          | Exercise         | Exercise          | Exercise         | Exercise        | Exercise      | Exercise      | Exercise      | Exercise          | Exercise      | Exercise          | Exercise         | Exercise      | Exercise      | Exercise          | Exercise         | Exercise                        | Exercise         |  |
| 23:00     |                             |                 |                      | 8 hr LH sampling  |                  |                   |                  |                 |               |               |               |                   |               |                   |                  |               |               |                   | 8 hr LH sampling |                                 |                  |  |

## Appendix A: Sample Patient Calendars

### Long-term follow-up (leptin naïve subjects, only)

| Study Day | 0     | 1                | 2                 | 3                | 4                 | 5                |
|-----------|-------|------------------|-------------------|------------------|-------------------|------------------|
| Time      |       |                  |                   |                  |                   |                  |
| 5:00      |       |                  |                   | Start tracers    |                   |                  |
| 6:00      |       | Fasting labs     | LH sampling       |                  |                   | Chamber          |
| 6:30      |       |                  |                   |                  |                   |                  |
| 7:00      |       | OGTT             | REE               |                  | Start 24 hr urine | Stop 24 hr urine |
| 7:30      |       | (NPO)            |                   |                  | Start skin temp   | DEXA             |
| 8:00      |       |                  |                   |                  | Chamber           |                  |
| 8:30      |       |                  |                   |                  |                   | Stop skin temp   |
| 9:00      |       |                  | Start Actigraph   |                  |                   |                  |
| 9:30      |       |                  | Skin temp monitor | Clamp            |                   | Stop Actigraph   |
| 10:00     |       |                  | Exercise test     |                  |                   |                  |
| 10:30     |       |                  |                   |                  |                   |                  |
| 11:00     |       |                  | Skin temp monitor |                  |                   | Discharge        |
| 12:00     |       |                  |                   |                  |                   |                  |
| 13:00     | Admit |                  |                   |                  |                   |                  |
| 14:00     |       |                  | MRI               |                  |                   |                  |
| 15:00     |       |                  |                   |                  |                   |                  |
| 16:00     |       |                  | Start HR monitor  | Stop HR monitor  |                   |                  |
| 17:00     |       |                  |                   | Start BP monitor | Stop BP monitor   |                  |
| 18:00     |       |                  |                   |                  |                   |                  |
| 19:00     |       |                  |                   |                  |                   |                  |
| 20:00     |       |                  |                   |                  |                   |                  |
| 23:00     |       | 8 hr LH sampling |                   |                  |                   |                  |

AM O (IRB approved 5/14/2018)

13-DK-0057

Submitted for CR 2019: 1/11/2019



## Appendix B: Investigator Qualifications and Roles

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

**Kong Chen, Ph.D.** is the director of the Human Metabolism and Body Weight Regulation Core, and a Clinical Investigator of the Diabetes Endocrinology, and Obesity Branch. Dr. Chen's role in this project will be to assist Dr. Brown with the measurements and data interpretation of energy expenditure, body composition, exercise efficiency, body temperature, and physical activity.

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

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

**Ahmed Gharib, M.D.**, is a board certified radiologist and clinical research in NIDDK. Dr. Gharib's role in this study will be to assist Dr. Brown with the measurement and interpretation of liver and muscle triglyceride.

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

**Kevin Hall, Ph.D.**, is a researcher in the Laboratory of Biological Modeling in NIDDK. His research focuses on using mathematical modeling to quantitatively integrate metabolism data with body weight and composition data. Dr. Hall's role in this project will be to assist Dr. Brown with the measurements and data interpretation of energy expenditure and body composition.

**Morey W. Haymond, M.D.** is a board-certified pediatric endocrinologist and Professor of Pediatrics and Medicine at Baylor College of Medicine. Dr. Haymond's role in this project will be to assist Dr. Rebecca Brown in the conduct of the clinical studies determining the rates of glucose production and gluconeogenesis in subjects with a variety of forms and severity of lipodystrophy. This will include the review of the protocol design, potentially the analysis of subject samples, review of the data derived and interpretation of the results.

**Megan Mattingly, RN, MPH** is a research nurse in the Diabetes, Endocrinology and Obesity Branch. Ms. Mattingly's role in this project will be to assist Dr. Brown with the coordination of patient visits, patient education and communication, as well as assist in measurement of insulin sensitivity using the euglycemic hyperinsulinemic clamp studies.

**Ranganath Muniyappa MD, PhD.** is a board-certified Endocrinologist and a Staff Clinician at DEOB, NIDDK, NIH. Dr. Muniyappa's role in this project will be to assist Dr. Brown with the assessment of insulin sensitivity/resistance using the euglycemic hyperinsulinemic clamp studies.

**Marc Reitman MD PhD.** is trained in internal medicine and endocrinology and metabolism and is currently a senior investigator and branch chief in intramural NIDDK. Dr. Reitman is an expert in leptin physiology and clinical trials. His role in this project will be to work with Dr. Brown on study strategy, design, and interpretation.

**Andrea Ramirez, M.D. M.S.,** is an Assistant Professor of Medicine in the Division of Diabetes, Endocrinology and Metabolism, with the Department of Medicine at Vanderbilt University School of Medicine in Nashville, TN. Dr. Ramirez earned her M.D. at Duke University and trained in Internal Medicine and Clinical Pharmacology at Vanderbilt University. She also earned her Masters of Science in Clinical Investigation at Vanderbilt University. Dr. Ramirez trained in Endocrinology, Diabetes, and Metabolism at the National Institutes of Health and is certified by the American Board of Internal Medicine in Internal Medicine and Endocrinology, Diabetes, and Metabolism. She has participated in multiple clinical protocols related to endocrinologic disease and studies the genetics of diabetes. Dr. Ramirez's role in this project will be to assist Dr. Rebecca Brown in determining the effects of leptin on circulating nucleic acids and pharmacoepigenomics of leptin therapy.

**Ravinder J. Singh, M.D., Ph.D.,** is a consultant Laboratory Medicine and Pathology at the Mayo Clinic located in Rochester, Minnesota. Dr. Singh's role in this project is to assist Dr. Rebecca Brown in the conduct of clinical studies by assessing whether steroid synthesis and metabolism is altered in patients with lipodystrophy and effected by metreleptin treatment. Dr. Singh will perform different measurements that he has developed using mass spectrometry using coded human plasma and urine samples, collected from the study subjects enrolled at NIH.

**Elizabeth Parks, Ph.D.,** is a professor of nutrition and exercise physiology at the University of Missouri School of Medicine. Dr. Parks' role in this project is to assist Dr. Rebecca Brown in the conduct of the clinical studies by examining the effects of leptin in patients with lipodystrophy on glucose and lipid metabolism and energy expenditure, independent of food intake. Dr. Parks plans to measure rates of de novo lipogenesis during periods in which patients do or do not receive leptin, using stable isotope tracer techniques.

**James Reynolds, M.D.,** is board-certified in endocrinology and nuclear medicine and a Scientist Emeritus at the National Institutes of Health and special volunteer in Radiology and Imaging Sciences Department. He is a Certified Clinical Densitometrist (ISCD) with 25 years' experience in bone densitometry and body composition studies. He will be assisting Dr. Rebecca Brown in providing data and analysis of whole body densitometry scans on patients with Lipodystrophy.

## Appendix C: Myalept Package Insert



package  
insert\_myalept\_acces

## **Appendix D: Waiver of Consent**

### **PURPOSE**

The purpose of this project is to understand more about the health problems experienced by people with lipodystrophy, so that we can understand how treatments such as leptin may improve or prevent those health problems. If we can show that patients who have not received leptin treatment have worse health problems than those who have received leptin treatment, this may eventually lead to wider availability of leptin treatment.

This project has four objectives:

- To describe the demographic and clinical characteristics of patients with generalized and partial lipodystrophy.
- To describe the severity of metabolic complications (e.g. hyperlipidemia, diabetes, steatohepatitis) of lipodystrophy and assess the association of disease severity markers (i.e., elevated glucose, triglycerides, low leptin levels) with survival.
- To characterize the burden (e.g., pain, organ damage, hyperphagia, school/work productivity) of lipodystrophy and assess the impact of disease severity markers (i.e., elevated glucose, triglycerides, low leptin levels)
- To assess the potential benefit of leptin replacement therapy on all of the above.

### **DATA COLLECTION**

Aegerion Pharmaceuticals, the current owner of leptin, through Analysis Group, Inc. would like to perform a one-time data abstraction from NIH to further understand the benefits of leptin.

The data sources that will be used by Analysis Group are:

1. Manual review of NIH medical records
2. Electronic download of NIH medical record data using BTRIS
3. Research records (not contained in CRIS or BTRIS) from the approved protocols covered by this consent waiver
4. Direct queries to investigators

Subjects whose data are abstracted for this project, will not be contacted about results pertaining to this amendment without prior approval by the IRB.

Below are the key variables to be abstracted:

- Demographics
- Diagnosis
- Anthropometric measurements (e.g., neck, waist, hip, arm circumferences; % fat, BMI)
- Family history
- Metabolic and lipid profile
  - Lab values (e.g., HbA1c, triglycerides)
  - Presence of diabetes or insulin resistance
- Liver profile
  - Lab values (including biopsy)

- Diagnoses of liver diseases
  - Liver transplant (new or historical)
- Kidney profile
  - Lab values (including biopsy)
  - Kidney transplant (new or historical)
- Pancreas profile
  - Diagnoses of pancreatic diseases
- Heart disease
  - Diagnoses of heart diseases
  - Heart transplant (new or historical)
- Medication use
  - Insulin
  - Metformin
  - Other diabetes medications
  - Triglycerides medications
  - Other lipid lowering medications
  - Antihypertensive medications
- Comorbidities (e.g., diabetes, hypertriglyceridemia, surgical history, malignancy, etc.)
- Visit information (including withdrawal from the study)
- Adverse events and associated resource utilization (e.g., emergency room visits, hospitalization, mortality and cause)
- Reproductive history
- Quality of life:
  - Hyperphagia history
  - Social/work productivity (as documented in Social History in medical records)
  - Pain/fatigue history
  - Psychological distress (e.g. related to physical appearance)

## **PROTECTION OF SUBJECT CONFIDENTIALITY**

The Analysis Group, Inc. has had all the relevant training to extract and review data. They are required to perform their services in accordance with HIPAA and other state/federal/privacy laws. The Analysis Group staff will receive NIH medical records system training. It is necessary for staff from Analysis Group to have access to PII while they are physically at NIH, because of the amount of effort that would be required by NIH staff to manually redact all PII from all patient medical records. All the data that leaves the NIH would be entered onto electronic case report forms (e-CRFs) housed in an electronic data base and would be completely de-identified. Each patient's data will be associated only with their study ID number. Only NIH research staff involved in these studies will be able to connect the study ID number to the patient's identity. No patient samples of any kind (e.g. blood, urine, DNA) will leave the NIH as part of this study. If data is lost or destroyed, we will report this to the NIDDK/NIAMS IRB.

## **CONSENT/ASSENT PROCEDURES**

Because the informed consent documents for this study did not specifically address this use of data, we have requested a waiver of informed consent.

All subjects agreed to participate in the study designed to test the safety and effectiveness of long-term leptin replacement. All patients started in 02-DK-0022 or 13-DK-0057. Additionally, most of the patients were also co-enrolled in 76-DK-0006 in which they consented to participate in studies the purpose of which was to understand the natural history of insulin resistance and lipodystrophy. Because

the purpose of the current project is identical to that expressed in the past consents, the rights and welfare of the patients are not altered.

The original and current consent for this protocol states, “Your stored samples and/or data may be sent to outside collaborating laboratories or shared with other NIH collaborating investigators. We will use these samples and data to study questions related to lipodystrophy and its complications (such as diabetes, lipids, and liver disease). Samples (blood or fluids) and data shared with collaborators or sent to commercial laboratories for research purposes will be labeled only with coded numbers, without personal identifiers.”

We would like to request a waiver of informed consent to proceed with this project.

NIH SOP Number 12 addresses the requirements for waiving the consent process. We wrote our response to each of the four points below.

## **12.10 WAIVING OR ALTERING ELEMENTS OF INFORMED CONSENT UNDER 45 CFR 46**

A. Circumstances in which the IRB may waive or alter elements of the informed consent procedure or waive the requirements to obtain informed consent: An NIH IRB may approve a consent procedure that does not include, or which alters some, or all, of the elements of informed consent set forth in 45 CFR 46.116(ab), or waive the requirements to obtain informed consent, provided the IRB finds and documents in the IRB meeting minutes:

1. The research involves no more than minimal risk to the subjects.

**Response:** Extracting the data from this protocol would involve no more than minimal risk to the subjects involved. The data will be de-identified and the team extracting the data is expected to perform their services per HIPPA and other relevant federal/state privacy laws.

2. The waiver or alteration will not adversely affect the rights and welfare of the subjects.

**Response:** Extracting the data from this protocol would not adversely affect the rights and welfare of the subjects. All subjects consented to participate in studies of which the purpose was to understand the natural history of insulin resistance and lipodystrophy as well as the efficacy of leptin over the long term. All subjects consented to share their data with other collaborating investigators as long as their data was de-identified. Because the purpose of the current project is identical to that expressed in the consents the rights and welfare of the patients are not altered.

3. The research could not practicably be carried out without the waiver or alteration, and

**Response:** Currently there are 28 patients enrolled in this protocol from across the United States, Canada and Argentina. They are on a one to two year visit cycle and re-consenting patients could not be completed in a timely manner.

4. Whenever appropriate, the subjects must be provided with additional pertinent information after participation (see 45 CFR 46.116(d), link also in **References**

**Response:** We do not think that this is relevant to this project.



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